

Endogenous Cardiac Steroids in Bipolar Disorder

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Contributor: Rif El-Mallakh , David Lichtstein

Type I bipolar disorder (BD) is a severe psychiatric illness that manifests as extreme variations in mood and energy, usually labelled as mania and depression, interspersed over a euthymic or dysthymic baseline. Bipolar disorder (BD) is a severe psychiatric illness with a poor prognosis and problematic, suboptimal, treatments. Treatments, borne of an understanding of the pathoetiological mechanisms, need to be developed in order to improve outcomes. Dysregulation of cationic homeostasis is the most reproducible aspect of BD pathophysiology. Correction of ionic balance is the universal mechanism of action of all mood stabilizing medications. Endogenous sodium pump modulators (collectively known as endogenous cardiac steroids, ECS) are steroids which are synthesized in and released from the adrenal gland and brain. These compounds, by activating or inhibiting Na^+ , K^+ -ATPase activity and activating intracellular signaling cascades, have numerous effects on cell survival, vascular tone homeostasis, inflammation, and neuronal activity.

bipolar disorder

endogenous cardiac steroids

endogenous ouabain

ouabain

Na^+

K^+ -ATPase

1. Introduction

Type I bipolar disorder (BD) is a severe psychiatric illness that manifests as extreme variations in mood and energy, usually labelled as mania and depression, interspersed over a euthymic or dysthymic baseline ¹. The disorder afflicts approximately 1% of people ^{2|3}, with documented suboptimal treatments and a host of undesirable outcomes related to both the disease and its treatment ^{4|5}. Despite over 60 years of directed effort, the pathoetiology of the illness remains unknown ³, but multiple clues have emerged that continue to inform ongoing research. The illness is viewed as multifactorial, involving elements (of development and neuroplasticity, inflammation, and aberrant modulation of brain function and circuitry) that are mediated by gene and environment interaction through multiple inherited genes and multiple altered epigenetic changes ^{3|6|7|8}. However, the absence of a centralized unifying model contributes to the fragmented, siloed, fashion of current pathophysiological research. In this article, the researchers propose a mechanism of pathophysiology that incorporates much of the translational and clinical data, and, in addition to focusing research efforts, also leads to potential novel treatment options.

Among the most reproducible findings in bipolar illness, the researchers observe the dysregulation of control of the electrically important ions sodium (Na^+), potassium (K^+), hydrogen (proton, H^+), and calcium (Ca^{2+}) ⁹. Ion

regulation spans across all of the proposed mechanisms pertaining to pathogenesis of abnormal moods in bipolar illness, and it spans across all successful treatment options. For example, in an analysis of susceptibility loci, Askland found that approximately 74% of known loci involve genes related to ion regulation, or what she refers to as neuroelectrical genes [10]. By comparison, genes involved in any neurotransmitter pathway account for only about 58% of the susceptibility loci, and the monoamines account for only 31% [10]. A similar conclusion is reached when genome wide association studies (GWAS) are explored [11]. The only animal models of bipolar illness, meeting all validity criteria, create ion transport abnormalities [12][13][14]. Additionally, nearly all interventions, effective in mania or mood stabilization, reduce intracellular sodium either directly or indirectly [15][16][17]. Changes in sodium pump activity are associated with changes in mood state [18][19], and endogenous cardiac steroids (ECS), that can determine sodium pump activity, are dysregulated in patients with BD [11][20].

2. Endogenous Cardiac Steroids in Bipolar Disorder

Cardenolides, such as ouabain and digoxin, and bufadienolides, such as bufalin, are steroids originally identified in plants (*Digitalis*, *Strophantus*) and toads (*Bufo*), which have been used for hundreds of years in Western and Eastern medicine to treat heart failure, arrhythmias, and other maladies. In the past 25 years, the cardenolides ouabain and digoxin, and the bufadienolides 19-norbufalin, marinobufagenin, and cinobufagenin were identified as normal constituents of mammalian tissues, including the brain [21][22][23][24]. These compounds, collectively termed endogenous cardiac steroids (ECS), are synthesized in the adrenal and hypothalamus of mammals [25][26][27], and they are considered a new class of hormones implicated in many physiological and pathophysiological mechanisms, including cell growth and cancer, vascular tone homeostasis, blood pressure, hypertension, natriuresis, heart contractility, and inflammation [22][24][28].

These mammalian ECS resemble non-mammalian compounds in their ionotropic effects, presumably due to inhibition of the sodium, and potassium-activated adenosine triphosphatase, or sodium pump (Na^+ , K^+ -ATPase) [29]. However, at low physiologic concentrations (nM), these compounds have been shown to stimulate pump activity [30][31][32][33]. Importantly, the interaction of ECS with the Na^+ , K^+ -ATPase results not only in the inhibition of the ion pumping function, but causes the activation of several signal transduction cascades, including mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK), protein kinase B, and orotoincogene tyrosine-protein kinase pathways [34][35]. In addition, a substantial body of studies has demonstrated that ECS also act by directly and indirectly affecting the activity of the nuclear receptor superfamily of transcription factors [36][37].

To date, the most studied ECS is the endogenous ouabain (EO); a compound that is similar or identical to the plant steroid. Based on its immunoreactivity with anti-ouabain antibodies, this compound has been shown to be present in mammalian brain and CSF, and it is considered a potential neuromodulator [22][38][39][40].

It is important to note that there remains some debate regarding the exact structure of EO [41]. Central to this debate, there is difficulty measuring the picomolar concentrations that exist in mammalian systems using direct

chemical methods [42]. Notably, the evidence for the existence of EO is quite overwhelming, and the dispute is focused on the exact structure of this steroid [27].

The synthetic pathways of ECS are not established, but it is known that cholesterol (which may be reduced in the brains of patients with bipolar illness [43]) is needed for ECS/EO production [27], and the pathway may involve pregnenolone and progesterone as intermediate steps [42]. Pregnenolone levels may be reduced in the cerebrospinal fluid of individuals with a diagnosis of mood disorder, and this reduction may be in relation to the severity of their symptoms [44]. Rapid elevations in plasma levels of ECS, as occurs with exhaustive exercise of normal controls [45], may occur due to the release of bound steroids from a carrier protein [46].

Despite the limited available data on the metabolism of ECS in general, and EO in particular, these compounds are referred to by some as “a hormone family” [21][27][47][48][49][50][51][52]. However, at the same time, as is evident from the lack of reference in textbooks and reviews, these steroids are ignored almost completely by mainstream biochemists, physiologists, and endocrinologists. The researchers pointed out the crucial importance of deciphering the biosynthetic pathway of ECS close to ten years ago [53], but this issue remained the Achilles heel of this field of research.

At *experimental* or *pharmacologic* micromolar concentrations, exogenously administered ouabain inhibits the activity of the sodium pump. However, at *physiologic* picomolar to nanomolar concentrations, EO *increases* sodium pump activity [30][31][32][33]. In notable cases, when there are documented increases of the plasma levels of EO, the total levels remain below concentrations that inhibit sodium pump activity [54]. At the low concentrations, EO also appear to activate second messengers of the Src kinase-, ERK1/2-, and Akt-mediated pathways or the sodium-proton exchanger 1 (NHE1) [54][55][56][57].

Activation of the sodium pump appears to be an essential feature in reducing central nervous system inflammation [58]. If sodium pump activity is blocked in glial cells, inflammatory pathways are activated in the presence of lipopolysaccharides [58].

2.1. Role of Exogenous CS and ECS in Animal Models of Mood Disorders

The process of modeling psychiatric disorders in animals is difficult because of the subjective nature of many psychiatric symptoms. Robbins and Sahakian set forth three requirements for an acceptable animal model of a psychiatric disorder [59]. First, they proposed “face validity,” in which they noted that the model must share the pathophysiological changes known to occur in the human condition. Second, they required “construct validity,” for which they required that the model display similar behavioral manifestations as the human disease. Finally, they defined “predictive validity,” in which the induced abnormal behavior responds to medications that improve the symptoms in afflicted humans [59].

The most commonly used model of mania pertains to administration of amphetamine to rats [60] or mice [61]. While this model does not fulfill Robbins and Sahakian’s criteria (it is actually an animal model of substance abuse), it has long been used as *the* animal model for mania. Mice receiving 5 mg/kg of amphetamine, by intraperitoneal (IP)

injection, developed hyperactivity immediately after injection; however, co-administration of intracerebroventricular (ICV) anti-ouabain antibodies normalized the amphetamine-induced hyperactivity [62]. ICV anti-ouabain antibodies alone had no effect [62]. Amphetamine was associated with a 300% increase in brain levels of ECS [62]. This may increase Na^+ , K^+ -ATPase activity in the brains of rats [63], or reduce Na^+ , K^+ -ATPase activity if the rats were fed a diet, rich in hydrogenated vegetable fats, which is rich in *trans* fatty acids [64]. Additionally, amphetamine, when administered to rhesus monkeys, upregulates the $\alpha 3$ isoform of the sodium pump [65]. In mice, ICV administration of anti-ouabain antibodies appears to inhibit the increase in brain ECS while preventing the associated hyperactivity [62].

Similarly, sterile inflammation appears to be a characteristic of bipolar disorder pathophysiology [66]. Chronic inflammation may induce oxidative stress [67]. In addition to playing a role in the genesis of symptoms, these conditions may also be responsible for comorbid medical conditions [66][68]. Cardiac steroids appear to mediate elaboration of oxidative stress [34], and the dramatic increase of EC in amphetamine treated rats may increase inflammation and oxidative stress as reflected by the increase of superoxide dismutase (SOD) activity in these animals [69]. Treatment with anti-ouabain antibodies normalizes SOD in amphetamine-treated mice [69].

Despite these findings pertaining to the amphetamine model, animal models in which the sodium pump is rendered dysfunctional, either by using ICV injections of ouabain [70][71][72], or by knocking down the α [73] or $\alpha 3$ [74][75][76] isoforms of the sodium pump, fulfill all three validity criteria for an animal model of a psychiatric illness [13]. These models mimic the abnormalities documented in bipolar patients, including reduced sodium pump activity, increased intracellular sodium [13], and CNS hypometabolism [77]. Importantly, these are the only animal models in which the same abnormality can produce both manic-like behaviors [71] and depressive-type behaviors [78]; thus, they are the best models the researchers have of BD [13].

In Flinders Sensitive Line (FSL) of genetically depressed rats, ICV administration of 50 μg of anti-ouabain antibodies significantly reduced depressive behaviors [79]. These same antibodies halved the concentration of ECS in ex vivo samples of human cerebrospinal fluid (SCF) [79].

A reduction of sodium pump activity, via administration of exogenous ouabain or by genetic knockout, serves as an animal model of mania and depression. Conversely, removal of ECS in animals with anti-ouabain antibodies appears to reduce manic symptoms. While these two observations appear to contradict each other, it should be noted that the former (reducing sodium pump) is being performed on a normal animal, while the later (removing EO with anti-ouabain antibodies) is being performed on a symptomatic animal.

2.2. Role of ECS in Humans Mood Disorders

ECS appear to play some role in the pathophysiology of BD. Patients with type I bipolar illness display reduced circulating EC when they are manic compared to healthy controls ($143.6 \pm \text{S.E.M. } 20.94$ vs. 296.6 ± 12.76 pg digoxin equivalents/mL, respectively, $F = 4.77$, $p < 0.05$), but not compared to euthymic bipolar subjects (213.8 ± 86.92 , $p = 0.77$) [80]. Furthermore, bipolar patients appear to be unable to increase production of EO based on physiologic need. They do not display the seasonal variation in EO levels that has been demonstrated in healthy

controls (higher concentrations in the Spring, Summer, and Fall, and lower levels in Winter, versus low levels all year long in patients with bipolar disorder [80]). More importantly, euthymic patients with bipolar illness do not increase circulating EO in the setting of exercise to exhaustion, as occurs with matched non-bipolar controls [81] or the general population [45]. It has been proposed that sleep deprivation, which is associated with increased production of EO and corticosterone in mice [82], might be a trigger for a manic switch in bipolar subjects [21][83]. As little as 24 h of sleep deprivation in humans is enough to increase serum cortisol [84][85][86] and manic-like symptoms [87].

There is direct evidence pertaining to the involvement of both the sodium pump and ECS in the CNS of patients with BD. An allelic association between BD and a Na^+ , K^+ -ATPase $\alpha 3$ subunit gene (*ATP1A3*) has been reported [88]. In comparison to non-psychiatric controls, the $\alpha 3$ subunit may be specifically under-expressed in neurons that release gamma aminobutyric acid (GABA) in the parietal cortex of patients with bipolar illness [89]. The parietal cortex of patients with bipolar illness may have fewer ouabain binding sites than non-psychiatric controls [90]. Subsequently, the researchers have demonstrated a significant association with BD of six single SNPs in the three genes of the Na^+ , K^+ -ATPase α isoforms. Haplotype analysis of the $\alpha 2$ isoform showed a significant association with two loci haplotypes pertaining to BD, suggesting that this enzyme plays a role in the etiology of the disease [91], and, indeed, this isoform appears to be reduced in the temporal cortex of individuals with bipolar disorder [92], but $\alpha 2$ and $\alpha 3$ may be increased in the prefrontal cortex [20].

When EO has been measured directly in the prefrontal cortex (PFC), it has been found to be at the same level as non-psychiatric controls; however, this finding appears to be driven by two outlier patients with EO levels more than three times higher than the average for the entire group [20]. Interestingly, smoking is associated with reduced PFC EO levels [20], and this is important because individuals with bipolar illness are more than twice as likely to smoke as the general population [93]; the smoking status of the two outliers is not known [20].

2.3. Hypothesis

Alterations of EO levels in bipolar disorder appear to be important in the pathogenesis of the illness. There is evidence for both an increase or a decrease in EO concentrations, and there are reasons to believe that each change can lead to the symptoms of the illness (**Figure 1**). This is an appropriate time to begin the process of translation from basic to clinical research.

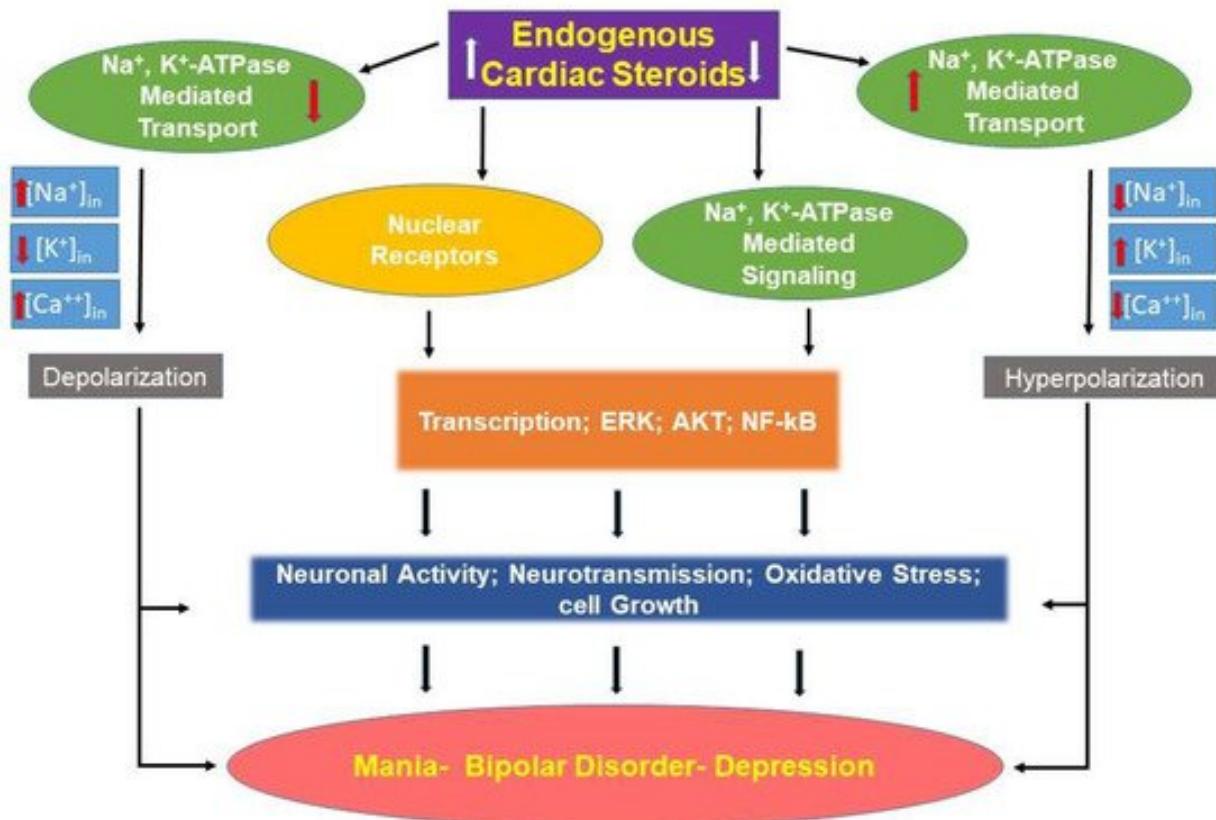


Figure 1. Schematic representation of the possible links between endogenous cardiac steroids and bipolar disorder. See text for details.

2.4. Translational Implications: Pathophysiologic Models

The data suggest two parallel but, possibly, competing models. In the first model, some factor (inflammation or physiologic stress) results in increased production of central nervous system EO.

As mentioned above, ECS are known to activate the Src, ERK, and AKT signaling pathways [22][94][95][96]. These are all signaling systems that have been implicated in BD [97][98][99][100]. Additionally, inhibition of sodium pump activity [70] and altering sodium pump isoform expression [72] have been demonstrated in animals receiving ICV ouabain. Activation of signaling pathways occur at levels that do not inhibit sodium pump activity, and this may be important in inducing symptoms of BD. Inactivation of EO with anti-ouabain antibodies would be a reasonable treatment option in this model of relative EO excess (Figure 1).

The second model may have a very similar physiology. In this model, some factor (inflammation or physiologic stress) results in a signal to increase central nervous system EO. However, patients with BD disorder are incapable of upregulating EO under conditions of high demand, and the body experiences a relative EO deficiency (Figure 1). Exogenous ouabain administration would be a reasonable treatment option in this model. Each one of these models will be discussed in additional detail.

2.5. EO Excess

Direct measurements of EO in the parietal cortex of postmortem samples have shown higher levels in BD patients, as compared to those with schizophrenia, major depression, and psychiatrically normal individuals [90]. The increased levels of EO in the brain of BD patients are likely to be brain-region specific, as this increase has not been witnessed in the temporal cortex [89]. Results from one of the researchers laboratories demonstrate that the reduction in brain EO (by intracerebroventricular administration of anti-ouabain antibodies) creates a dramatically protective effect pertaining to a depressive-like behavior in rats [90], with concomitant alterations in catecholamine levels in specific brain regions [79]. Furthermore, a reduction in brain EO also protects against a manic-like behavioral response in an amphetamine (AMPH)-induced mania model [62], with a concomitant reduction of oxidative stress markers [69].

In view of the above, the researchers predicted that the reduction of circulating EO in BD patients might ameliorate their symptoms. Indeed, in a preliminary study, Digibind (Digoxin Immune Fab fragment used for the treatment of digoxin intoxication) was given to six inpatients who were hospitalized for a recurrent episode of bipolar depression. The clinical rating of depression was ascertained using the Montgomery—Asberg Depression Rating Scale (MADRS), before the intervention, and 6, 24, 48, and 72 h after the intervention. In the results of this experiment, there was an observed decrease in the MADRS score, which reached a peak (8.3 points) 24 h after the administration of Digibind, followed by a gradual return towards baseline scores over the following two days (Zilberstein, A., Krivoy, N., Klein, E. and Lichtstein, D. unpublished observation). These preliminary results, which need to be characterized and extended to more patients, strongly suggest that increased levels of EO are involved in the pathophysiology of the disease and, consequently, their reduction will have beneficial effect.

The mechanisms underlying BD are extremely complex, and the increased ECS levels may be involved in this pathology at various stages and in different biochemical pathways. As mentioned above, it is well established that ECS cause both the inhibition of Na^+ , K^+ -ATPase transport activity and the activation of the Ras-Raf-MEK-ERK signaling cascade, resulting in the activation of MAPK [34]. These two events lead to an increase in intracellular Ca^{2+} [101], resulting in the opening of mitochondrial ATP-sensitive K^+ channels and the generation of reactive oxygen species (ROS) [102]. The increase in ROS levels, beyond the capacity of antioxidant defense mechanisms in the brain, damages cellular components, leading to a disruption of normal neuronal function, and this may contribute to the pathological behavioral changes which are characteristic of BD.

2.6. Relative EO Deficiency

As previously noted, work in one of the researchers laboratories has identified reduced plasma levels of EO in unmedicated manic patients versus unmedicated controls [80]. Additionally, individuals with BD lack the seasonal variation of EO levels that has been noted in the control subjects who display higher levels in the spring, summer, and autumn (in the range of 400–600 pg digoxin equivalents/mL), and lower levels in the winter (around 150 pg digoxin equivalents/mL), with levels in the range of 150 pg digoxin equivalents/mL throughout the entire year [80]. More importantly, euthymic patients with BD do not increase EO levels when they exercise to exhaustion, as has been noted to occur in non-bipolar controls [45] (EO at 60 min of exercise $0.007 \pm \text{S.D. } 0.02 \text{ ng/mL}$ in bipolar vs. $0.075 \pm 0.06 \text{ ng/mL}$ in normal control subjects, $p = 0.029$, but not significant at peak exercise, $0.009 \pm \text{S.D. } 0.02$

ng/mL in bipolar vs. 0.131 ± 0.21 ng/mL in normal control subjects, $p = 0.15$ [81]). In these studies, BD patients have measurable EO, but it does not vary with physiologic need.

It is proposed that physiological elevations in EO levels that may normally occur with inflammation, severe illness [103], or sleep deprivation [82], do not occur in subjects with BD, and they experience a relative EO deficiency. Considering that physiologic levels of EO activate Na^+ , K^+ -ATPase activity, the relative deficiency results in sodium pump hypofunction in relation to the need, and consequent depolarization of neural tissues [18][21].

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