Why isn’t endogenous ouabain more widely accepted?

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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
digoxin data (55). Ouabain could have different effects, so I long ignored the
were indisputable, but I didn’t understand how digoxin and
extended to salt-sensitive hypertension (26, 63). These results
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observations appeared as an abstract in 1993 (38); the full
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blood pressure in ouabain-infused rats. In direct contradiction
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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
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were indisputable, but I didn’t understand how digoxin and
ouabain could have different effects, so I long ignored the
digoxin data (55).

The Na+ pump catalytic subunit-α, which contains the
ouabain binding site, has four isoforms. All cells express
pumps (αβ-dimers) with an α1-isofrom and pumps with one
other α-isofrom (5, 33); cardiac and smooth muscles express
α1 and α2 Na+ pumps, but the large majority, ~75–80%, are
α1 (13, 53, 61). Rodent α1 pumps are notoriously resistant to
ouabain (EC50 ~ 100 µM) (5, 41); they cannot be the physi-
ological receptors for EO because plasma EO levels are nor-
mally subnanomolar (17, 36, 50). Accordingly, Lingrel and col-
leagues (12, 13) generated mice with mutant, ouabain-resistant, α2
Na+ pumps. These α2WR mice do not develop hypertension when
injected chronically with ouabain or adrenocorticotropic hor-
mone, whereas wild-type (ouabain sensitive) α2 mice do (12,
13, 34). Mice in which α2 Na+ pumps are selectively knocked
out in the cardiovascular system are also resistant to adreno-
corticotropic hormone -induced hypertension (50). Addition-
ally, recent studies indicate that either cardiac-specific overexp-
ression (8) or knockout (51) of α2 Na+ pumps attenuates the
development of pressure overload-induced cardiac hypertro-
phy and dysfunction. Clearly, the α2 Na+ pump and its endog-
igenous ligand play a crucial role in at least some forms of
hypertension and heart failure: additional remarkable, but
largely ignored, data that are complementary to, but independ-
ent of, EO measurements.

In 2010, Golovina and colleagues (48) reported that expres-
sion of several Ca2+ transporter proteins is markedly increased
in arterial smooth muscle (ASM) from rats with ouabain-
induced hypertension. The proteins include Na+/Ca2+ ex-
changer-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 afore-
mentioned 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 wis-
dom 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 activ-
ated 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 obser-
vations, 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-
endoGENOUS OUABAIN: WHY THE MYSTERY?

A Arteries

\[ \text{Plasma EO} \]

\[ \downarrow \alpha_2 \text{Na}^+ \text{pump} \]

\[ \uparrow [Na^+]_{\text{CYT}} \]

\[ \uparrow \alpha_2 \text{Na}^+ \text{pump (non-transport function)} \]

\[ \uparrow [Ca^{2+}]_{\text{CYT}} \]

\[ \uparrow \text{SERCA2b} \]

\[ \uparrow [Ca^{2+}]_{\text{SR}} \]

\[ \uparrow \text{NCX1} \]

\[ \uparrow \text{TRPC6}, \text{SERCA2b expression} \]

\[ \uparrow \text{Arterial Constriction} \]

B Heart

\[ \text{Plasma EO} \]

\[ \downarrow \alpha_2 \text{Na}^+ \text{pump} \]

\[ \uparrow [Na^+]_{\text{CYT}} \]

\[ \uparrow \text{NCX1} \]

\[ \uparrow [Ca^{2+}]_{\text{CYT}} \]

\[ \downarrow [Ca^{2+}]_{\text{SR}} \]

\[ \uparrow \text{SERCA2a} \]

\[ \uparrow [Ca^{2+}]_{\text{SR}} \]

\[ \downarrow \text{Arterial Constriction} \]

\[ \uparrow \text{Cardiac Contraction} \]

\[ \downarrow \text{Cardiac Contraction} \]

The most widely cited, long-term trial of the therapeutic effectiveness of digoxin in heart failure (10). Yet, ouabain-digoxin antagonism implies that patients with low- and high-ambient EO are likely to have different outcomes when treated with digoxin. Thus a golden opportunity to predict who might benefit most from digoxin, the primary goal of the trial, was lost. Furthermore, it is now difficult to dismiss the striking correlation between the highest plasma EO levels and the worst morbidity and mortality statistics in patients with heart failure and related cardiovascular diseases (4, 21, 46, 54, 57). Surely, it is time to bring EO, a key neuroendocrine and cardiovascular hormone, in from the cold.

Fig. 2. Proposed effects of EO on the arteries (A) and heart (B). The well-documented acute action of EO (inhibition of \( \alpha_2 \text{Na}^+ \text{pumps} \)), but not its chronic effect (\( \alpha_2 \text{Na}^+ \text{pump-mediated activation of the C-Src, ERK1/2, MAPK, and protein kinase cascade} \)), is mimicked by digoxin. Sustained elevation of plasma EO leads, apparently via the protein kinase cascade, to increased expression of several arterial \( \text{Ca}^{2+} \) transporter proteins. These include \( \text{Na}^+/\text{Ca}^{2+} \) exchanger-1 (NCX1), transient receptor potential cation channel, subfamily C, member 6 (TRPC6; component of receptor-operated channels), and the sarcoplasmic reticulum (SR) \( \text{Ca}^{2+} \) 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 \( \text{Na}^+ \) pump inhibition by EO raises the cytosolic \text{Na}^+ concentration (\([\text{Na}^+]_{\text{CYT}}\)). This reduction in the \text{Na}^+ electrochemical gradient, in turn, promotes net \text{Ca}^{2+} \text{gain via NCX1, and a rise in both cytosolic and SR calcium concentrations, } [\text{Ca}^{2+}]_{\text{CYT}} \text{and } [\text{Ca}^{2+}]_{\text{SR}} \text{, respectively, and, thus, enhances } \text{Ca}^{2+} \text{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 } \text{Ca}^{2+} \text{extrusion and promote relaxation during diastole (3)}, \text{whereas in arteries, its main role seems to be to mediate } \text{Ca}^{2+} \text{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.}

AJP-Heart Circ Physiol • doi:10.1152/ajpheart.00404.2014 • www.ajpheart.org
ACKNOWLEDGMENTS

I thank J. M. Hamlyn, F. H. H. Leenen, and W. G. Wier for helpful critiques of a preliminary version of this manuscript and H. Song for help with the figures.

GRANTS

This study was supported by National Heart, Lung, and Blood Institute Grants R01-HL-045215 (to M. P. Blaustein and J. M. Hamlyn) and R01-HL-107555 (to M. P. Blaustein).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

M.P.B. conceived and designed research; analyzed data; interpreted results of experiments; prepared figures; and drafted, edited, revised, and approved final version of manuscript.

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