ELSEVIER

Atherosclerosis 166 (2003) 345–349

ATHEROSCLEROSIS

www.elsevier.com/locate/atherosclerosis

Aldosterone induces contraction of the resistance arteries in man

Paola Romagni, Fabio Rossi, Lara Guerrini, Chiara Quirini, Vittorio Santiemma *

Dipartimento di Fisiopatologia Medica, V Clinica Medica, Policlinico Umberto I, Facoltà di Medicina, Università di Roma 'La Sapienza', Viale del Policlinico, 00161 Rome, Italy

Received 17 July 2002; received in revised form 20 September 2002; accepted 27 September 2002

Abstract

Very rapid nongenomic effects of aldosterone in vitro have been described in recent years and in vivo evidence has been reported as well. In the present study, we investigated the rapid effect of aldosterone on resistance arteries in vivo in man. We performed a randomized, placebo-controlled, double-blind crossover study on ten healthy male volunteers. Forearm blood flow (FBF) was measured using venous occlusion plethysmography in both forearms. FBF was reduced by administration of aldosterone 2.5 pmol/min at min 4 (from 4.45 ± 0.03 to 3.3 ± 0.25 ml/100 ml tissue) and reached its nadir at min 12 (from 4.45 ± 0.03 to 1.6 ± 0.08 ml/100 ml tissue, $P < 0.001$). Our study documents a direct nongenomic effect of aldosterone on the resistance arteries in vivo in man. The rapid vasoconstrictive effect of aldosterone at physiological concentrations opens the way to investigations on the vascular role of this steroid in several disorders, such as hypertension, characterized by elevated peripheral vascular resistance.

Keywords: Aldosterone; Nongenomic; Plethysmography; Vascular resistance; Forearm blood flow

1. Introduction

In addition to genomic steroid actions, very rapid, nongenomic effects of steroids have been described during recent years. Non-genomic effects, not involving DNA transcription and ex novo protein synthesis, differ from genomic steroid actions by their short time lag of action, occurring in vivo within a few minutes and in vitro within seconds after the addition of steroids. These effects have been well documented for aldosterone [1].

In vitro rapid effects of aldosterone have been reported also in vascular cells. In vascular smooth muscle cells (VSMC) and endothelial cells, aldosterone affects the intracellular concentrations of sodium, potassium, calcium, cell volume and the sodium-proton-antiport within some minutes after addition [2,3]. Not being inhibited by the antagonists of the classical mineralcorticoid receptors, these nongenomic effects seem to imply the existence of distinct receptors, even though recent evidence suggests that this may not be the case [4].

In vivo evidence for rapid aldosterone actions has been reported as well [5–8]. In a double-blind placebo-controlled trial on patients with suspected coronary heart disease, the effect of aldosterone intra cava on cardiovascular function was assessed during cardiac catheterization [6]. Among the hemodynamic parameters evaluated, systemic vascular resistance (SVR) and cardiac index were significantly changed, compared with the placebo group. The effect of aldosterone dissipated within 10 min, indicating their likely non-genomic nature, which was further substantiated by another clinical study [7] which showed that i.v. injection of aldosterone increased SVR within 5 min in healthy male volunteers. In addition, aldosterone non-genomic cardiovascular effects were shown to interact with the adrenergic system [8].

However, these studies could not rule out a systemic, as distinct from a direct, effect of aldosterone on vascular cells because of the route of aldosterone administration.

To assess a direct nongenomic aldosterone effect on vascular cells in vivo, in the present study we investigated the rapid effects of aldosterone on resistance

* Corresponding author. Tel.: +39-06-4997-0704; fax: +39-06-4905-30

E-mail address: vittorio.santiemma@uniroma1.it (V. Santiemma).

0021-9150/02/$ - see front matter © 2002 Published by Elsevier Science Ireland Ltd.
PII: S0021-9150(02)00363-5

arteries in man, using strain-gauge plethysmography with a venous occlusion technique.

2. Materials and methods

We performed a randomized, placebo-controlled, double-blind crossover study on ten healthy male volunteers (age range: 25–35 years; mean ± S.D.: 30 ± 5) recruited from our university campus. All subjects were non smokers and medication free and were judged to be healthy by a medical examination and all were normotensive, defined as the absence of familial history of essential hypertension and blood pressure $< 140/80$ mmHg. All subjects gave written informed consent according to procedures approved by the ethics committee of our university.

Before experimental sessions, all participants received a standardized diet equilibrated in protein, fat and carbohydrate and divided into 5.7 (dinner), 12.8 (breakfast) and 10 (lunch) cal/kg/BW, with 1.89 mmol/kg per day sodium and 1.49 mmol/kg per day potassium for 2 weeks and were required to abstain from engaging in severe physical exercise for at least 2 days.

All experiments were performed at 09:00 h after an overnight fast, with subjects lying supine in a quiet 22 ± 1 °C temperature- and humidity-controlled room.

An intravenous catheter was inserted into an antecubital vein of the dominant arm and maintained patent with a slow drip of 0.9% sterile saline solution. After a rest period of 30 min and at the end of infusion, blood was drawn through a stopcock for subsequent analysis. Samples were immediately centrifuged and stored at − 80 °C until analysis. Then, all the participants in the study, after catheterization with a polyethylene cannula (21 gauge, BD) that was inserted into the brachial artery (nondominant arm) under local anesthesia (2% lidocaine), were administered 0.9% NaCl solution for 20 min. After this, they were randomly subjected to infusion either of aldosterone 2.5 pmol/min or of 0.9% NaCl solution for 10 min in two distinct sessions 1 week apart.

The administration of placebo or aldosterone (30 ml/h infusion rate) was performed with a syringe infusion pump perfusor (Perfusor Compact B. Braun). Noninvasive measurements of forearm blood flow (FBF), expressed as milliliters per 100 ml of tissue per minute (NIVP3, D.E. Hokanson, Inc.), were performed during the equilibration and test time.

FBF was measured, as previously described [9], using venous occlusion strain-gauge plethysmography in both forearms (experimental and contralateral forearms). Both hands were slightly supported above heart level and a calibrated mercury-in-silastic strain-gauge was placed around the mid forearm to record changes in volume. The gauges were connected to a plethysmograph (EC6 Hokanson, Inc.). A blood pressure cuff placed on the upper arm and inflated to 50 mmHg prevented venous outflow from the extremity. Circulation of the hand was excluded 1 min before each FBF measurement by inflating a pediatric cuff around the wrist at suprasystolic blood pressure. Each FBF determination comprised four separate measurements performed at 15-s intervals. Measurements were made under baseline condition every 5 min and then during the infusion every 4 min. Heart rate and arterial blood pressure were measured before each measurement.

2.1. Drugs

Aldosterone (mixed micelles) for clinical research was obtained from Clinalfa AG (Läufelfingen, CH). The aldosterone solution was prepared according to the instructions of the provider with 0.9% NaCl solution. Aldosterone concentration was checked by analyzing the concentration of drug at the end of the polyethylene cannula.

2.2. Hormonal analysis

Plasma levels of aldosterone were measured in duplicate by commercially available radioimmunoassay kit (Chematil srl SA, Italy). The minimal detectable quantity of aldosterone was 25 pg/ml. The intra and interassay coefficients of variation were 4.7 and 8.5%, respectively.

2.3. Statistical analysis

Results are expressed as mean ± S.D. Analysis of variance for repeated measure and Bonferroni post-hoc test were used to compare the means of blood flow in the infused (non dominant) arm and in the control (dominant) arm before and after aldosterone infusion. Other parametric data, expressed as mean ± S.D., were compared by Student's t-test. $P < 0.05$ was taken as statistically significant.

3. Results

Baseline characteristics of the subjects before each session of the study are detailed in Table 1. Systolic and diastolic blood pressure and heart rate showed no changes during and after aldosterone infusion and during saline solution infusion, (Tables 2 and 3). Plasma aldosterone concentration was not different before and at the end of aldosterone administration (Table 4). Forearm blood flow under baseline conditions and during saline solution infusion was consistent throughout the study with mean values of 4.45 ± 0.03 ml/100 ml

Table 1
Clinical characteristics and laboratory parameters of the study volunteers

Parameter	Before aldosterone infusion (mean±S.D.)	Before placebo infusion (mean±S.D.)
Wt (kg)	70±8	70.5±6
Na+ (mmol/l)	143±0.6	142±0.4
K+ (mmol/l)	4.18±0.05	4.20±0.05
Protein (mg/dl)	7.45±0.15	7.40±0.23
Basal aldosterone (pg/ml)	189±11.2	175±10.1
Basal renin (pg/ml)	10.3±0.9	11.4±1.7
Urinary sodium (mmol/24 h)	161±23	159±22
Urinary creatinine (mg/24 h)	2125±183	2150±206

Table 2
Cardiovascular parameters (mean±S.D.) during aldosterone 2.5 pmol/min i.a. infusion (0.5 ml/min infusion rate)

Min.	SBP (mmHg)	DBP (mmHg)	Hr (beats/min)
−10	127±4	80±2	73±5
−5	127±4	80±5	76±4
0	130±5	78±4	75±5
4	125±4	78±4	74.5±3
8	128±3	78±4	80±4
12	130±2	80±3	74±5
16	128±5	80±3	76±6
20	127±3	75±3	75±6
24	128±2	82±3	75±4
28	130±3	80±5	76±4
32	128±2	76±4	76±4
36	130±5	80±2	75±5

SBP, systolic blood pressure; DBP, diastolic blood pressure; Hr, heart rate.

Table 3
Cardiovascular parameters (mean±S.D.) during 0.9% sterile saline solution i.a. infusion (0.5 ml/min infusion rate)

Min.	SBP (mmHg)	DBP (mmHg)	Hr (beats/min)
−10	128±2	80±4	75±5
−5	130±5	76±4	80±4
0	127±4	78±4	74±5
4	128±5	79±2	76±6
8	130±4	80±3	75±6
12	130±3	76±3	78±6
16	126±3	75±3	76±4
20	127±3	80±2	75±5
24	124±5	76±4	74±3
28	127±3	78±2	76±3
32	128±2	80±5	73±4
36	129±3	80±3	76±5

SBP, systolic blood pressure; DBP, diastolic blood pressure; Hr, heart rate.

Table 4
Plasma aldosterone concentration (pg/ml, mean±S.D.) before and at the end of aldosterone administration

Subjects No.	Aldosterone administration	
	Start	End
1	201±6	198±3
2	183.5±5	209±2
3	206±3	198±4
4	184±3	208±4
5	181±4	154±4
6	155±3	154±3
7	140±3	144±4
8	195±3	227±5
9	213±4	209±3
10	220±2	200±2

tissue per min without significant differences between the two arms.

The i.a. administration of 2.5 pmol/min aldosterone induced FBF changes. FBF was significantly reduced at min 4 (from 4.45±0.03 to 3.3±0.25 ml/100 ml/min, $P < 0.01$) and reached its nadir at min 12 (from 4.45±0.03 to 1.6±0.08 ml/100 ml/min $P < 0.001$). FBF did not change in the control arm (Fig. 1).

Corrected forearm blood flow (infused arm minus control arm) was −1.2±0.3 ml/100 ml/min ($P < 0.01$) at

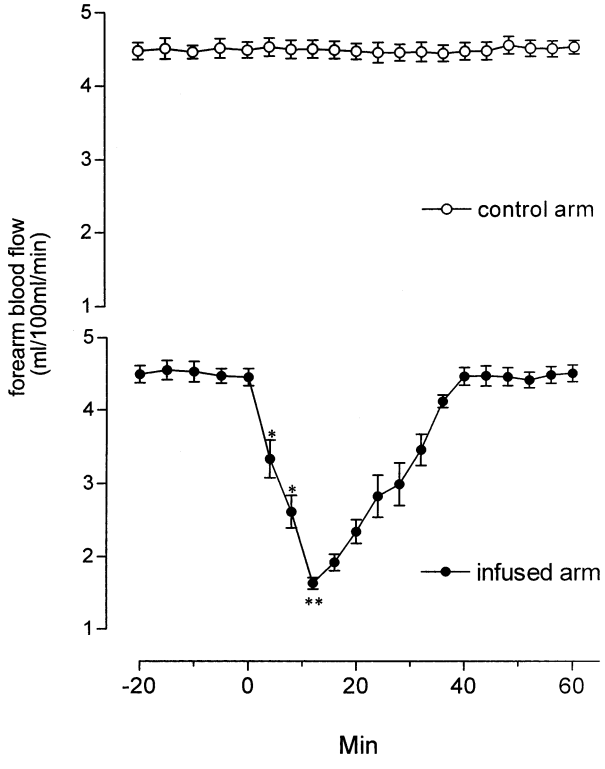

Fig. 1. Forearm blood flow, expressed as ml/100 ml/min, in infused (●) and control arm (○) before, during and after 10 min aldosterone (2.5 pmol/min) infusion. Each point is expressed as the mean of four measurements performed at 15-s intervals in the ten subjects; bars show S.E.M., *$P < 0.01$, **$P < 0.001$.

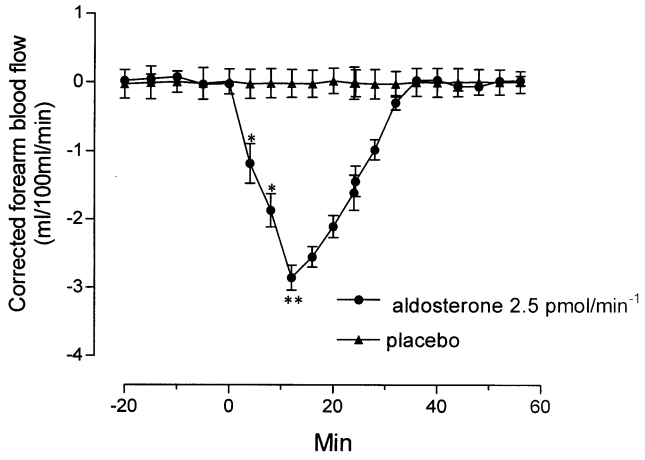

Fig. 2. Corrected forearm blood flow (infused arm minus control arm), expressed as ml/100 ml/min, before, during and after 10 min 2.5 pmol/min aldosterone infusion (●) and during saline solution infusion (▲). Each point is expressed as the mean of the ten subjects; bars show S.E.M., $*P < 0.01$, $**P < 0.001$.

min 4 and -2.86 ± 0.2 ml/100 ml/min ($P < 0.001$) at min 12 during aldosterone infusion and was unchanged during placebo infusion (Fig. 2).

The maximal effect of 2.5 pmol/min aldosterone, expressed as percent reduction in FBF ratio infused/control arm, was $\approx 70\%$ at min 12 (Fig. 3).

4. Discussion

The present investigation documents that aldosterone, at physiological concentrations (inducing $\approx 100\%$ increase over basal levels in the brachial artery), is able to induce contraction of the resistance arteries in man. The aldosterone administration into the brachial artery and the venous occlusion plethysmography assessment of forearm blood flow permit the evaluation of the steroid direct vascular effect.

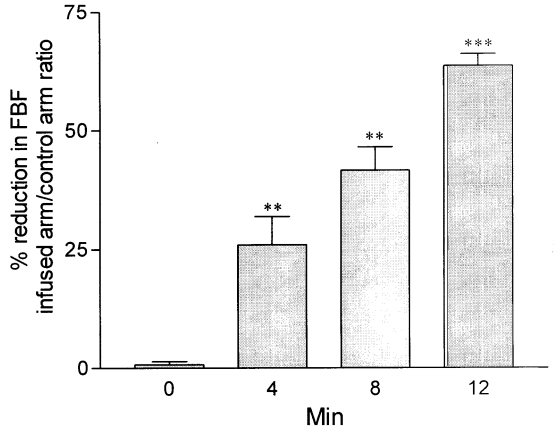

Fig. 3. Percent reduction in FBF infused arm/control arm ratio. Data are normalized assuming as 100% the mean control FBF. Each point is expressed as the mean of four measurements performed at 15-s intervals in the ten subjects; bars show S.E.M., $*P < 0.01$, $**P < 0.001$.

Providing relevant information on the regulation of vascular tone in vivo, venous occlusion plethysmography has been used in numerous investigations on vascular endothelium function and dysfunction. This technique has proven very effective in the evaluation of the vascular response to several vasoactive compounds (e.g. acetylcholine, bradychinine, N^G-monomethyl-L-arginine, angiotensin II and endothelin-1) both in normal subjects [10–12] and in subjects with cardiovascular disorders and endothelial dysfunction [13–19].

In the investigations on the in vivo nongenomic aldosterone effects reported up to now [5–8], aldosterone was administered by an intravenous route at pharmacological doses and induced systemic effects, including changes of cardiac output and vascular resistance. Venous occlusion plethysmography evaluation of FBF and brachial artery administration of physiological aldosterone amounts allowed us to document nongenomic, direct effects of aldosterone on FBF in the absence of systemic effects, as evidenced by the unmodified BP, heart rate and forearm blood flow in the contralateral forearm.

The documented direct and rapid vasoconstriction induced by aldosterone has to be added to the already known vascular genomic effects of aldosterone, including increased vasoconstrictive effect of catecholamines [20], impaired acetylcholine-induced vasodilation [21], upregulation of β-adrenergic and angiotensin II (AngII) receptors [22]. Aldosterone may enhance the vascular effects of AngII by promoting the production of AT1 receptors [23] and of Na channel proteins [24], intracellular alkalinization and subsequent activation of the mitogenesis in VSMC [25].

The variety of aldosterone effects suggests that this hormone plays a role in the complex regulation of the resistance artery tone by vasoconstrictor and vasorelaxant agonists and may be pathophysiologically relevant in cardiovascular disorders, such as hypertension, characterized by progressively elevated peripheral vascular resistance.

In addition, data compatible with paracrine vascular effects of aldosterone have been reported [26–29]. Vascular cells seem capable of local aldosterone production [26,27]. CYP11B2 mRNA, encoding aldosterone synthase, the key enzyme for aldosterone biosynthesis, has been detected in both endothelial cells and smooth muscle cells cultured from human pulmonary artery [28]. Moreover, aldosterone-specific MR-1 receptors and 11 hydroxysteroid dehydrogenase (11HSD) have been reported in VSMC [29]. Increased aldosterone vascular synthesis has been reported in experimental models of hypertension [30] and it could further enhance the amplifying effect of aldosterone on AngII-induced vasoconstriction and smooth muscle cell proliferation.

The finding that aldosterone is able to induce vasoconstriction indicates that circulating aldosterone may directly affect vascular tone and may be part of the vasoconstriction response to gravity. In addition, it opens the way to investigations on the balance between endocrine and putative paracrine effects of aldosterone on vascular cells.

However, the relevance of aldosterone in the mechanisms involved in vascular remodelling and increased vascular tone present in the hypertensive state needs to be evaluated by further investigations specifically oriented to address that issue.

As far as the nongenomic effects of aldosterone are concerned, solving the problem of the receptors involved—new ones to be characterized or the old ones through different signal transduction pathways—seems to be one of the crucial steps.

Acknowledgements

This work was partially supported by 60 and 40% MURST grants.

References

[1] Wehling M. Specific, nongenomic actions of steroid hormones. Annu Rev Physiol 1997;59:365–93.
[2] Christ M, Wehling M. Rapid actions of aldosterone: lymphocytes, vascular smooth muscle and endothelial cells. Steroids 1999;64:35–41.
[3] Wehling M, Ulsenheimer A, Schneider M, Neylon C, Christ M. Rapid effects of aldosterone on free intracellular calcium in vascular smooth muscle and endothelial cells: subcellular localization of calcium elevations by single cell imaging. Biochem Biophys Res Comm 1994;204:475–81.
[4] Funder JW. Non-genomic actions of aldosterone: role in hypertension. Curr Opin Nephrol Hypertens 2001;10:227–30.
[5] Zange J, Muller K, Gerzer R, Sippel K, Wehling M. Nongenomic effects of aldosterone on phosphocreatine levels in human calf muscle during recovery from exercise. J Clin Endocrinol Metab 1996;81:4296–300.
[6] Wehling M, Christoph HS. Rapid cardiovascular action of aldosterone in man. J Clin Endocrinol Metab 1998;83:3517–22.
[7] Schmidt B, Montealegre A, Martin J, et al. Short term cardiovascular effects of aldosterone in healthy male volunteers. J Clin Endocrinol Metab 1999;84:3528–33.
[8] Schmidt B, Georgens C, Martin N, Tillmann H, Feuring M, Christ M, Wehling M. Interaction of rapid nongenomic cardiovascular aldosterone effects with the adrenergic system. J Clin Endocrinol Metab 2001;86:761–7.
[9] Bejamin N, Calver A, Collier J, Robinson B, Vallance P, Webb D. Measuring forearm blood flow and interpreting the responses to drugs and mediators. Hypertension 1995;25:918–23.
[10] Haynes WG, Clarke JG, Cockcroft JR, Webb DJ. Pharmacology of endothelin-1 in vivo in humans. J Cardiovasc Pharmacol 1991;17(7):S284–6.
[11] Ritter JM, Cockcroft JR, Sciberras DG, Goldberg MR. Clinical pharmacology of angiotensin and bradykinin in human forearm vasculature. J Hypertens 1993;11:S59–61.
[12] Benjamin N, Calver A, Collier J, Robinson B. Measuring forearm blood flow and interpreting the response to drugs and mediators. Hypertension 1995;25:918–23.
[13] Panza JA, Quyyumi AA, Brush JE, Epstein SE. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. New Engl J Med 1996;323:22–7.
[14] Angus JA. Role of endothelium in the genesis of cardiovascular disease. Clin Exp Pharmacol Physiol 1996;23:S16–22.
[15] Baan J, Jr, Chang PC, Vermeij P, Pfaffendorf M, van Zwieten PA. Effects of angiotensin II and losartan in the forearm of patients with essential hypertension. J Hypertens 1998;16:1299–305.
[16] Taddei S, Virdis A, Ghiadoni L, Mattei P, Salvetti A. Effects of angiotensin converting enzyme inhibition on endothelium-dependent vasodilatation in essential hypertensive patients. J Hypertens 1998;16:447–56.
[17] Goldsmith SR, Garr M, McLaurin M. Regulation of regional norepinephrine spillover in heart failure: the effect of angiotensin II and beta-adrenergic agonists in the forearm circulation. J Card Fail 1998;4:305–10.
[18] Hogikyan RV, Galecki AT, Pitt B, Halter JB, Greene DA, Supiano MA. Specific impairment of endothelium-dependent vasodilation in subjects with type 2 diabetes independent of obesity. J Clin Endocrinol Metab 1998;83:1946–52.
[19] McAuley DF, McGurk C, Nugent A, Hanratty C, Hayes JR, Johnston GD. Vasoconstriction to endothelin-1 is blunted in non-insulin-dependent diabetes: a dose-response study. J Cardiovasc Pharmacol 2000;36:203–8.
[20] Weber MA, Purdy RE. Catecholamine-mediated constrictor effects of aldosterone on vascular smooth muscle cells. Life Sci 1982;30:2009–17.
[21] Taddei S, Virdis A, Mattei P, Salvetti A. Vasodilation to acetylcholine in primary and secondary forms of human hypertension. Hypertension 1993;21:929–33.
[22] Schiffrin EL, Franks DJ, Gutkwoska J. Effect of aldosterone on vascular angiotensin II receptors in the rat. Can J Physiol Pharmacol 1985;63:1522–7.
[23] Ullian ME, Fine JJ. Mechanics of enhanced angiotensin II-stimulated signal transduction in vascular smooth muscle by aldosterone. J Cell Physiol 1994;16:201–8.
[24] Ebata S, Muto S, Okada K, Nemoto J. Aldosterone activates Na/H exchanges in vascular smooth muscle cells by nongenomic and genomic mechanisms. Kidney Int 1999;56:1400–12.
[25] Xiao F, Puddefoot JR, Vinson GP. Aldosterone mediates angiotensin II-stimulated rat vascular smooth muscle cells proliferation. J Endocrinol 2000;165:533–6.
[26] Takeda Y, Miyamori I, Yoneda T, et al. Regulation of aldosterone synthase in human vascular endothelial cells by angiotensin-II and adrenocorticotropin. J Clin Endocrinol Metab 1996;81:2797–800.
[27] Hatakeyama H, Miyamori I, Takeda Y, Yamamoto H. The expression of steroidogenic enzyme genes in human vascular cells. Biochem Mol Biol Int 1996;40:639–45.
[28] Hatakeyama H, Miyamori I, Fijita T, Takeda Y, Takeda R, Yamamoto H. Vascular aldosterone. Biosynthesis and link to angiotensin-II-induced hypertrophy of vascular smooth muscle cells. J Biol Chem 1994;269:24316–20.
[29] Kornel L. Colocalization of 11-beta-hydroxysteroid dehydrogenase and mineral corticoid receptors in cultured vascular smooth muscle cells. Am J Hypertens 1994;7:100–3.
[30] Rocha R, Stier CT, Jr. Pathophysiological effects of aldosterone in cardiovascular tissues. Trends Endocrinol Metab 2001;12:308–14.