IJC Heart & Vessels 3 (2014) 49–56

Contents lists available at ScienceDirect

IJC Heart & Vessels

journal homepage: http://www.journals.elsevier.com/ijc-heart-and-vessels

Corticosteroids increase intracellular free sodium ion concentration via glucocorticoid receptor pathway in cultured neonatal rat cardiomyocytes☆,☆☆,★

Daisuke Katoh *, Kenichi Hongo, Keiichi Ito, Takuya Yoshino, Yosuke Kayama, Makoto Kawai, Taro Date, Michihiro Yoshimura

Division of Cardiology, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo 105-8461, Japan

ARTICLE INFO

Article history:
Received 10 February 2014
Accepted 3 March 2014
Available online 13 March 2014

Keywords:
Intracellular sodium
Cardiomyocytes
Corticosteroids
Glucocorticoid receptor
Hypertrophy

ABSTRACT

Background: Glucocorticoids as well as mineralocorticoid have been shown to play essential roles in the regulation of electrical and mechanical activities in cardiomyocytes. Excess of these hormones is an independent risk factor for cardiovascular disease. Intracellular sodium ($[Na^+]_i$) kinetics are involved in cardiac diseases, including ischemia, heart failure and hypertrophy. However, intrinsic mediators that regulate $[Na^+]_i$ in cardiomyocytes have not been widely discussed. Moreover, the quantitative estimation of altered $[Na^+]_i$ in cultured cardiomyocytes and the association between the level of $[Na^+]_i$ and the severity of pathological conditions, such as hypertrophy, have not been precisely reported.

Methods and results: We herein demonstrate the quantitative estimation of $[Na^+]_i$ in cultured neonatal rat cardiomyocytes following 24 h of treatment with corticosterone, aldosterone and dexamethasone. The physiological concentration of glucocorticoids increased $[Na^+]_i$ up to approximately 2.5 mM (an almost 1.5-fold increase compared to the control) in a dose-dependent manner; this effect was blocked by a glucocorticoid receptor (GR) antagonist but not a mineralocorticoid receptor antagonist. Furthermore, glucocorticoids induced cardiac hypertrophy, and the hypertrophic gene expression was positively and significantly correlated with the level of $[Na^+]_i$. Dexamethasone induced the upregulation of Na^+/Ca^{2+} exchanger 1 at the mRNA and protein levels.

Conclusions: The physiological concentration of glucocorticoids increases $[Na^+]_i$ via GR. The dexamethasone-induced upregulation of NCX1 is partly involved in the glucocorticoid-induced alteration of $[Na^+]_i$ in cardiomyocytes. These results provide new insight into the mechanisms by which glucocorticoid excess within a physiological concentration contributes to the development of cardiac pathology.

1. Introduction

The sodium ion (Na^+) is the primary determinant of the distribution of body fluids. While the extracellular Na^+ ($[Na^+]_o$) is ~140 mM, the concentration of free intracellular Na^+ ($[Na^+]_i$) is normally 4–16 mM, as exquisitely maintained by a series of ion channels and transporters [1–6]. This transsarcolemmal Na^+ gradient is a key regulator of various intracellular ions and metabolites. In the heart, $[Na^+]_i$ has been shown to increase in the presence of cardiac diseases, including ischemia, heart failure and hypertrophy [2–6]. Although the molecular mechanisms by which $[Na^+]_i$ increases in pathological conditions and the causal relationship between $[Na^+]_i$ and cardiac disease remain controversial, some reports have suggested that elevated $[Na^+]_i$ induces unfavorable effects in cardiomyocytes. For example, an ionophore monensin, which transports ions across the cell membrane and increases $[Na^+]_i$, has been reported to activate the hypertrophic gene expression via salt-inducible kinase 1 (SIK1), a kinase known to be critical for cardiac development, in a myocyte cell line [7]. On the other hand, elevated $[Na^+]_i$ increases the mitochondrial formation of reactive oxygen species in failing cardiac myocytes [8,9]. Moreover, a rise in $[Na^+]_i$ reduces Ca^{2+} extrusion via the Na^+/Ca^{2+} exchanger (NCX), which induces diastolic Ca^{2+} overload [5]. These reports suggest that elevated $[Na^+]_i$ in cardiomyocytes is a trigger for the development of pathological conditions in the heart.

Corticosteroids, including aldosterone and cortisol (in humans)/corticosterone (in rodents), regulate the absorption of sodium ions in

☆ All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.
☆☆ There was no financial support for this study.
★ The authors report no conflicts of interest.
* Corresponding author at: Division of Cardiology, Department of Internal Medicine, The Jikei University School of Medicine, 3-25-8 Nishi-shinbashi, Minato-ku, Tokyo 105-8461, Japan. Tel.: +81 3 3433 1111; fax: +81 3 3459 6043.
E-mail address: dkato@jikei.ac.jp (D. Katoh).

http://dx.doi.org/10.1016/j.ijchv.2014.03.001

renal tubules via a member of the steroid receptor superfamily, mineralocorticoid receptor (MR). An excess of these hormones induces sodium retention, thereby causing hypertension [10,11]. Moreover, high levels of cortisol and aldosterone are known to be independent risk factors for cardiovascular events [11–13]. This may be partly due to the direct alteration of mineralocorticoid receptor (MR) signaling in the cardiovascular system in addition to secondary systemic activities of these hormones, such as the induction of hypertension, as MR antagonists are responsible for marked prognostic improvements in patients with heart failure [14–16]. In cardiomyocytes, MR is expressed, while 11beta-hydroxysteroid dehydrogenase type 2, which converts glucocorticoids to their inactive metabolites, is not [17,18]. Hence, under physiological conditions, most MRs are presumably occupied by cortisol/corticosterone. On the other hand, glucocorticoid receptor (GR), which displays high sequence homology with MR and binds glucocorticoids with higher affinity than the mineralocorticoid aldosterone, is also expressed in cardiomyocytes. However, the role of specific GR signaling in the cardiovascular system is poorly understood.

We and others have recently reported that aldosterone induces $[Na^+]_i$ elevation in cultured cardiomyocytes and that this effect is rapid and non-genomic and occurs in a mineralocorticoid receptor-independent fashion [19,20]. Although there is a previous report that 24-hour treatment with aldosterone activates Na^+/H^+ exchange (NHE) and increases $[Na^+]_i$ in cardiomyocytes using a sodium fluorescent indicator [21], the absolute value of the alteration in $[Na^+]_i$ is not available. To our knowledge, the effects of glucocorticoid/GR signaling on $[Na^+]_i$ handling in cardiomyocytes have not been previously reported. Moreover, the relationship between the severity of a pathological status, such as cardiac hypertrophy, and the level of $[Na^+]_i$ in cardiomyocytes has not been discussed.

The present study was conducted to identify the role of corticosteroids, including corticosterone, aldosterone and synthesized glucocorticoid dexamethasone, in the regulation of $[Na^+]_i$ in cardiomyocytes. The results suggest that NCX1 is involved, at least in part, in the pathogenesis of altered sodium ion handling under conditions of glucocorticoid excess via the GR pathway.

2. Materials and methods

2.1. Reagents

Corticosterone, dexamethasone, eplerenone and RU486 were purchased from Sigma-Aldrich. Aldosterone was purchased from Wako

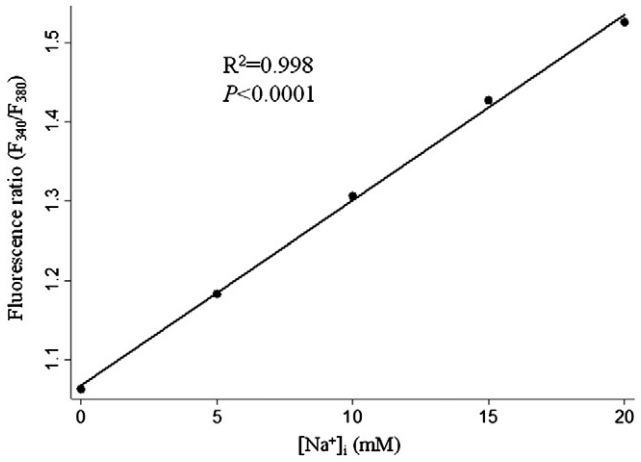

Fig. 1. *In vivo* calibration of SBFI. The *in vivo* calibration of SBFI was accomplished by exposing NRVM to various extracellular $[Na^+]$. Between 0 and 20 mM $[Na^+]_i$, the SBFI fluorescence ratio (F_{340}/F_{380}) exhibited a linear relationship with $[Na^+]_i$ ($R^2 = 0.998$, $P < 0.0001$).

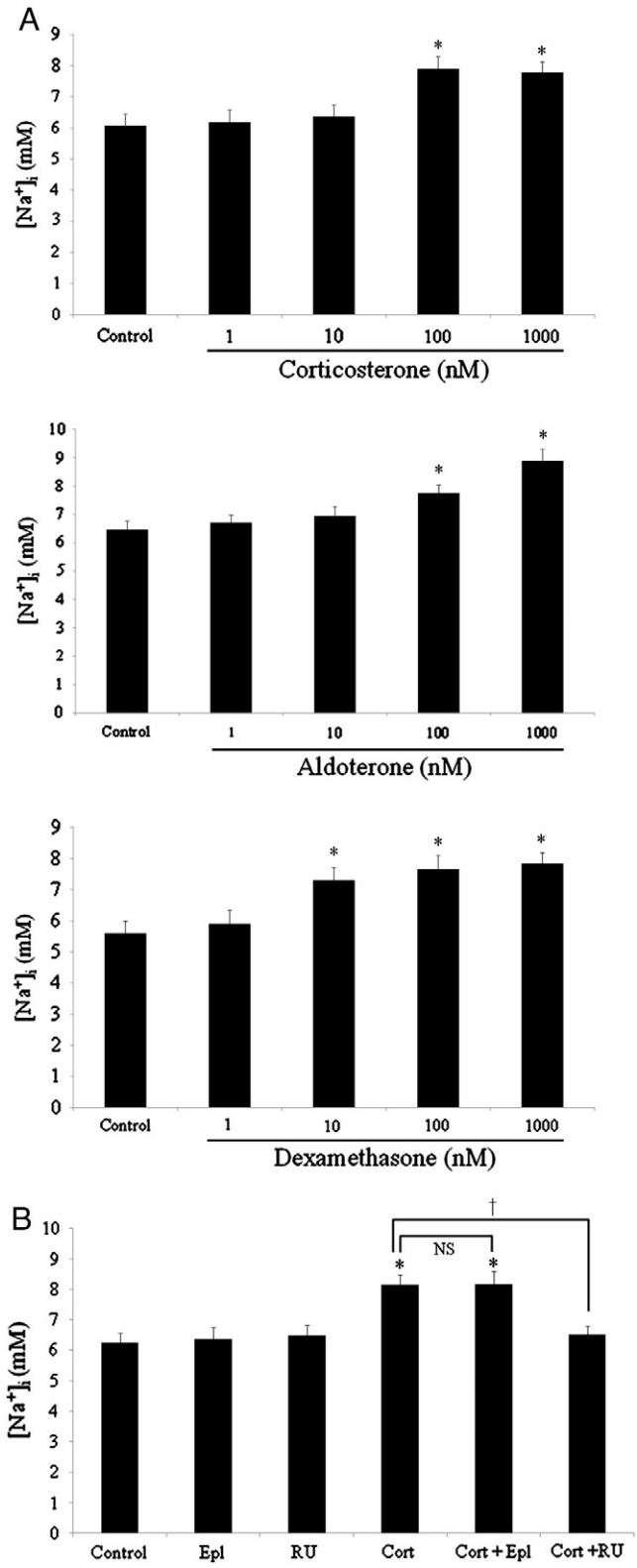

Fig. 2. Corticosteroids increase $[Na^+]_i$ via GR in NRVM. NRVM were treated with corticosterone, aldosterone and dexamethasone at concentrations of 1 to 1000 nM in the presence or absence of 1 μM of RU486 or 10 μM of eplerenone for 24 h. The quantitative estimation of $[Na^+]_i$ was carried out after treatment. A: corticosterone, aldosterone and dexamethasone increased $[Na^+]_i$ in a dose-dependent manner. The data represent the mean ± SEM of six to nine independent experiments. B: the corticosterone-induced $[Na^+]_i$ elevation was blocked by RU486 but not eplerenone. The data represent the mean ± SEM of seven independent experiments. Cort, 100 nM of corticosterone; Epl, 10 μM of eplerenone; RU, 1 μM of RU486. *$P < 0.05$ versus control group, †$P < 0.05$.