Why isn’t endogenous ouabain more widely accepted?

Mordecai P. Blaustein
Departments of Physiology and Medicine and the Center for Heart, Hypertension and Kidney Disease, University of Maryland School of Medicine, Baltimore, Maryland

Submitted 11 June 2014; accepted in final form 30 June 2014

A new idea is the most quickly acting antigen known to science.—Wilfred Trotter

During the past few years, several distinguished senior colleagues who visited our department and inquired about the status of endogenous ouabain (EO) have asked, “How come I didn’t know about these [published] data?” And, “Why isn’t endogenous ouabain more widely accepted?” I usually simply shrug and express disappointment that EO is not yet a “mainstream hormone,” even though the seminal reports published more than two decades ago in leading journals (cited below) have been confirmed and supplemented (6, 7, 22). This has now happened often enough to give me pause.

Fierce competition for resources undoubtedly slowed the evolution of the EO story, with its several surprises and multiple components, making it difficult to sustain attention outside the immediate field. Research has also been stymied because the biosynthetic pathway has been only partly elucidated, and there are no commercial EO assays or clinically approved antagonists.

Upon further reflection, however, I have concluded that there is at least a partial explanation based on well-known human behavior to which we, as scientists, are particularly prone (28). We are all very wary of new, paradigm-shifting ideas that don’t fit our preconceived notions. By profession, we are skeptics and we demand proof, but we are also often quick to express negative opinions without carefully assessing the data. We tend to ignore ideas and data that don’t fit our preconceptions; in fact, we often don’t even read (or digest) articles that fall outside our comfort zone. “Mea culpa!” As Darwin noted, established investigators are usually set in their ways; new ideas are for newcomers (9). And we are in good company (although this isn’t a rationale): recall Einstein’s famous put down of Heisenberg’s uncertainty principle, “He [God] does not throw dice” (15).

Below I illustrate the problem with some examples, primarily from my own slow journey of recognition and acceptance of groundbreaking observations about which I was initially very skeptical. And, if I was so suspicious, how could I expect others to rapidly grasp and accept ideas and data that ostensibly don’t fit existing dogma? Nevertheless, if the facts are inconsistent with the prevailing paradigm, progress requires a new one. This is “scientific revolution” (28).

In 1991, Hamlyn and colleagues (23) purified an endogenous cardiotonic steroid from human plasma and analytically identified it as ouabain. The following year, Doursout and coworkers (14) and, a year later, Yuan and colleagues (60) demonstrated that prolonged ouabain administration induces hypertension in normal rats. These remarkable observations have been replicated in a number of highly respected laboratories [reviewed in Blaustein et al. (7)]. The early studies were rapidly followed by reports that circulating EO is elevated in many patients with essential hypertension and mineralocorticoid hypertension (52) and in those with congestive heart failure (21). As in any research field, however, a few investigators failed to replicate the original findings, or reported false positives, and some have continued to question the validity of the original reports [e.g., Baecher et al. (2), Doris et al. (11), and Nicholls et al. (40). It is inappropriate to critique those studies here, but see, for example, Manunta et al. (35).] Thus it might seem understandable that individuals working outside the immediate field may be left in a quandary and would therefore simply ignore these data and arguments, but this is not the whole story.

Leenen’s 1992 discovery of “brain ouabain,” including its key roles in salt-sensitive hypertension (25) and heart failure (31), was another seminal contribution. Numerous subsequent reports from that laboratory confirmed and extended those results by showing that brain ouabain was a distal component of a hypothalamic chronic renin-angiotensin II (ANG II)-aldosterone-epithelial Na\(^+\) channel-brain ouabain pathway (Fig 1) (7, 18, 30). This slow brain pathway, which is activated by high salt and/or ANG II modulates central sympathoexcitatory neurons (i.e., the acute mechanisms) that are involved in both hypertension and heart failure (16, 29, 30, 43, 62). Note that high dietary salt/salt retention suppresses the peripheral renin-angiotensin-aldosterone system (RAAS) (1, 36), but, paradoxically, activates the brain RAAS (18, 43). The more proximal components of the slow pathway, ANG II and ANG II type 1 receptors, aldosterone and mineralocorticoid receptors, and epithelial Na\(^+\) channel (19, 20, 44), are often mentioned in the literature, but brain ouabain has been ignored. Indeed, even though I was aware of it, I, too, long disregarded brain ouabain; it didn’t fit my biased, vasculocentric view of how EO works in hypertension. Finally, in 2010, John Hamlyn and I sat down with Frans Leenen and listened to each other’s ideas. We agreed that the brain EO data and plasma EO data were both valid and needed to be reconciled. The outcome was the joint proposal of a new, comprehensive view of the pathogenesis of salt- and ANG II-dependent hypertension that involves both brain EO and circulating EO and links the kidneys, brain, and arteries (7). This led to collaboration and to the discovery that the chronic brain pathway regulates the plasma EO level (24).

Following the demonstration that ouabain induces hypertension (14, 60), Hamlyn, Manunta, and associates performed another key experiment: they infused digoxin into rats; after all, ouabain and digoxin are both cardiotonic steroids that indistinguishably inhibit Na\(^+\) pumps (5, 59). Astonishingly, digoxin did not induce hypertension; in fact, it normalized
blood pressure in ouabain-infused rats. In direct contradiction to more than 60 years of pharmacological dogma, digoxin could also behave as an “ouabain antagonist.” These amazing observations appeared as an abstract in 1993 (38); the full paper wasn’t published until 2000 (37), largely because of reviewer skepticism, but the results have been confirmed and extended to salt-sensitive hypertension (26, 63). These results were indisputable, but I didn’t understand how digoxin and ouabain could have different effects, so I long ignored the data that are complementary to, but independent of, EO measurements.

In 2010, Golovina and colleagues (48) reported that expression of several Ca2+ transport proteins is markedly increased in arterial smooth muscle (ASM) from rats with ouabain-induced hypertension. The proteins include Na+/Ca2+ exchanger-1 (NCX1); transient receptor potential cation channel, subfamily C, member 6 (TRPC6, component of some receptor-operated channels, ROCs); and sarcoplasmic reticulum Ca2+ ATPase pump-2 (SERCA2). Furthermore, they and others found that NCX1 and several other Ca2+ transporters are overexpressed in ASM in many common hypertension models [reviewed in Blaustein et al. (7) and Pulina et al. (47)]. These proteins are also upregulated in primary cultured normal rodent and human ASM cells incubated with nanomolar ouabain for 72–96 h (32, 48). In contrast, digoxin does not upregulate these proteins either in vivo or in vitro; in fact, digoxin antagonizes the effects of ouabain (Fig. 2) (63), consistent with the aforementioned blood pressure data (26, 37, 38). Ouabain-digoxin antagonism could no longer be overlooked simply because it did not fit the dogma that all cardiotonic steroids act only as Na+ pump inhibitors (5, 56). Also, another key discovery about ouabain that is inconsistent with this conventional wisdom could no longer be disregarded; namely, reports from Askari and colleagues (39, 45) that ouabain binding to Na+ pumps activates a C-Src-dependent protein kinase signaling cascade. In ASM, both in vivo and in vitro, this cascade is activated by ouabain, an effect antagonized by digoxin (Fig. 2) (63). This verifies ouabain-digoxin antagonism [and see Song et al. (55)] and implies that activation of the cascade and the protein upregulation do not depend on Na+ pump inhibition and NCX-mediated enhancement of Ca2+ signaling. Thus the Na+ pump is both an ion transporter and a hormone receptor (Fig. 2). The binding of EO and digoxin have similar short-term, but different long-term, effects.

In heart failure, the RAAS is activated (16, 29, 62) and ANG II-stimulated, EO-dependent mechanisms (Figs. 1 and 2) may also contribute to cardiac remodeling. In the heart, ouabain stimulates extracellular matrix formation (27, 49) and activates C-Src (39), and NCX1 overexpression is a common, but unexplained, feature of heart failure that, paradoxically, may impair cardiac contractility (42, 58).

The several aforementioned independent and seminal observations, together, reveal a new axis that links all these factors directly to hypertension (7) and heart failure (see above). The components include (Figs. 1 and 2) a stimulus (ANG II and/or high salt), the ANG II-stimulated central control system (the brain slow neurohumoral regulatory pathway), an hormonal messenger (EO), “biased” EO receptors (α2 Na+ pumps, which exhibit ouabain-digoxin antagonism), an EO-activated trans-
Arteries

A. Proposed effects of EO on the arteries (A) and heart (B). The well-documented acute action of EO (inhibition of α2 Na\(^+\) pumps), but not its chronic effect (α2 Na\(^+\) pump-mediated activation of the C-Src, ERK1/2, MAPK, and protein kinase cascade), is mimicked by digoxin. Sustained elevation of plasma EO leads, apparently via the protein kinase cascade, to increased expression of several arterial Ca\(^{2+}\) transporter proteins. These include Na\(^+/\)Ca\(^{2+}\) exchanger-1 (NCX1), transient receptor potential cation channel, subfamily C, member 6 (TRPC6; component of receptor-operated channels), and the sarcoplasmic reticulum (SR) Ca\(^{2+}\) ATPase pump-2b (SERCA2b). These chronic effects of EO are blocked by digoxin (26, 37, 63), as indicated; digoxin may either mimic (not shown) or antagonize the acute effect of EO, depending upon the relative concentrations (55). (Note: ouabain-digoxin antagonism has been demonstrated in arteries and neurons, but not yet in heart, as indicated by the question marks in B.) Chronically elevated plasma EO may also increase NCX1 expression in the heart by a C-Src-activated protein kinase cascade (39, 45). Acute Na\(^+\) pump inhibition by EO raises the cytosolic Na\(^+\) concentration ([Na\(^+\)]\(_{\text{cyt}}\)). This reduction in the Na\(^+\) electrochemical gradient, in turn, promotes net Ca\(^{2+}\) gain via NCX1, and a rise in both cytosolic and SR calcium concentrations, [Ca\(^{2+}\)]\(_{\text{cyt}}\) and [Ca\(^{2+}\)]\(_{\text{SR}}\), respectively, and, thus, enhances Ca\(^{2+}\) signaling and contraction in both the heart and arteries [acute (green) pathways in A and B]. With increased NCX1 expression due to chronically elevated plasma EO, however, the heart and arteries apparently respond differently. The main role of NCX1 in the heart is to mediate Ca\(^{2+}\) extrusion and promote relaxation during diastole (3), whereas in arteries, its main role seems to be to mediate Ca\(^{2+}\) entry and maintain tone (61). Thus NCX1 upregulation in the heart should decrease contractility (B, chronic pathway), but in arteries it should increase contractility (A, chronic pathway). In other words, the acute and chronic effects of EO should be synergistic in arteries but antagonistic to one another in the heart. See text for further details.

Heart

B. Proposed effects of EO on the arteries (A) and heart (B). The well-documented acute action of EO (inhibition of α2 Na\(^+\) pumps), but not its chronic effect (α2 Na\(^+\) pump-mediated activation of the C-Src, ERK1/2, MAPK, and protein kinase cascade), is mimicked by digoxin. Sustained elevation of plasma EO leads, apparently via the protein kinase cascade, to increased expression of several arterial Ca\(^{2+}\) transporter proteins. These include Na\(^+/\)Ca\(^{2+}\) exchanger-1 (NCX1), transient receptor potential cation channel, subfamily C, member 6 (TRPC6; component of receptor-operated channels), and the sarcoplasmic reticulum (SR) Ca\(^{2+}\) ATPase pump-2b (SERCA2b). These chronic effects of EO are blocked by digoxin (26, 37, 63), as indicated; digoxin may either mimic (not shown) or antagonize the acute effect of EO, depending upon the relative concentrations (55). (Note: ouabain-digoxin antagonism has been demonstrated in arteries and neurons, but not yet in heart, as indicated by the question marks in B.) Chronically elevated plasma EO may also increase NCX1 expression in the heart by a C-Src-activated protein kinase cascade (39, 45). Acute Na\(^+\) pump inhibition by EO raises the cytosolic Na\(^+\) concentration ([Na\(^+\)]\(_{\text{cyt}}\)). This reduction in the Na\(^+\) electrochemical gradient, in turn, promotes net Ca\(^{2+}\) gain via NCX1, and a rise in both cytosolic and SR calcium concentrations, [Ca\(^{2+}\)]\(_{\text{cyt}}\) and [Ca\(^{2+}\)]\(_{\text{SR}}\), respectively, and, thus, enhances Ca\(^{2+}\) signaling and contraction in both the heart and arteries [acute (green) pathways in A and B]. With increased NCX1 expression due to chronically elevated plasma EO, however, the heart and arteries apparently respond differently. The main role of NCX1 in the heart is to mediate Ca\(^{2+}\) extrusion and promote relaxation during diastole (3), whereas in arteries, its main role seems to be to mediate Ca\(^{2+}\) entry and maintain tone (61). Thus NCX1 upregulation in the heart should decrease contractility (B, chronic pathway), but in arteries it should increase contractility (A, chronic pathway). In other words, the acute and chronic effects of EO should be synergistic in arteries but antagonistic to one another in the heart. See text for further details.
REFERENCES

44. Pare MC, Maltais S, Escher E. The neurogenic origin of hypertension in SHR may be mediated by angiotensin II through a receptor different from AT1 and AT2. Regul Pept 47: 81–86, 1993.