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ENDOGENOUS DIGITALIS-LIKE FACTORS. AN HISTORICAL OVERVIEW

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1. ABSTRACT

The sodium pump is a ubiquitous cell surface enzyme, a Na/K-ATPase, that maintains ion gradients between cells and the extracellular fluid. The extracellular domain of this enzyme contains a highly conserved receptor for a plant-derived family of compounds, the digitalis glycosides, used in the treatment of congestive heart failure, and certain cardiac arrhythmias. The concept that an endogenous modulator of this enzyme, analogous to the cardiac glycosides, emerged from work on two separate areas: the regulation of extracellular fluid (ECF) volume by a natriuretic hormone (NH), and the regulation of peripheral vascular resistance by a circulating inhibitor of vascular Na/K-ATPase. These two areas merged with the hypothesis that natriuretic hormone and the vascular Na/K-ATPase inhibitor were the same factor, and furthermore, that this factor played a causative role in the pathophysiology of certain types of hypertension. In this communication, the development of this field from its beginnings is traced; evidence for the existence of and efforts to identify the structure of this factor are briefly reviewed, and suggestions for future development of the field are put forward.

2. INTRODUCTION

The digitalis glycosides are a class of plant-derived steroids (cardenolides) used in the treatment of heart failure whose main effect is to inhibit the ubiquitous cell surface enzyme Na/K-ATPase. Na/K-ATPase is the "sodium pump" which transports sodium (Na) ions out of cells and potassium (K) ions into cells, thus maintaining the ion gradients between intracellular and extracellular compartments necessary for normal cell function. Na/K-ATPase has two subunits, alpha and beta, with the extracellular domain of the alpha subunit containing a stereospecific-binding site for the digitalis glycosides and related steroids (1).

The identification of the sodium pump as Na/K-ATPase, and the effect of digitalis glycosides to inhibit the sodium pump, occurred in the 1950s (2). Before the discovery of Na/K-ATPase, Ringer in 1885 suggested the possibility of an endogenous compound that stimulated cardiac contraction in a manner similar to the digitalis glycosides (3). The modern development of the concept of endogenous digitalis-like factors (EDLF) began in the late 1970s with the convergence of two lines of investigation:

the regulation of renal sodium excretion by extracellular fluid (ECF) volume, and the pathophysiology of volume expanded models of hypertension.

3. ECF VOLUME REGULATION

3.1. Natriuretic Hormone

In a classic article in 1957, Homer Smith reviewed the existing evidence on the regulation of renal sodium excretion. Based on this review, he postulated the existence of a receptor-integrator-effector reflex by which changes in ECF volume caused appropriate changes in renal sodium excretion (4). Based on evolutionary considerations, he postulated that the proposed effector, which he called "Hormone X", was an anti-natriuretic hormone, analogous to antidiuretic hormone, which evolved to conserve sodium as our primitive ancestors made their "ascent through the brackish waters of the estuary/to the salt poor lakes and ponds" (from a poem by Dr. Maurice Strauss). Aldosterone had been identified in 1953 by Luetscher and Johnson (5), so Smith's Hormone X was clearly proposed as an additional volume sensitive sodium retaining hormone, decreased levels of which would cause natriuresis in response to increased ECF volume.

Four years later, the controversy as to whether the natriuresis caused by increased ECF volume was all due to an increase in GFR or to a decrease in tubular sodium reabsorption was resolved in favor of the latter in a classic paper by deWardener and colleagues published in 1961 (6). They showed that natriuresis caused by saline infusion in dogs was not abolished when GFR was reduced below initial levels by constriction of the aorta above the renal arteries. Furthermore, they showed that blood circulated from volume-expanded dogs (donor) to euvolemic dogs (recipient) caused natriuresis in the recipient. Based on these studies, deWardener and colleagues suggested that volume expansion increased the circulating level of some natriuretic substance, and the concept of "natriuretic hormone" was born.

Although the early cross circulation studies were interpreted as indicating the presence of a natriuretic substance, the results could have been due to suppression of an anti-natriuretic factor as suggested by Smith. The first actual demonstration that the effector substance of the volume expansion "reflex" might be natriuretic, rather than anti-natriuretic, was by Cort and Lichardus (7-9) in the mid-1960s. They showed that extracts of plasma from cats undergoing natriuresis after carotid artery occlusion (which they considered to be a model of central blood volume expansion that avoided the problem of plasma dilution caused by physiological saline infusions) caused natriuresis in assay rats and inhibited sodium transport in frog skin. Other investigators soon showed that plasma, plasma extracts and urine from volume-expanded animals caused natriuresis when injected into other animals (10-14), the mechanism of which drew immediate interest. The question was whether the factor caused changes in renal hemodynamics or inhibited sodium transport systems. The first studies suggesting the latter were performed by Bricker, *et al.* in which inhibition of PAH transport by rabbit kidney cortical slices was inhibited by plasma from volume expanded subjects (15). Inhibition of transport in renal tubular epithelium was subsequently shown in isolated tubular cells (16).

As noted, Cort and Lichardus reported inhibition of sodium transport as measured by Ussing's short circuit current (SCC) technique in isolated frog skin by deproteinized, concentrated plasma extracts with very high sodium concentrations. In more extensive studies using plasma ultrafiltrates with physiological salt concentrations from volume-expanded dogs, Buckalew and colleagues in 1970 showed similar effects on toad bladder SCC of toads (*bufo marinus)* (17). Ussing and others had demonstrated that the SCC in anuran membrane was due to active sodium transport, and could be inhibited by ouabain. The demonstration that the putative natriuretic hormone inhibited SCC thus set the stage for investigation of the effect of this factor, or factors, on Na/K-ATPase. The initial attempts to relate natriuretic hormone to Na/K-ATPase inhibition were unsuccessful. In the best documented studies, Katz and Genant were unable to show inhibition of the enzyme in renal cortical microsomes from volume-expanded dogs and rats, or an effect of plasma dialysates from these animals on renal microsomal Na/K-ATPase isolated from euvolemic animals (18). However, Gonick and colleagues subsequently reported that a natriuretic fraction extracted from renal tissue and plasma of volume-expanded animals inhibited ouabain sensitive Na/K-ATPase isolated from whole rat kidney (19, 20).

Numerous refinements of the cross circulation studies were published that addressed various criticisms of the early studies, such as the role of plasma dilution, and the fact that the natriuresis in the recipient was much less than that in the donor animal. The former was effectively dealt with by expanding the donor with blood from a reservoir equilibrated with the donor (21). The latter was never entirely explained, but some interesting observations were made. For example, response in the recipient was increased by infusing blood from the donor into the aorta just above the renal arteries (22), suggesting a short biologic half-life of the circulating natriuretic factor. Also, recipient response was enhanced by preventing the donor from excreting the administered volume load, suggesting some effect of "sustained" volume expansion, a poorly defined concept that has not been explored further (23).

A number of investigators explored the effect of plasma, plasma extracts, and urine extract of volume-expanded subjects on sodium excretion in assay animals, usually rats. Two basic patterns were demonstrated that differed primarily in time to peak and duration of effect (24). The shorter acting pattern showed an immediate onset, a peak effect in 40-60 minutes, and duration of about 120 minutes. The longer-acting pattern exhibited a delay in onset of 10-60 minutes, a peak effect in 2-3 hours, and duration longer than 3 hours. Some initial purification studies indicated that the more rapidly acting factor was found in fractions containing low molecular weight substances, and the longer acting factor appeared in

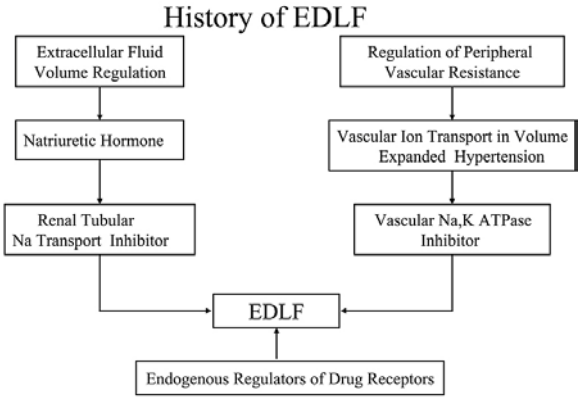

Figure 1. The concept of an endogenous natriuretic hormone that inhibits Na/K-ATPase in a manner similar to the cardiac glycosides developed from two lines of investigation (see text). The concept was further refined by the hypothesis that the presence of highly specific drug receptors in mammalian organisms implied the existence of endogenous analogs of those drugs (see text).

fractions containing high molecular weight substances (24). However, no final purification and identification of these factors were ever published.

Two major developments in the late 70s and early 80s caused a shift in the direction of natriuretic hormone research. The discovery in 1981 of atrial natriuretic factor (ANF) (25) and its subsequent characterization as a peptide cascade present in many organs displaced most other lines of investigation with regard to the existence and nature of a natriuretic hormone. Arguments were advanced that natriuretic hormone systems other than ANF might be important in volume regulation (24). However, very little further work on this hypothesis was performed except as regards the second major development, namely that natriuretic hormone might be an inhibitor of vascular Na/K-ATPase that could also be a causative factor in certain types of hypertension (see next section).

3.2. Volume Expanded Models of Hypertension

The suggestion that some types of hypertension, especially those associated with ECF volume expansion, might be due to a circulating inhibitor of Na/K-ATPase evolved from studies of the phenomenon of potassium (K) induced vasodilation in the late 60s and early 70s. Overbeck and colleagues showed that the dilator response to K, but not to other agents, was suppressed in the forelimb of the rat with two kidneys, one clip hypertension and the dog with one-kidney, one-wrap hypertension (26, 27). Subsequent studies showed that K-induced vasodilation was completely blocked by ouabain, leading to the hypothesis that the vasodilation was due to stimulation of vascular smooth muscle Na/K-ATPase. According to this hypothesis, stimulation of the electrogenic sodium pump led to hyperpolarization, decreased voltage sensitive influx of calcium, and hence vascular relaxation (28).

Reduced serum K produced identical effects in the opposite direction. That is, hypokalemia was associated with vasoconstriction and suppressed Na pump activity suggesting a cause and effect relationship. As predicted by this paradigm, vascular depolarization was found in several volume-expanded hypertension models. Thus, the hypothesis was proposed that vasoconstriction leading to hypertension might be caused by generalized inhibition of vascular Na/K-ATPase activity (29, 30). In a further refinement of the hypothesis in 1976, based on a review of then existing evidence for a humoral factor that slowly increased blood pressure in both animal models and humans with hypertension, Haddy and Overbeck proposed that Na/K-ATPase inhibition in vascular tissue, and hence vasoconstriction, might be due to a circulating factor (31). They, in fact, proposed in that review that the postulated circulating inhibitor of Na/K-ATPase might be "natriuretic hormone". Thus, the two fields of ECF volume regulation, and regulation of vascular tone in volume-expanded models of hypertension, were brought together in the search for a common, explanatory factor (Figure 1).

An alternative explanation connecting vascular tone to the sodium pump was proposed by Blaustein in 1977, a theory that has been widely discussed and stimulated a large number of papers (32). The theory was based on the discovery of a Na-Ca exchanger located in the plasma membrane, driven by the intracellular-extracellular Na gradient. According to the hypothesis, inhibition of the sodium pump by the natriuretic hormone would decrease the Na gradient, inhibiting the outward transport of calcium, thereby increasing vascular smooth muscle calcium concentration, causing vasoconstriction.

The natriuretic hormone hypothesis of hypertension raised the question of how volume regulation by a potentially vasoconstrictor natriuretic hormone occurred in normal versus hypertensive subjects. Volume-expanded models of hypertension involved some manipulation that reduced the ability of the kidney to excrete sodium. This approach was based on the concept proposed by Guyton and colleagues (33) that all hypertension was caused by an abnormal relationship between blood pressure and renal sodium excretion. According to this theory, in normal subjects, renal adaptation to increases and decreases in sodium intake occur without any, or with only small, changes in blood pressure. Thus, renal sodium excretion plotted as a function of blood pressure is nearly perpendicular over a large range of sodium intake. In hypertensive subjects, the set point of this relationship is shifted to the right, with either the same slope or a decrease in slope. In either case, increased blood pressure is required to maintain ECF volume regulation in the presence of impaired renal sodium excretion through the phenomenon of “pressure diuresis”. Guyton postulated the rise in pressure was due to a volume-induced increase in cardiac output and the consequent “long term autoregulation” (i.e. vasoconstriction) that ensued. Thus, ECF volume is maintained at the expense of increased peripheral vascular resistance and high blood pressure.

Based on this theory, several investigators proposed a unifying hypothesis incorporating natriuretic

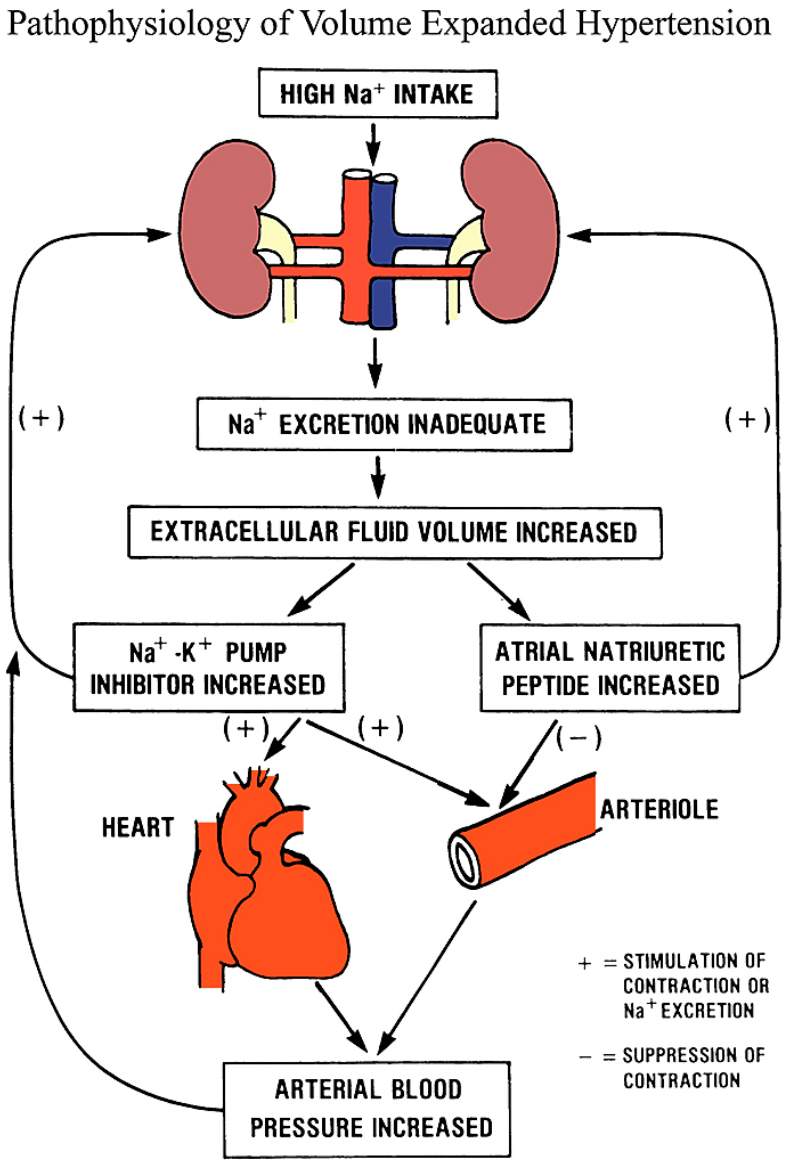

Figure 2. Diagram of a proposed pathophysiology of hypertension in which a defect in renal sodium excretion leads to volume expansion increased circulating levels of an inhibitor of Na/K-ATPase, and atrial natriuretic factor (ANF). The Na pump inhibitor increases cardiac contractility and peripheral vascular resistance, raising blood pressure. This effect is modulated but not overcome by ANF. The increased blood pressure and the pump inhibitor feed back to overcome the defect in renal sodium excretion, preserving ECF volume at a higher than normal blood pressure. (With permission from Kidney International).

hormone that explained many observations then existing in the literature (Figure 2) (34, 35). According to this formulation, the defect in renal response to increases in sodium and water intake in hypertensive subjects leads to increases in natriuretic hormone, vascular Na/K-ATPase inhibition, vasoconstriction and increased blood pressure. Volume homeostasis is maintained in the presence of a defect in renal sodium excretion by both the rise in blood pressure through the mechanism of pressure natriuresis, and by the effect of the natriuretic hormone to inhibit renal tubular sodium reabsorption. The difference between hypertensive and normotensive subjects was, as suggested by deWardener and MacGregor, that the former would be in a "state of continuous correction of a slightly expanded extracellular volume", resulting in a sustained elevation of natriuretic hormone (35).

4. ENDOGENOUS DIGITALIS-LIKE FACTOR

Because of the suggestion that natriuretic hormone might be an inhibitor of Na/K-ATPase it was subsequently referred to as "ouabain-like" or "digitalis-like". This terminology became more than nomenclature as the field turned to proving the true digitalis-like nature of the circulating factor.

4.1. The concept of endogenous drug-like compounds

The demonstration that the specificity and actions of some drugs were due to drug binding to stereospecific receptors led to speculation that naturally occurring endogenous compounds existed that bound specifically to these receptors (36). The discovery of endogenous opioids was a direct result of this hypothesis (37). In 1976, Ginzler *et al.* proposed, as an extension of this concept, that antigen-antibody binding specificity might be analogous to drug-receptor binding specificity (38). That is, an antibody specific for a drug might recognize the same structure as the specific receptor for that drug, and could act as a "surrogate" receptor. This hypothesis had at least two interesting implications. First, antibodies to drugs might recognize endogenous compounds that utilize the same receptor as the drug; and second, antibodies to drugs (or endogenous compounds) might be used to block the effects of those compounds by displacing them from their receptor. The second possibility had already been anticipated by a number of investigators including the demonstration that digoxin antibodies would reverse the clinical manifestations of digoxin intoxication (39).

Based on these concepts, Gruber, *et al.* showed in 1980 that plasma of volume-expanded dogs contained a factor that cross reacted with digoxin antibodies in a specific fashion; i.e. the dose response curve in the digoxin radioimmunoassay (RIA) of the endogenous factor was parallel to that of authentic digoxin (40). Furthermore, plasma extracts containing the digoxin immunoreactive compound inhibited Na/K-ATPase, providing further evidence for a true EDLF that had some structural and functional similarity to digoxin. The finding also suggested that digoxin RIA's could be used to study plasma levels of this factor and numerous studies of mammalian "digoxin-like" factor were soon published. However, studies using this approach are subject to non-specific cross reactivity of various interfering substances in the digoxin RIA and have led to some confusion (41).

4.2. Characterization of endogenous digitalis-like factors

Subsequent to the work briefly described above, numerous attempts were made to purify and identify the principle factor responsible for the EDLF demonstrated in volume-expanded subjects. Numerous candidate structures were proposed, including steroids, lipids, peptides and a variety of other novel compounds (41, 42). A complete review of these reports is beyond the scope of this paper, but a few highlights should be noted.

In 1991, Hamlyn *et al*. reported purification of a compound indistinguishable from ouabain by mass spectroscopy from 300 liters of human plasma (43). Subsequent work has amply confirmed this observation and indicated that mammalian ouabain is present in multiple body fluids and tissues, is probably synthesized by the adrenal gland, and its secretion is subject to hormonal-type regulation (44, 45). Its role in volume regulation and the pathophysiology of hypertension continues to be debated (see below).

Marinobufogenin, a steroid belonging to a class of compounds called bufadienolides, first identified in toads, has been purified and proposed as a physiological regulator of renal sodium excretion and a hypertension-promoting factor by Bagrov and his associates (46). Amphibian species have been known for many years to synthesize a number of different bufodienolides that inhibit Na/K-ATPase in a manner similar to the cardenolides. Dienolides differ from cardenolides in the structure of the lactone ring, which contains six members and two unsaturated double bonds compared to five members and one double bond in the cardenolides (47). Both cardenolides and dienolides have a 14-beta hydroxyl group and a cis tertiary configuration of the C/D ring junction. Lichstein and colleagues published a series of studies of the bufodienolides in toads on the possibility that mammalian EDLF might be a similar compound (48, 49). They identified a compound in toad skin and plasma as resibufagenin and showed that adaptation of its concentration in toad skin was regulated by the salt content of its aquatic environment.

Bagrov and colleagues purified a compound from toad venom which they identified as a previously described dienolide marinobufagenin (MBG), developed a polyclonal antibody to toad MBG, and demonstrated increased concentration of a compound recognized by that antibody in plasma of volume-expanded dogs (46), rats (50) and patients with preeclampsia (51). Subsequent purification by high-pressure liquid chromatography confirmed a structure indistinguishable from authentic MBG (49). Using an antibody specific for ouabain, they further confirmed that the compound they purified was different from ouabain, and that mammalian plasma contains both ouabain and MBG. Subsequent work has demonstrated that the MBG-like compound meets essentially all the criteria originally postulated for the EDLF-type NH described above (52).

Recent work from Takahashi and collaborators has extended the observations of Bagrov's group. Using a highly sensitive liquid chromatography-mass spectrometry technique, they have purified and identified four digitalis-like steroids from mammalian sources as follows: ouabain and digoxin (53), and MBG and telocinobufagin (TCB) (54). TCB differs from MBG by one hydrogen in the 14β position (54). Thus, TCB has an -OH group at the 14β position, while MBG has an epoxy group at the 14-15 position. Quantitative analysis of these two bufadienolides in human plasma showed that the concentrations of both were correlated, but the level of TCB was approximately two-fold higher than MBG (54). The reason for this discrepancy needs further clarification.

Despite efforts by many laboratories, the structure of the endogenous factor or factors that cross reacts with digoxin antibodies has never been firmly established (41). A compound with the mass spectroscopic appearance of authentic digoxin was isolated from 100 tons of human urine, but this finding is complicated by the fact that numerous foods contain compounds that are structurally similar to digoxin, and some of these

accumulate in the adrenal glands (41, 47). As noted, purification of EDLF from various mammalian fluids and tissues has demonstrated compounds indistinguishable from ouabain, MBG, and as noted above, TCB (46,47, 54). Since ouabain and MBG (and presumably TCB) cross-react with digoxin antibodies to some extent, digoxin antibodies might recognize all and are unlikely to distinguish among them (46). Thus, the endogenous digoxin-like factor originally described could be digoxin, ouabain and/or MBG/TCB. It should be noted, however, that binding of one polyclonal digoxin antibody to MBG was 50 times greater than to ouabain (46).

As noted, several lipids were purified that had activity in various assays for digitalis-like activity, and the concentration of one, lysophosphatidyl choline (LPC) was shown to increase in plasma of volume-expanded dogs and to have some weak natriuretic activity (24). However, it was shown that the mechanism of the digitalis-like effects of these lipids was clearly different from that of the glycosides and they were never shown to have any function as physiological regulators. The possibility that EDLF might be a peptide was seriously considered by several laboratories (55). Despite considerable effort, none were ever completely identified. Peptide fragments of pro-opiomelanocortin (POMC), particularly gamma-MSH, were shown to have natriuretic and pressor activity, but they did not inhibit Na/K-ATPase (24). The possibility remains, however, that some of the mammalian digitalis-like activity in mammalian tissues might be due to a peptide or peptides.

4.3. EDLF and hypertension

Using multiple assays for EDLF, numerous studies have attempted to show some correlation between plasma EDLF levels and the blood pressure in hypertension, details of which have been previously reviewed and are beyond the scope of this paper (41, 42, 56, 57,). Briefly, some studies have shown a correlation, while others have not, and no clear picture has emerged as to what factors account for this variability. With the identification of ouabain and MBG/TBG as candidate structures for EDLF, studies of plasma levels of these compounds using specific antibody assays have been reported. Endogenous ouabain levels have been found to be elevated in hypertensive subjects (56-58). More recently, blood levels of ouabain were found to be increased by acute volume depletion and not by volume expansion, and ouabain levels did not distinguish between salt sensitive and salt insensitive hypertension (59). On the other hand, a series of studies from Bagrov's group have shown that MBG levels are elevated in plasma, urine and tissue of hypertensive Dahl salt-sensitive rats (60) and in preeclampsia (51). In addition, MBG-like substance is increased and MBG antibody blunts the natriuresis of acute ECF volume expansion in Dahl rats (60).

These data support the hypothesis that endogenous MBG or MBG-like steroids more closely fits the postulated characteristics of the hypertension promoting natriuretic hormone than ouabain. However, there may be a role for endogenous ouabain in certain types of hypertension through a central pathway. EDLF has been demonstrated in hypothalamic and pituitary extracts, a compound (or compounds) that cross-reacts with a polyclonal anti-ouabain antibody (61). Extensive studies by Leenen and coworkers have shown increases in this compound in the hypothalamus of spontaneously hypertensive rats (SHR) (62, 63), Dahl salt-sensitive rats (64), and normal rats in which blood pressure is increased by an increase in cerebrospinal fluid sodium concentration (65). The critical role of brain DLF in each of these models was demonstrated by prevention of the rise in blood pressure by central administration of a commercially available FAB fragment of an antidigoxin antibody known to cross react with EDLF (Digibind®) (63-66) (see below).

An integrated role for both endogenous cardenolides and bufadienolides in hypertension in Dahl salt-sensitive rats has been proposed by a study suggesting that release of MBG is controlled by the central ouabain pathway discussed above (67). Further studies on the interaction of this central pathway with mediators of peripheral resistance should be of interest.

4.4. Reversal of hypertension by digoxin antibodies

If elevated circulating levels of a factor that cross reacts with digoxin antibodies is contributing to elevated blood pressure in some subjects, it is possible that blood pressure might be lowered by administration of exogenous (or production of endogenous) digoxin antibodies. A substantial literature has accumulated that has tested this hypothesis, much of which describes studies using a commercially available product Digibind®. Digibind® is a purified FAB fragment of a sheep anti-digoxin antibody developed for the treatment of digoxin intoxication (68) and was based on the concept that binding of free digoxin by antibody in plasma, with renal excretion of the antibody-drug complex, shifts the equilibrium between receptor bound and free drug toward the latter, reversing the effects of excess drug.

Studies using Digibind as a probe to assess the possible role of EDLF in hypertensive subject assume that it will cross react with EDLF and that, in large enough doses, will displace EDLF from its receptor, analogous to its effect in digoxin toxicity. A number of studies are compatible with this formulation. Krep *et a*l showed that Digibind reduced blood pressure in the DOCA-salt rat model (69). Kaide *et al* obtained the same results in a 5/6-reduced renal mass model (70). In the latter study, no effect of Digibind on blood pressure was observed in sham-operated controls, suggesting that the blood pressure reduction was not due to some non-specific or toxic effect of Digibind such as an anaphylactoid reaction. Mann *et al* had suggested the latter, but their studies were done with commercial preparations other than Digibind (71). One other model in which Digibind lowered blood pressure is that of ACTH-induced hypertension (72). This is a particularly interesting finding since it suggests that ACTH stimulates secretion of EDLF. In addition to these in vivo studies, Krep *et al* showed that Digibind reversed the contraction response of isolated aorta to an EDLF isolated from peritoneal dialysis fluid (73). Digibind has also been

reported to reduce blood pressure in several hypertension models when given directly into the central nervous system (see above) (74, 75), to lower blood pressure in patients with preeclampsia (76), and to block the natriuresis of saline infusion in dogs (47).

Antibodies against other glycosides have also been shown to lower blood pressure in animal models. Immunization against ouabain prevented the development of hypertension in Dahl salt-sensitive rats (77). Also, administration of MBG antibodies lowered blood pressure in Dahl salt-sensitive rats (60). These studies suggest that whatever EDLF might be, whether single or multiple compounds, it cross-reacts with antibodies against several cardenolides.

5. SUMMARY IN RETROSPECT AND FUTURE DIRECTIONS

The search for a factor that regulates renal sodium excretion and also plays a causative role in certain types of hypertension has produced a huge body of literature which can no longer be reviewed in a single article. In this review I have highlighted those papers which seem to me to be the most important, without any attempt to be comprehensive. The review emphasizes the origins of the search, with some reference to the current state of the field. The latter is of course explored in more depth by the other contributions to this edition. The many details of the various substances that were isolated by laboratories that purified material having one or more of the biologic activities expected by the NH/DLF hypothesis are not reviewed.

The fact is that, after all the work briefly summarized here, three compounds have emerged as objects of current interest, atrial natriuretic (ANF) and its analogs, ouabain, and MBG/TCB. ANF is clearly a volume-sensitive natriuretic hormone, but its physiological role in regulation of the circulation or renal function has never been established. The only current interest in this factor is the use of one of its analogs as a diagnostic test for congestive heart failure (CHF)(78), and as a treatment for acute CHF (79). Ouabain and MBG continue to be investigated as putative physiological regulators of renal and cardiovascular function. MBG appears to fit the criteria for the factor proposed by the original natriuretic hormone hypothesis (Figure 2) far better than ouabain. Further investigations of the synthesis, physiological regulation, and effects of MBG should be encouraged. If it plays a role in certain hypertensive states, as current evidence indicates, interference with its synthesis or its effects should provide a new target for antihypertensive drugs. The intrarenal mechanism by which MBG causes natriuresis should be of interest.

Further studies should be done to determine whether additional EDLF-like factors may exist in mammalian tissues other than ouabain and MBG. Whatever EDLF may ultimately turn out to be, "it" appears to cross react with the polyclonal antibodies to digoxin contained in the commercial preparation Digibind®. Digibind appears to be a useful probe for the role of EDLF in various physiological and pathophysiological conditions and further studies using this preparation should be encouraged.

The concept of a NH/EDLF factor has been controversial from its beginnings. Despite many reports of false leads and non-reproducible findings, resulting in alternating peaks and valleys of enthusiasm and skepticism, the concept has survived. The contents of this volume attest to its viability and suggest many new approaches to its study. Many who have worked long and hard in this difficult field are pleased to see this new burst of enthusiasm.

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