

Endogenous Ouabain Changes Rapidly During Cardiac Pulmonary by Pass

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Abstract

Objective: Endogenous Ouabain (EO) is a cardiac glycoside secreted from the adrenal glands that plays a role sodium homeostasis with hemodynamic and renal effects. It is considered a stress hormone. The role of EO during critical illness is unknown.

Aim: to study 1. the time course of EO during cardio pulmonary bypass (CPB) and 2. the ability of renal replacement therapy (RRT) to remove EO.

Methods: in 11 patients undergoing mitral valve repair were performed an intraoperative time course with serial blood samples for EO, serum creatinine, NT-proBNP and catecholamines. During surgery blood samples were repeated every 15 minutes. Then all these biomarkers were dosed at the end of surgery, 4 hours and 24 hours later. In the 15 patients undergoing EO time course during RRT, EO plasma levels were measured when AKI (Acute Kidney Injury) occurred (at R of RIFLE and 24 hours after this moment).

Results: In patients undergoing mitral valve repair EO levels increased 15 minutes after the beginning of CPB reaching the peak 4 hours after surgery (from 198 ± 10 to 350 ± 130 pmol/L, $p < 0.0001$). Circulating catecholamine (Norepinephrine and Epinephrine) levels increased immediately after CPB. NT-proBNP increased only 4 hours after surgery, reaching high plasma levels when EO decreased. Plasma EO and creatinine levels resulted significantly directly related ($r = 0.45$, $p = 0.01$) after surgery. Continuous RRT did not modified circulating EO in AKI patients.

Conclusion: EO may be considered a stress hormone that changes rapidly during acute volume expansion and blood pressure fall during CPB.

Keywords: Na pump inhibitor; Cardiac surgery; Cardiac glycosides; Adrenal gland; Hormone

Abbreviations: ACTH: Adreno Cortico Tropic Hormone; ANG II: Angiotensin II; AKI: Acute Kidney Injury; CPB: Cardio Pulmonary Bypass; CYP11A1: Cholesterol side-chain cleavage; EO: Endogenous Ouabain; EuroSCORE : European System for Cardiac Operative Risk Evaluation; HSD3B: β -hydroxysteroid dehydrogenase; NT-proBNP: N-terminal pro-brain-type natriuretic peptide; RRT: Renal Replacement Therapy; SNP: Single Nucleotide Polymorphism

Introduction

Acute kidney injury (AKI) is now recognized as a major public health problem affecting millions of patients worldwide and leading to decreased survival or increased progression of underlying chronic kidney disease, and, sometimes, to new onset of chronic kidney disease [1]. Furthermore, AKI is not a single disease but rather a syndrome comprising multiple clinical conditions. Outcomes in AKI are influenced by the underlying disease causing the condition, as well as by the severity and duration of renal impairment and by the baseline condition of the patient [2]. AKI occurs in as many as 40% of patients after cardiac surgery and requires dialysis in 1% of cases [3,4]. AKI is associated with an increased risk of mortality and morbidity, predisposes patients to a longer hospitalization, requires additional treatments, and increases the hospital costs. In 2005, Chertow and colleagues [5] demonstrated that small changes in serum creatinine, like a rise in serum creatinine by 0.3 mg/dl, were indicative of significant renal dysfunction. They also find an association between high in hospital serum creatinine levels and mortality. Although serum creatinine is typically used for diagnosis of AKI, it is an insensitive and unreliable biomarker during acute changes of renal function. In fact serum creatinine does not increase until about half of the kidney function is lost [6]. For this reason there is need for new early biomarkers for AKI. New biomarkers are likely to be

useful in facilitating early diagnosis, guiding targeted interventions and monitoring disease progression.

Endogenous Ouabain (EO) is an adrenal stress hormone with potential hemodynamic and renal effects, secreted by adrenal gland [7]. EO is a cardiac glycoside, structurally similar to digoxin, that modulates the activity of membrane bound Na^+/K^+ -ATPase pump and induces signal transduction via a second messenger system [8]. A growing number of clinical and experimental evidences identified high concentrations of this hormone in human and animal adrenal glands, which are proposed to be a primary source of EO in mammals [8,9]. Furthermore, EO modulates Na^+/K^+ -ATPase in the vascular bed decreases vascular compliance by increasing smooth muscle tone [7,10] and this effect is ouabain specific [11]. In the heart, EO activated though Na^+/K^+ -ATPase generated Src-kinase second messenger pathway promotes cardiomyocyte hypertrophy [12]. Observational data in naïve hypertensive patients revealed that elevated EO concentrations are associated with increased diastolic blood pressure and left ventricular hypertrophy [13]. Prolonged ouabain infusion in rats causes an increase in plasma creatinine, blood pressure and tubular Na reabsorption.

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[7], Recently [14], we have shown the relationship between EO and impaired glomerular filtration rate as direct consequence of long exposure to increased level of plasma EO in hypertensives. Therefore, we conducted these prospective observational studies to test the time course of circulating EO changes during cardio pulmonary bypass (CPB), where acute volume and blood pressure modification occurs. We also test whether post-operative AKI may influence by EO and we verify the ability of renal replacement therapy (RRT) to clear EO.

Materials and Methods

In the setting of cardiac surgery, after Ethical Committee approval and patients' written consent, were enrolled in the study 11 consecutive patients undergoing mitral valve repair and 15 consecutive patients undergoing postoperative AKI threaded with RRT.

For the 11 patients undergoing mitral valve repair inclusion criteria were: age between 18 and 75 years old, mitral valve repair, pre-operative ejection fraction > 60% and pre-operative end-diastolic diameter < 60 mm. Exclusion criteria were no written consent, other than mitral valve repair in median sternotomy, any preoperative comorbidity and perioperative need for inotropes or vasoconstrictors. Disease severity was scored according to European System for Cardiac Operative Risk Evaluation (EuroSCORE) [15].

For the 15 patients treated with continuous renal replacement therapy (CRRT) the inclusion criteria were: age > 18 years old, cardiac surgical intervention and AKI treated with CRRT. The exclusion criteria were no written consent and other type of surgery (ex vascular surgery).

All patients underwent preoperative clinical evaluation, routine blood tests and instrumental examinations (resting ECG, echocardiography).

All patients received a standardized anesthetic management. Premedication (morphine 0.1 mg/kg intramuscularly; scopolamine 0.003 mg/kg intramuscularly) was administered 1 hour before surgery; general anesthesia was induced with fentanyl (10-20 mcg/kg) and propofol (2 to 4 mg/kg). To facilitate endotracheal intubation, rocuronium 0.5 mg/kg was administered. Anesthesia was maintained with propofol (2 to 4 mg/kg/h), or sevoflurane (end-tidal concentration >1 MAC) and additional doses of fentanyl when required. All operations were performed by one of six surgeons. All patients underwent median sternotomy. The surgery is performed through the cardiopulmonary bypass (CPB), a heart-lung machine that replaces the function of these organs. The heart must be cold, empty and still. The heart is stopped with a cardioplegic solution.

Standard CPB technique was used as previously reported [16]. According to the needs of the patients were infused fluids administered diuretics and inotropes, respectively to maintain urinary output above 1ml/kg/he and to ensure a mean arterial pressure of 70 mmHg.

After surgery, all patients were transferred to intensive care unit and therated according to standardized protocols. In all patients postoperative renal function was monitored with serum creatinine and urinary output and AKI was assessed using the RIFLE classification [17]. When AKI occurred, the first treatment was hemodynamic optimization with fluid challenge and inotropes to rise a Mean Blood Pressure > 70 mmHg and a urinary output \geq 1 ml/kg/h.

Renal Replacement Therapy

In our institution, continuous renal replacement therapy (RRT) is the preferred option in acutely ill patients and the only initial treatment

option. For the purpose of this study, indications for RRT were: worsening of renal function despite hemodynamic optimization, refractory fluid overload, hyperkalemia (plasma potassium concentration >6.5 meq/L) or rapidly rising potassium levels and metabolic acidosis (pH less than 7.1) refractory to medical therapy.

All patients signed informed consent before surgery and preoperative blood sample for EO were drawn. In the 11 patients undergoing mitral valve repair were performed an intraoperative time course with serial blood samples for EO, serum creatinine, NT-proBNP and catecholamines (epinephrine and norepinephrine). During surgery blood samples were repeated every 15 minutes. Than all these biomarkers were dosed at the end of surgery, 4 hours and 24 hours later. In the 15 patient undergoing EO time course during RRT, EO plasma levels were measured when AKI occurred (at R of RIFLE and 24 hours after this moment), at the beginning of CRRT and during the treatment (1 hours, 3 hours, 6 hours, 12 hours, 24 hours, 36 hours, 48 hours). During RRT EO was dosed also in dialysate. In all patients were monitored vital signs and renal function (serum creatinine, GFR and urinary output).

Statistical Analysis

Descriptive statistics are presented with mean and SD or median as appropriate, ordinal data are presented with number and percentage. Comparisons of nominal data were by means of the chisquare test or Fisher's exact test where indicated.

Results

EO Time Course During Mitral Valve Repair

The 11 patients, undergoing EO time course during mitral valve repair, were 45 ± 3.5 years old (Table 1). In patients undergoing mitral valve repair EO levels increased 15 minutes after the beginning of CPB reaching the peak 4 hours after surgery (from 198 ± 10 to 350 ± 130 pmol/L, $p < 0.0001$, Figure 1 A panel), while serum creatinine remained unmodified (Figure 1, C panel). Plasmatic sodium levels (not shown) and haematocrit decreased (-35% Figure 1 B panel) during CPB, simultaneously with the acute circulating volume expansion. Circulating catecholamine (Norepinephrine ad Epinephrine) levels increased immediately after CBP (Figure 2, B and C panel respectively). NT-proBNP increased only 4 hours after surgery (Figure 2, E panel), reaching high plasma levels when EO decreased.

EO and Plasma Creatinine

In thirty-one the plasma creatinine 48 hours after cardiac surgery reached plateau values (Figure 3, A panel). However plasma EO showed a top increase after 24 hours and started to decrease in 2nd day after surgery (Figure 3, B panel). Furthermore, plasma EO and creatinine levels resulted significantly directly correlate ($r=0.45$, $p=0.01$) after surgery.

EO Time Course During RRT

The most important preoperative characteristics are shown in Table 2.

EO levels remained high from the preoperative sample to all the treatment with continuous RRT (Figure 4 A panel). We also dosed EO in the dialysate, but it was not detectable. These findings suggest that EO has no dialytic clearance during RRT. Also hematocrit levels (Figure 4 B panel), after a decrease in the first postoperative hours explainable with intraoperative blood loss, remained unchanged. Mean serum

	Mean/Median/Percentage	SD/IQ range
Age (years)	74	6.5
Female (N; %)	7; 46%	-
EuroScore (%)	15	(10.3-20.9)
Previous cardiac surgery (N; %)	7; 46%	-
Other than isolated CABG (N;%)	11; 73%	-
NYHA 3 and 4 (N;%)	10; 66%	-
Ejection Fraction (%)	50.5	12.3
End diastolic diameter (mm)	55.2	8.2
Hypertension (N;%)	8; 53%	-
Cronic Renal Failure (N;%)	8; 53%	-
Diabetes Mellitus (N;%)	4; 26%	-
Serum creatinine (mg/dL)	1.32	0.59
EO (pmol/L)	281	(181.7-482.3)

Table :1 Preoperative characteristics.

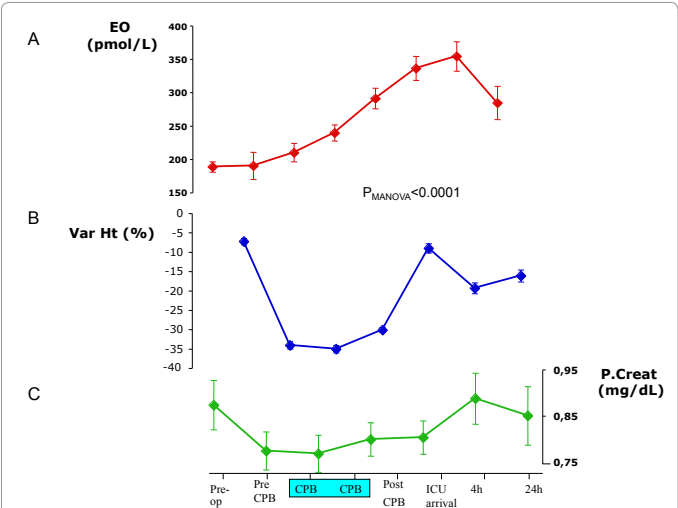


Figure 1: EO time course during CPB. *Panel A:* 15 min after general anesthesia was induced plasma EO (red) start to rise achieving the maximum increase 4 hour after CPB. (MANOVA repeated measure $p < 0,0001$ after correction for SEX, AGE, BMI, Euroscore). *Panel B:* the haematocrit (bleu) expressed as variation percent (Var Ht (%)), decrease 35% from baseline suggesting an acute volume espansione before CEC. *Panel C:* plasma creatinine (green) durino volume expansion decreased as consequence of plasma dilution.

creatinine (Figure 4 C panel) rise progressively until it reaches a peak at the start of RRT, then gradually decrease due to dialysis clearance of serum creatinine. The mean arterial pressure (Figure 4 D panel) reaches the minimum average of 24 hours after R of RIFLE, then fluctuated between 73 mmHg and 80 mmHg of mean value during the dialysis treatment continuously.

Discussion

The most striking observation in this study is the marked and rapid change of circulating plasma EO after induction of anesthesia. Neither catecholamines nor NT-proBNP can be a stimulus for EO release, because their increase is delayed compared to that of EO (Figure 1). EO rapid increase could be induced by acute volume expansion due to cardioplegia and blood pressure fall, after general anesthesia induction and during CPB [18]. AKI in cardiac surgery is mainly related to the adverse effects of CPB and cardioplegia, which causes dramatic hemodynamic changes, including marked volume expansion and change from pulsatile to continuous flow. CPB and cardioplegia are necessary for cardiac surgery, because they make possible to work on

a cold, empty and still heart. A long series of experimental and clinical data supports the notion that endogenous glycoside is secreted in the circulation in response to volume expansion [7,19,20].

Endogenous Ouabain may be considered a stress hormone secreted by adrenals glands as supported by:

- I. In an early study in patients with primary aldosteronism, EO levels in mixed inferior vena cava blood were more than fivefold higher than in normal controls [21]. We suggested recently that a step up in adrenal venous plasma of about threefold was likely in that study [22]. Further studies using methods based on mass spectrometry are needed to confirm this impression and to provide crucial proof of the appropriate venous gradients in humans. In work with conscious, afebrile dogs with surgically placed adrenal venous catheters, the EO content of the adrenal venous effluent was about fivefold to sixfold higher than that of arterial blood [23].
- II. Elevated plasma levels of EO were found in two rare hypertensive patients with nonclassic adrenocortical tumors [24]. Removal of the tumors was associated with a normalization of plasma EO levels and the remission of hypertension. The results were compatible with those in other patients with aldosterone-secreting tumors [25], which also hyper secrete EO and cause hypertension.
- III. Cultured human and bovine adrenocortical cells secrete EO into the culture fluid [26]. The secretion is augmented by angiotensin II, adrenocorticotrophic hormone (ACTH), and possibly vasopressin, as well as α_1 adrenoreceptor agonists [22,26]. With contemporary dietary Na^+ intakes and with plasma renin activity largely suppressed, both plasma K^+ and ACTH appear to be key regulators of circulating EO in humans [27,28]. The adrenal biosynthesis of EO involves cholesterol side-chain cleavage (CYP11A1) and 3β -hydroxysteroid dehydrogenase (HSD3B) with sequential metabolism of pregnenolone and progesterone [29,30].
- IV. The renal excretion of cardiac glycosides is mediated in part by the organic anion transporter (SLCO4C1) at the basolateral membrane [31] and in part by the Pglycoprotein (PGP, encoded by MDR1) [32] at the apical membrane of the nephron. Accordingly, a single nucleotide polymorphism (SNP) and haplotype-based association study was performed with a total of 26 informative SNPs in a large cohort of hypertensive patients. In that study, CYP11A1 and MDR1 loci were associated with circulating EO and diastolic BP, likely reflecting their influence on EO synthesis and transmembrane transport, respectively [33].
- V. The acute increase of EO has been previously report under stress condition. Bauer et al. [34] have shown that the marked and rapid change of plasma EO in humans and dogs during physical exercise. Moreover, in dogs the increase in EO release is completely abolished by β -blockade and ACE inhibition suggesting that both β_1 -adrenergic stimulation and the renin-angiotensin system are immediately involved in EO release during exercise. Furthermore, an increase in plasma ANG II causes neuronal activation in hypothalamic nuclei and a pressor response, presumably by increasing sympathetic drive. Leenen et al. [35] postulated that the activation of a neuromodulatory pathway, involving aldosterone and "ouabain," is involved in these responses.

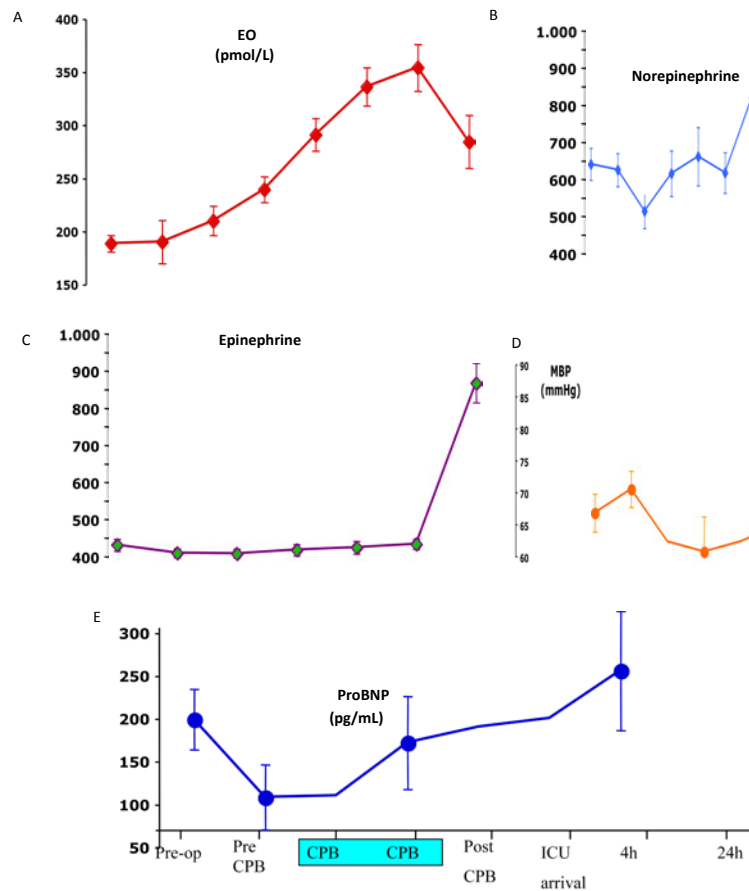


Figure 2: Norepinephrine (orange, B panel), epinephrine (bleu, C panel) and ProBNP (green, E panel) as expected a late rise was observed when EO decreased. However a significant fall in blood pressure (bluette, D panel) during CPB was present.

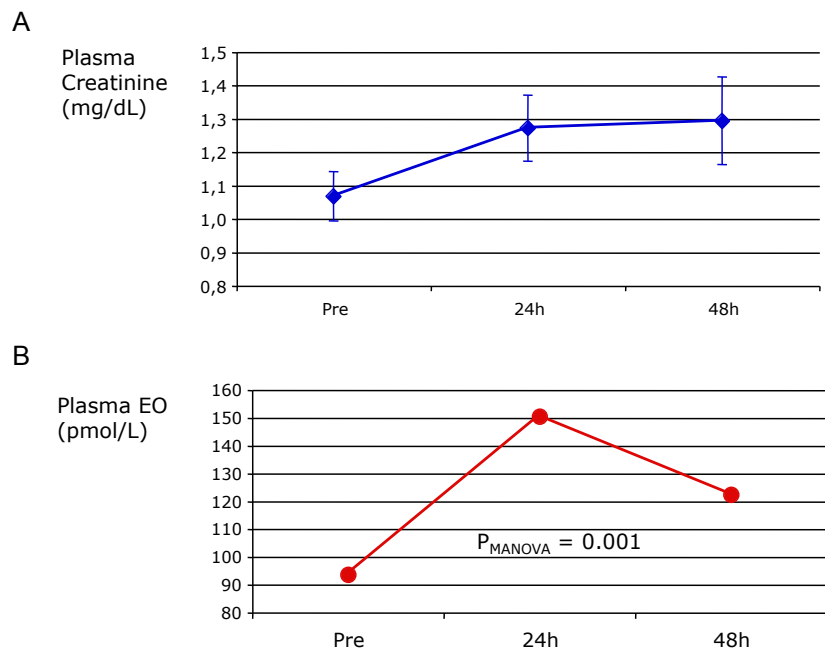


Figure 3: Plasma creatinine (A panel) and Endogenous Ouabain (B panel) 24 and 48 hours after CPB.

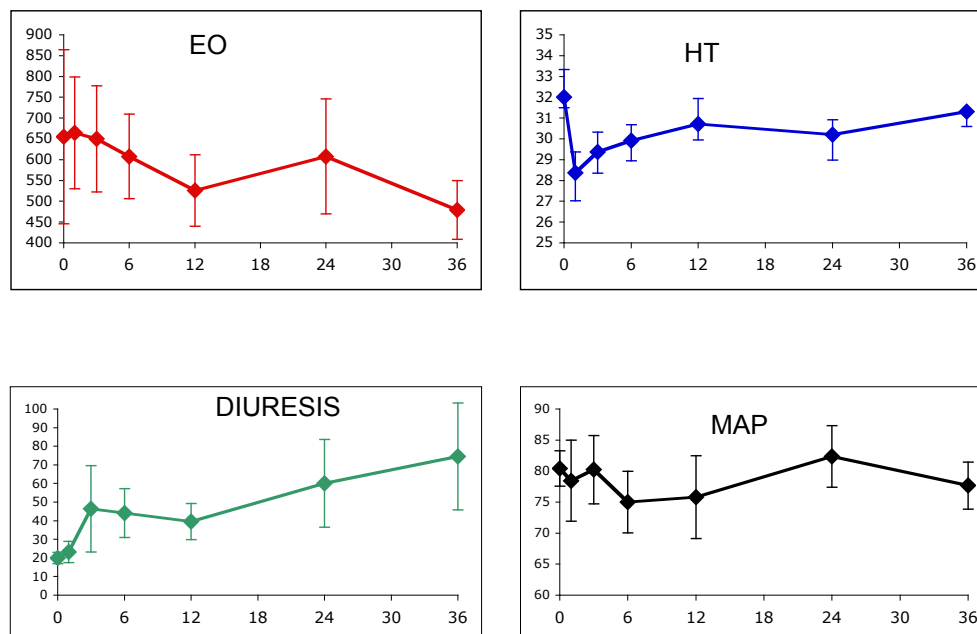


Figure 4: RRT. During renal replacement therapy EO did not changes significantly as well as hematocrit (HT), mean arterial pressure (MAP). However, urinary out-put increase as effect of continous RRT

	Mean/Median/Percentage	SD/IQ range
R of RIFLE serum creatinine (mg/dl)	2.47	0.93
R of RIFLE EO levels (pmol/L)	354	(331-579)
Serum creatinine at RRT start (mg/dl)	3.40	0.77
EO levels at CRRT start (pmol/L)	473	(249-837)
Length of RRT (hours)	114	(39-108)
Predilution (N;%)	8; 53%	-
Postdilution (N; %)	7; 47%	-
Ultrafiltration (ml/h)	128	46
ICU stay (days)	16	(9-23)
Length of Hospital Stay (days)	37	(14-44)
30-days Mortality (N;%)	6; 40%	-

Table :2 Postoperative characteristics

The observation that EO is not removed by RRT is in agreement with our previous data in dialysis patients [36]. Plasma levels of EO were largely unaffected by treatment modality (haemodialysis and chronic ambulatory peritoneal dialysis) as well as by haemodialysis procedure.

Conclusion

EO may be considered a stress hormone that changes rapidly during acute volume expansion and blood pressure fall during CPB. Future studies will determine whether the use of EO as preoperative marker that will predict the incidence of postoperative AKI in cardiac surgery patients.

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