ORIGINAL ARTICLES

Positive linear regression between left ventricular contraction duration and plasma aldosterone levels in healthy anesthetized rabbits. A hypothesis generating relationship for a new action of aldostrerone

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ABSTRACT: *Introduction*: Aldosterone (Aldo), besides its genomic renal and cardiac effects [cardiac fibrosis and left ventricular (LV) enlargement], elicits also various rapid nongenomically mediated effects such as increase of myocardial monophasic action potential duration within minutes after intravenous application.

Methods: Investigating the effects of endogenous vasoactive substances on cardiovascular and hormonal parameters in 25 healthy anesthetized New Zealand White male rabbits, a serendipitous positive correlation between LV contraction duration and plasma Aldo levels came to our attention. From LV pressure (LVP) tracings (taken invasively), maximal and minimal change over time in LVP (LVmaxdp/dt and LVmindp/dt) and Δd (time interval between LVmaxdp/dt and LVmindp/dt = LV contraction duration) were estimated.

Results: A positive linear regression was found between Aldo (measured by RIA) and Δd (Δd = 0.1126 + 0.00019, r = 0.47, p = 0.018). Stepwise regression analysis demonstrated that peripheral blood pressure and Aldo were the most important determinants of Δd and that the effect of Aldo was independent of confounding factors.

Conclusion: Since electrical and mechanical events in cardiac muscle overlap in time, it may be speculated that the nongenomically-mediated increase in monophasic action potential duration by Aldo could result in increase of myocardial contraction duration, a hypothesis supported indirectly by the found regression.

Key Words: Aldosterone, Left ventricular function, Left ventricular contraction duration, Rabbits.

INTRODUCTION

In addition to its well-known renal effects aldosterone (Aldo) exerts cardiac actions such as blood pressure-independent cardiac fibrosis[1] and left ventricular (LV) enlargement[1]. Apart from these presumably genomic effects, Aldo elicits rapid nongenomically-mediated effects[2]; it acutely increases systemic vascular resistance[3], decreases cardiac output[4] and increases atrial myocardial monophasic action potential duration within minutes after intravenous iv application[5].

We like to report a serendipitous finding that came to our attention, a positive correlation and a linear regression between LV contraction duration and plasma Aldo levels that do not seem to have yet been reported in the literature. This correlation was found during our investigation of the effects of endogenous vasoactive substances [Atrial Natriuretic Peptide (ANP) and Brain Natriuretic Peptide (BNP)] on cardiovascular and hormonal parameters in healthy anesthetized male New Zealand White (NZW) rabbits.

In this paper we describe the experimental protocol as well as the findings and the statistical results that guided us to the above correlation and we attempt its interpretation on the basis of other investigators results for Aldo actions.

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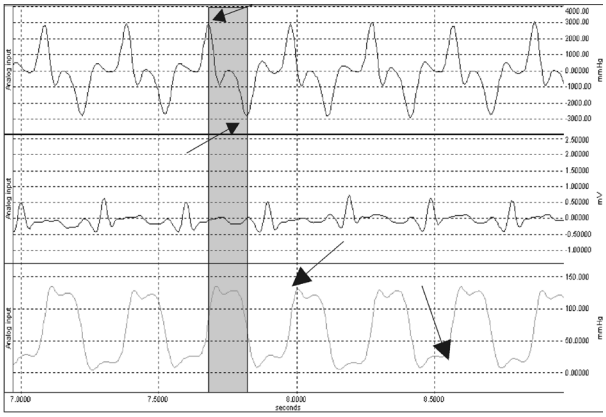

Figure 1. Concurrent recordings of ECG, LVP and its derived dp/dt tracing, for the measurement of LVmaxdp/dt, LVmindp/dt, Δd and LVEDP. (LVmaxdp/dt = upper tracing, upper arrow, LVmindp/dt = upper tracing, lower arrow, Δd = dashed area between LVmaxdp/dt and LVmindp/dt, LVEDP = lower tracing, arrow).

METHODS

Twenty five New Zealand White (NZW) male rabbits with body weight (BW): 3600 ± 485 g [corresponding to an age range of 16 to 26 wk[6]] and hematocrit (Ht): 33±6% ($\overline{X}$ ± SD) were used, raised in our Department in all-metal wire floor-type hutches and given food and water ad libitum. The calculated daily Na^+ intake was 23 - 30 mEq. Under urethane (Sigma®) anesthesia (1.5g/kg BW iv) the left ventricle of the heart and the left carotid and femoral arteries were cannulated for left ventricular pressure (LVP) and blood pressure (BP) recordings, with concurrent ECG tracing. Under urethane anaesthesia both the parasympathetic and the sympathetic sections of the autonomic nervous system are tonically active in controlling resting cardiovascular parameters[7]. Central venous pressure (CVP) was also recorded and blood samples for ANP, plasma renin activity (PRA) and Aldo levels were taken from the cannulated right jugular vein. The catheters used were siliconized nasogastric radiopaque tubes 6CH filled with heparinized saline 1,000U/ml.

Carotid and femoral BP and LVP were recorded simultaneously via Gould P23XL pressure transducers in a Vital Signs Monitor (Physiocontrol® VSM4), while a TSD 104A blood pressure transducer of Biopac® was used for CVP recording. Data acquisition was performed by the aid of MP100 Work Station for Windows (MP100WSW-Biopac®). All tracings were recorded by a sample rate of 200 samples/sec and lasted 15 sec. From LVP, the left ventricular maximal (systolic) pressure (LVPmax), the left ventricular end diastolic pressure (LVEDP), the maximal and the minimal change over time in LV pressure (LVmaxdp/dt and LVmindp/dt) and the Δd [time interval between LVmaxdp/dt and LVmindp/dt = contraction duration, measured as was described by Yamamoto et al[8]] were estimated by the aid of Acqknowledge 3.2.4® software. LVPmax was estimated as the mean LVPmax of a 15 sec tracing and LVEDP was calculated as the pressure corresponding to the beginning of the LVP upstroke (mean value of LVEDP of 5 consecutive cardiac cycles). LVmaxdp/dt (index of LV contractility during isovolumic contraction) and LVmindp/dt (index of isovolumic relaxation) were estimated from the 1st derivative (dp/dt) of the LVP (a new tracing resulting, by the aid of Acqknowledge 3.2.4®, from the LVP tracing) (Figure 1). By the aid this software all maximal positive peaks and all maximal negative peaks of the 15 sec tracing were added and LVmaxdp/dt and LVmindp/dt were estimated as their mean values respectively. From the carotid artery blood pressure tracing, the left ventricular ejection time (LVET) was also estimated, as the time from the upstroke of the left carotid arterial pulse to dicrotic notch (mean value of LVET of 5 consecutive cardiac cycles) and the Double Product [DP, (heart rate X systolic blood pressure- HR x SBP)] was calculated as index of heart work. Heart rate (HR) was calculated as the mean HR of a 15sec ECG tracing, after smoothing of the ECG with a digital filter [Low Pass Finite Impulse Response (FIR) filter] Blackman -67db (cutoff frequency 20Hz), for noise removal. The ECG was monitored from standard EEG lead II. Mean blood pressure (MBP) was calculated as the ratio of the integral of pulse pressure (PP) tracing by the duration of the cardiac cycle [integral of all pulse pressures of the 15 sec tracing divided by the recording time (15 sec)].

Pulse wave velocity (PWV), which is inversely related to distensibility, was used as parameter of aorta stiffness. PWV was calculated according to the method of Hirata et al[9] as the ratio of the distance from the carotid artery to the femoral artery (distance between the recording sites: 30-37 cm according to animal size), to the time required for the pulse wave to travel from the carotid to the femoral artery, i.e. the differ-

ence of two times, the time from the beginning of the QRS to the beginning of the left femoral arterial pulse and the time from the beginning of the QRS to the upstroke of the left carotid arterial pulse.

A solution of 0.9% NaCl [normal saline (N/S)] was iv infused (by an ear vein) to the extent of 1% of body weight as priming infusion and then was followed by sustaining infusion at a rate 0.5 ml/kg/min during the whole experimental period. After the cannulation of the vessels and the ventricle, an acclimatization period of 30 min was allowed and thereafter recordings and blood samples for hormone measurement were taken. Blood (3,0 ml) was taken in prefrozen tubes containing 1mg/ml K_3EDTA and 500 U/ml aprotinine (protease inhibitor) and was centrifuged at 1,040 g for 15 min at 4°C to provide plasma for hormone measurement. The plasma was then removed and stored at -20 ^{0}C for subsequent analysis by RIA. ANP levels were measured by hANP (1-28) RIA kit of Bachem® (specificity 100% for hANP and rabbit ANP, sensitivity 5 pg/tube, intrassay variation 6.5%, interassay variation 10.5%) while PRA and Aldo were measured by RIA kits of Radim® (PRA RIA kit specificity 100% for angiotensin I, sensitivity 0.15 ng/tube, intrassay variation 5.4%, interassay variation 7.7%, Aldo RIA kit specificity 100% for Aldo, sensitivity 8.8 pg/tube, intrassay variation 6.6%, interassay variation 8.4%). All experiments were done at the same diurnal time interval (10.00-14.00).

Local Veterinary Ethical Committee approved the study, as it was in accordance with the 86/609 European Union Council order.

Descriptive statistics was used for presenting data. All values are expressed as mean ± standard deviation ($\overline{X}$ ± SD). Correlations of Δd and Aldo with other cardiovascular parameters were performed as well as stepwise regression analysis to evaluate potentially confounding factors independently contributing to Δd. Statistical significance accepted at the α = 5% level except for stepwise regression procedure, where an α = 10% boundary was set to minimize type II errors. Statistical analysis was performed using the JMP IN statistical computer program.

RESULTS

The results of cardiovascular parameters and vasoactive hormone levels are shown in Table 1.

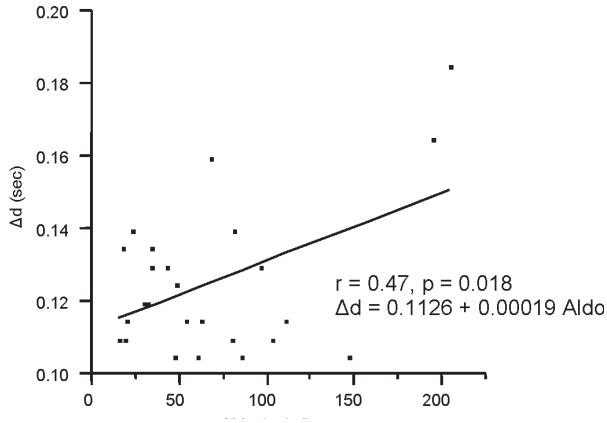

Figure 2. Linear regression between Δd and Aldo in healthy male rabbits.

Correlations of Δd with other cardiovascular parameters are shown in table 2, whereas in table 3 correlations of Aldo with other cardiovascular parameters are shown. There is a linear regression between Δd and Aldo given by the equation Δd = 0.1126 + 0.00019 Aldo, $r^2 = 0.22$ (r = 0.47), p = 0.018 (Figure 2).

To define factors that may independently contribute to Δd, stepwise regression analysis was performed. Factors included in the analysis were other measured parameters of cardiovascular function, none of which involve time as the primary variable. From the potentially confounding factors BW, Ht, HR, SBP (carotid and femoral), DBP (carotid and femoral), LVEDP, LVmaxdp/dt, LVmindp/dt, PWV, CVP, ANP, PRA, Aldo and Aldo/PRA ratio that may contribute to LV contraction duration (Δd) (that best correlated with Aldo), femoral DBP ($r^2 = 0.30$, p = 0.004) followed in tandem by femoral SBP ($r^2 = 0.39$, p = 0.08), Aldo ($r^2 = 0.48$, p = 0.067), BW ($r^2 = 0.56$, p = 0.067), Ht ($r^2 = 0.64$, p = 0.05), LVmindp/dt ($r^2 = 0.74$, p = 0.01) and PWV ($r^2 = 0.77$, p = 0,18) entered the analysis increasing r^2 from 0.30 to 0.77. Therefore from the 77% of the explained variance of Δd, 30% is attributed to femoral DBP, 9% to femoral SBP, 9% to Aldo, 8% to BW, 8% to Ht, 10% to LVmindp/dt and 3% to PWV. This means that Aldo is a determinant of Δd and that its effect is independent of confounding factors.

DISCUSSION

Cardiovascular and hormonal parameters

Basal cardiovascular parameters were found to be

Table 1. Cardiovascular and hormonal parameters measured.

Cardiovascular and hormonal parameters (n = 25)	$\overline{X}$ + SD
Heart rate (beats/min)	194 ± 25
Carotid (central) SBP (mmHg)	124 ± 16
Carotid (central)DBP (mmHg)	91 ± 13
Carotid (central)MBP (mmHg)	107 ± 15
Carotid (central) (PP) (mmHg)	31 ± 10
Femoral (peripheral) SBP (mmHg)	123 ± 15
Femoral (peripheral) DBP (mmHg	96 ± 13
Femoral (peripheral) MBP (mmHg)	108 ± 14
Femoral (peripheral) (PP)(mmHg)	26 ± 9
LVPmax (mmHg)	145± 27
LVEDP (mmHg)	8 ± 3
LVmaxdp/dt (mmHg/sec)	3370 ± 872
LVmin dp/dt (mmHg/sec)	-2889 ± 862
Δd (sec)	0.125 ± 0.012
LVET (sec)	0.125 ± 0.013
DP (beats / min x mmHg)	23834 ± 3014
CVP (mmHg)	-1 ± 2
PWV (cm/sec)	598 ± 120
ANP (pg/ml)	168 ± 101
PRA (ng/ml/h)	21 ± 7
Aldo (pg/ml)	69 ± 52
Aldo to PRA ratio (Aldo/PRA)	3.73 ± 2.81

within the limits reported by other investigators[10-12]. Basal levels of PRA (21 ± 7 ng/ml/h) and plasma Aldo (69 ± 52 pg/ml) were found to be within the limits reported by previous studies: PRA: 29.1 ± 5.7ng/ml/h[13] and Aldo: 27±9 pg/ml to 99±23 pg/ml[13-16]. Basal plasma ANP levels (168 ± 101 pg/ml) were found to be about two to three-fold higher than in other studies in anesthetized and conscious rabbits: 53.3 ± 4.3pg/ml[17], 61 ± 7 pg/ml[18], 59 ± 9.1 pg/ml[19], 79 pg/ml[20], 76.6 ± 18 pg/ml[21]. These increased basal levels may be attributed to the relative volume expansion from the iv infused N/S solution during the experiment, as well as to the irritation of the heart chambers wall by the maneuvers for the insertion of the catheter.

Correlation of Δd with Aldo

In the present study LV contraction duration was measured using the time interval between LVmaxdp/dt and LVmindp/dt (Δd), as was described by Yamamoto et al[8]. This interval measures the systolic time involved between just after the closure of mitral valve (point of maximal isovolumic contraction rate) and just before the closure of aortic valve (point of maximal relaxation rate - protodiastole). Classical systolic time intervals are the pre-ejection period (PEP), the left ventricular ejection time (LVET), and total electromechanical systole (QS_2) that have already been measured non-invasively in humans [critically reviewed by Lewis et al[22]] as well as in rabbits[12]. Since invasive methods are used in our study, it was more convenient to use Δd as LV contraction duration. Though QS_2 equals PEP + LVET, Δd incorporates part of PEP and the major part of LVET and is therefore minor to QS_2.

An interesting finding of our study is the positive correlation and the linear regression of Δd with Aldo as well as the fact that Aldo contributes by 9% as a completely independent factor to Δd (stepwise regres-

Table 2. Correlation of Δd with other measured parameters.

Parameter	r	p
Femoral (peripheral) DBP (mmHg)	-0.55	0.004
Femoral (peripheral) MBP (mmHg)	-0.42	0.034
Femoral Pulse Pressure (PP) (mmHg)	0.37	0.069
Aldo (pg/ml)	0.47	0.018
PRA (ng/ml/h)	0.08	0.69
Aldo to PRA ratio (Aldo/PRA)	0.42	0.038
LVET (sec)	0.71	0.0001

Table 3. Correlation of Aldo with other measured parameters.

Parameter	r	p
Femoral (peripheral) SBP (mmHg)	-0.38	0.061
Femoral (peripheral) DBP (mmHg)	-0.40	0.046
Femoral (peripheral) MBP (mmHg)	-0.37	0.067
LVEDP (mmHg)	-0.66	0.0003
Δd (sec)	0.47	0.018
LVET (sec)	0.57	0.003

sion analysis). Moreover there is no correlation of Δd to PRA but only to Aldo/PRA ratio. Lazurova et al[23] found also positive correlation between plasma Aldo and LV structural and functional echocardiographic parameters (IVSd, PWd, LVIDd and E/A) in hypertensive patients. However no correlation between PRA and LV structure and function was found.

In addition to its well-known renal effects, Aldo exerts cardiac actions such as blood pressure-independent cardiac fibrosis[1,24] and LV enlargement[1]. Apart from these presumably genomic effects (requiring >10 min to be expressed), Aldo elicits rapid (requiring <10 min to be manifested) nongenomically mediated effects[2,25,26]; it acutely increases systemic vascular resistance[3], decreases cardiac output[4], affects intracellular second messengers such as calcium and inositol triphosphate[2], induces positive inotropic and vasoconstrictor effects in rat heart[27] but negative inotropic response in human trabeculae[28] and in rabbit cardiomyocytes[29], decreases the rate of repolarisation of atrial muscle fibers[30] and increases monophasic action potential duration within minutes after iv application[5]. Furthermore, as the observed prolongation in monophasic action potential is rapid and waned quickly[5] and other study[31] has suggested that non-genomic effects lessen despite the continued presence of Aldo, acute surges of endogenous Aldo rather than persistently elevated levels may be important for these effects[32]. The genomic effects of Aldo are mediated via mineralocorticoid receptor (MR). These effects involve binding of Aldo to the intracellular MR and the translocation of the steroid–MR complex to the nucleus, where it acts as a transcriptional regulator, inducing effects after several hours[33]. The rapid 'nongenomic' effects of Aldo (occurring within minutes) are likely to be transmitted via specific membrane receptors[33]. They occur at subnanomolar levels of Aldo and involve, among others, inositol 1,4,5-triphosphate (IP3), protein kinase C and Ca2+ [34,35]. The identity of the receptor responsible for the nongenomic effects is currently not known.

Since electrical and mechanical events in cardiac muscle overlap considerably in time[36] it might be speculated that the aforementioned nongenomically mediated increase of monophasic action potential duration by Aldo would result, by analogy, in (nonge-

nomically mediated) increase of myocardial contraction duration (Δd). The non-correlation of Δd to PRA suggests that the effect may be attributed directly to Aldo. Therefore our unexpected finding, from the statistical analysis for Aldo, may provide insight into another (eventually non-genomic) mechanism of its cardiac action, a hypothetical at best, supported only indirectly by the found regression. Certainly experimental confirmation of the suggested action is needed by iv application of increasing doses of Aldo versus cardiovascular parameters over time, including pretreatment with classical mineralocorticoid antagonists, e.g. spironolactone.

CONCLUSION

In the present study a positive correlation and a linear regression were found between Aldo and myocardial contraction duration (Δd) in healthy NZW anesthetized male rabbits. Peripheral blood pressure (diastolic and systolic) and Aldo were the most important determinants of Δd and the effect of Aldo was independent of confounding factors. The correlation may provide insight into another (eventually nongenomically-mediated) action of Aldo, but this remains to be established experimentally. Thus, any conclusion about mechanisms or causality must be strictly identified as hypothetical and as a stimulus for further research.

Θετική συσχέτιση επιπέδων αλδοστερόνης και διάρκειας συστολής αριστερής κοιλίας σε φυσιολογικά κουνέλια

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ΠΕΡΙΛΗΨΗ: *Εισαγωγή*: Η αλδοστερόνη εκτός από τις γνωστές γονιδιακές επιδράσεις [νεφρικές και καρδιακές (ίνωση και διάταση)] έχει και μη γονιδιακές δράσεις όπως αύξηση της διάρκειας μονοφασικού μυοκαρδιακού δυναμικού δράσης. Κατά την έρευνα της επίδρασης ενδογενών αγγειοδραστικών ουσιών σε καρδιαγγειακές και ορμονικές παραμέτρους σε 25 φυσιολογικά αρσενικά λευκά κουνέλια Νέας Ζηλανδίας (NZW), παρατηρήθηκε ως τυχαίο εύρημα θετική συσχέτιση μεταξύ διάρκειας συστολής αριστερής κοιλίας (ΑΚ) και επιπέδων αλδοστερόνης.

Υλικό και μέθοδοι: Από την καμπύλη πίεσης της ΑΚ υπολογίσθηκε η μέγιστη και η ελάχιστη ανά μονάδα χρόνου μεταβολή της πίεσης (LVmaxdp/dt και LVmindp/dt) και μετρήθηκε ο χρόνος κοιλιακής συστολής δηλ. το χρονικό διάστημα μεταξύ LVmaxdp/dt και LVmindp/dt (Δd).

Αποτελέσματα: Διαπιστώθηκε θετική γραμμική συσχέτιση μεταξύ αλδοστερόνης (που μετρήθηκε με RIA) και Δd (Δd = 0,1126 + 0,00019, r = 0,47, p = 0,018). Η ανάλυση πολλαπλών παραγόντων έδειξε ότι οι σημαντικότεροι παράγοντες που καθορίζουν τη Δd είναι η (περιφερική) αρτηριακή πίεση και η αλδοστερόνη, της οποίας η επίδραση είναι ανεξάρτητη των άλλων παραγόντων.

Συμπέρασμα: Επειδή ο χρόνος μυοκαρδιακής συστολής διαρκεί όσο και το δυναμικό δράσης, μπορεί να υποτεθεί ότι η μη γονιδιακά προκαλούμενη αύξηση της διάρκειας μονοφασικού μυοκαρδιακού δυναμικού δράσης από την αλδοστερόνη, επιφέρει αύξηση της διάρκειας της μυοκαρδιακής συστολής, υπόθεση η οποία υποστηρίζεται από τη συσχέτιση που βρέθηκε.

Λέξεις Κλειδιά: Αλδοστερόνη, Διάρκεια συστολής αρ. κοιλίας, Κουνέλια, Λειτουργία αρ. κοιλίας.

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