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Relationships among endogenous ouabain, α -adducin polymorphisms and renal sodium handling in primary hypertension

Paolo Manunta^a, Marc Maillard^b, Cristina Tantardini^a, Marco Simonini^a, Chiara Lanzani^a, Lorena Citterio^a, Paola Stella^a, Nunzia Casamassima^a, Michel Burnier^b, John M. Hamlyn^c, and Giuseppe Bianchi^a

^aDivision of Nephrology, Dialysis and Hypertension, Scientific Institute San Raffaele, University 'Vita-Salute' San Raffaele, Milan, Italy ^bDivision of Nephrology, CHUV, Lausanne, Switzerland

^cDepartment of Physiology, School of Medicine, University of Maryland, Baltimore, Maryland, USA

Abstract

Objective—The basolateral Na pump drives renotubular reabsorption. In cultured renal cells, mutant adducins, as well as sub-nanomolar ouabain concentrations, stimulate the Na–K pump.

Methods—To determine whether these factors interact and affect Na handling and blood pressure (BP) *in vivo*, we studied 155 untreated hypertensive patients subdivided on the basis of their plasma endogenous ouabain or α -adducin genotype (ADD1 Gly460Trp-rs4961).

Results—Under basal conditions, proximal tubular reabsorption and plasma Na were higher in patients with mutated Trp ADD1 or increased endogenous ouabain ($P = 0.002$ and 0.05 , respectively). BPs were higher in the high plasma endogenous ouabain group ($P = 0.001$). Following volume loading, the increment in BP (7.73 vs. 4.81 mmHg) and the slopes of the relationship between BP and Na excretion were greater [0.017 ± 0.002 vs. 0.009 ± 0.003 mmHg/(μ Eq min)] in ADD1 Trp vs. ADD1 Gly carriers ($P < 0.05$). BP changes were similar, whereas the slopes of the relationship between BP and Na excretion were lower [0.016 ± 0.003 vs. 0.008 ± 0.002 mmHg/(μ Eq min)] in patients with low vs. high endogenous ouabain ($P < 0.05$). In patients with high endogenous ouabain, volume loading increased the BP in the ADD1 Trp group but not in the Gly group ($P < 0.05$). Thus, patients with ADD1 Trp alleles are sensitive to salt and tubular Na reabsorption remains elevated after volume expansion.

Conclusion—With saline loading, BP changes are similar in high and low endogenous ouabain patients, whereas tubular Na reabsorption increases in the high endogenous ouabain group. Saline loading unmasks differences in renal Na handling in patients with mutant adducin or high endogenous ouabain and exposes an interaction of endogenous ouabain and Trp alleles on BP.

Keywords

functional genomic; high blood pressure; Na pump inhibitors; Na transport; ouabain-like

Introduction

Abnormalities in renal sodium handling have been proposed as a major cause of primary hypertension. The pivotal function of the kidney in regulating body sodium and blood pressure (BP) is achieved via the interactions among various hormones (angiotensin II, aldosterone, vasopressin, endogenous ouabain, and dopamine, etc.) and the genetically determined constitutive capacity of the renal tubules to reabsorb Na. The latter is affected by the polymorphisms of the genes coding for various proteins involved in the regulation of Na transport in the renal plasma membranes [1–3]. Therefore, the individual long-term body sodium and BP set points reflect the normal coordination between these intrinsic and extrinsic mechanisms. In humans, the specific molecular mechanisms that mediate the link between salt intake, body sodium, and BP remain unclear.

Previous studies in cultured renal cells have shown that the renal Na–K pump activity, the key ion transporter regulating the driving force for tubular Na reabsorption is modulated by at least two hypertension-promoting factors. One is a hormonal factor, the endogenous ouabain [4], whereas the other is genetic, the cytoskeletal protein adducin [5]. Both human and rat mutated variants of adducin produce similar effects on the Na–K pump, even though the missense mutations occur on different coding sites in the two species [6,7]. Long-term exposure of renal cells to nanomolar ouabain [8] or to cell transfection with the mutated adducin variants from either rats or humans enhances the expression and activity of the renal Na–K pump in the basolateral membrane [5,9,10]. Augmentation of Na pump activity increases the constitutive capacity of the tubular cells to transport Na and is expected to enhance renal Na retention [11].

In view of the similar effect of ouabain and the mutated adducin on the renal cell Na–K pump [5–7,8,12,13], we investigated the extent to which these factors affected BP and/or renal Na handling in patients with primary hypertension under basal conditions and after an acute saline load.

Methods

Clinical measurements

The study was conducted in 155 patients with untreated mild hypertension who were referred to the ‘Outpatient Clinic for Hypertension’ of San Raffaele Hospital of Milan. The Ethics Committee of the San Raffaele Hospital approved the study and informed consent was obtained from each individual. Patients underwent clinical examination, routine biochemistry, and secondary hypertension was excluded by routine methods.

To avoid the large day-to-day variation in salt intake, the patients were asked to follow a controlled diet with a known content of about 150 mmol per day for at least 15 days before the study period. A 24-h urine collection was obtained to verify dietary compliance.

All patients underwent 24-h ambulatory BP monitoring (24-h ABPM; Spacelab 90207; Spacelab Medical Inc., Redmond, Washington, USA). Blood samples for Na, creatinine, renin activity (PRA), aldosterone (Aldo), endogenous ouabain, genotype characterization (ADD1 Gly460Trp-rs4961) [11], and 24-h urine collections for Na, K, and creatinine were obtained the day before the 24-h ABPM recording.

Acute protocol saline infusion—A subset of the whole study group of 124 hypertensive patients, who had given signed informed consent, were included in this study. The acute Na loading protocol used was similar to that previously reported [14].

Biochemical and renal parameters—Serum and urinary sodium were measured by flame photometry, serum and urinary creatinine by an automated enzymatic method, and plasma renin activity and plasma aldosterone by radioimmunoassay (RIA; Medical System, Genova, Italy). Plasma endogenous ouabain was determined by RIA on C-18 extracted samples using a specific antiserum as previously described [15]. Endogenous trace lithium was determined with an electrothermal atomic absorption spectrophotometer (model 1100B) with a HGA-700 graphite furnace (Perkin-Elmer Inc., Boston, Massachusetts, USA) [9].

Clearances (C) were calculated as $C_x = U_x \times V/P_x$, where U_x and P_x are the urinary and plasma concentrations of the solute x , respectively, and V is the urine flow rate measured in milliliter per minute. Fractional excretion of sodium (FENa) and lithium (FELi) were calculated as $P_x \times U_{\text{crea}}/P_{\text{crea}} \times U_x$, where U_x and P_x are the urinary and plasma concentrations of the solute x , respectively, and U_{crea} and P_{crea} are the urinary and plasma concentrations of creatinine, respectively [9].

The slope of the relationship between BP and Na or lithium excretion [mmHg/(μ Eeq min)] was calculated by plotting the lithium or Na excretion on the Y -axis as a function of mean blood pressure (MBP), on the X -axis, observed both under basal conditions and after 2 h of saline infusion, as discussed elsewhere [14].

Statistical analysis

For database management and statistical analysis, we used the SPSS software package (SAS Institute, Cary, North Carolina, USA), version 11. In basal conditions, quartile display values correspond to the 25th, 50th, and 75th percentiles. The two lower and the two higher quartiles were grouped in the subset of patients that carried out the acute Na load because of the smaller size of this cohort. We performed analysis of variance to compare means between groups. Our statistical methods also included single and multiple linear regressions. We searched for possible covariates of the arterial and renal phenotypes; age, BMI and sex were considered for entry into the model.

Results

Baseline conditions

Table 1 summarizes the baseline clinical characteristics of the 155 untreated patients with mild hypertension who were subdivided into two groups according to their ADD1 genotypes (Table 1a) and into four groups according to their plasma endogenous ouabain quartiles (Table 1b). Only PRA and diastolic blood pressure (DBP) differed in the ADD1 and endogenous ouabain groups, respectively. Systolic BP showed a similar trend without reaching statistical significance due to the small group size. All other parameters measured were similar among the groups.

As shown in Fig. 1, the patients with the highest levels of plasma endogenous ouabain or those who carried the Trp ADD1 allele had higher DBP, plasma Na, and proximal tubular reabsorption (detected by the decrease of FELi) compared with the low (<157, and 157–236) endogenous ouabain groups. Similarly, patients with one or more ADD1 Trp alleles have higher plasma Na and augmented proximal tubular reabsorption of lithium than those with ADD1 Gly alleles. In Fig. 1, statistical significance was achieved for the parameters shown except for the DBP between Trp and Gly ADD1 carriers where the trend was evident but not significant ($P = 0.09$). In addition, under baseline conditions, we observed no significant interaction or additive effect between the ADD1 genotypes and plasma endogenous ouabain on either BP or tubular Na reabsorption when analyzed as described below (data not shown).

Acute Na loading protocol and renal Na handling—Of the 155 patients studied, 124 entered the sodium loading protocol. Due to the relatively lower size of this cohort, the patients were subdivided into two groups according to their ADD1 genotypes (Table 2a) and their endogenous ouabain levels. Only three patients were homozygous for Trp so they were included in the Gly/Trp subgroup. For endogenous ouabain, we grouped the patients in the lower two endogenous ouabain quartiles as the low group and those in the higher endogenous ouabain quartiles as the high group (Table 2b).

The saline load increased BP significantly in all patient subgroups ($P < 0.001$). The BP increase was similar in the high and low endogenous ouabain groups; whereas those patients who carried the mutated Trp ADD1 allele displayed a larger increase in DBP and MBP (not shown) compared with the Gly ADD1 homozygotes. The difference in the BP response to the saline load was not explained by variations in the Na or lithium excretion rates.

Following 2-l infusion of saline, the renal electrolyte handling can be affected by changes in blood and kidney perfusion pressures. Thus, a more appropriate evaluation of the influence of the ADD1 genotype or endogenous ouabain levels on renal electrolyte handling should ideally consider the simultaneous changes in BP by calculating the slope of the relationship between BP and Na (or Li) excretion both under basal conditions and at the end of the infusion period. As shown in Table 2, the slopes of the pressure–natriuresis relationship were higher for Na and Li in patients who carried mutated Trp ADD1 alleles than in control patients that carried the Gly ADD1 homozygote. This indicates that a larger increase in BP was needed in the Trp carriers to excrete the same amount of Na and Li. Conversely, the slope of the pressure–natriuresis relationship was significantly lower in the patient group with high endogenous ouabain compared to those with low endogenous ouabain levels. Thus, during saline loading, patients with high endogenous ouabain seem to excrete more Na at any given level of BP.

An interaction between plasma endogenous ouabain and the ADD1 Trp genotype was found to cause changes in DBP after an acute Na load (Fig. 2, panel a; $P = 0.028$). The difference in the BP increase between carriers of the mutated Trp ADD1 allele and Gly/Gly homozygotes was observed only in the presence of high circulating endogenous ouabain. Moreover, the hypertensive effect of the adducin Trp allele and plasma endogenous ouabain combination was not explained by differences in renal Na excretion; FELi and FENa were not different between the two subgroups of patients with the lowest and largest increase in BP (Fig. 2).

Discussion

The major new results of the present study are as follows. First, under basal conditions, untreated patients with mild hypertension who carry the mutated ADD1 or have elevated circulating levels of endogenous ouabain show significantly higher DBP, higher plasma Na concentrations, and increased proximal tubular reabsorption compared with their relevant controls (Table 1; Fig. 1). Previous studies [7,16] from our laboratory using a larger sample of untreated hypertensive patients showed a statistically different DBP between ADD1 genotypes. Although evident (Fig. 1), the same trend was not significant in the present study.

Second, we found no evidence for additive effects between endogenous ouabain and the ADD1 genotype among the patients at baseline for the renal function parameters measured. Following saline infusion, however, the slopes of the pressure natriuresis (MBP/Na or Li excretion) relationship were positive in all groups (Table 2). Moreover, there were significant differences in the slopes for patients who carried the ADD1 Trp allele compared

with those with high circulating endogenous ouabain. For example, the tendency to retain sodium was persistent in the Trp carriers. In contrast, sodium excretion in patients with high endogenous ouabain was higher at any given BP compared with those with low circulating endogenous ouabain.

Third, following the saline load, significant BP effects were found that reflect the interaction between the ADD1 Trp genotype and circulating endogenous ouabain ($P = 0.028$). Among those patients with high circulating endogenous ouabain, saline infusion produced a greater hypertensive response in the Trp allele carriers (Fig. 2) and the pressor response was clearly unrelated to renal cation excretion as determined from the similar values for FELi and FENa.

One intriguing observation of our study is the significantly higher plasma Na concentration among patients under basal conditions with either high circulating endogenous ouabain levels or the mutated ADD1 (Fig. 1). This concordance is striking especially because both endogenous ouabain and Trp alleles have been proposed to augment renal Na pump activity and have anti-natriuretic effects. Previous studies [17–21] have observed significant changes in plasma sodium following an increase in dietary sodium or between hypertensive and normotensive subjects. Moreover, the changes in plasma sodium may have a number of functional consequences including activation of sympathetic nerve activity [21]. Sustained changes in plasma sodium imply an alteration in the relationship between cation balance and water homeostasis.

Increased proximal tubular reabsorption has been proposed as an independent determinant of the BP response to salt [22,23]. The molecular mechanisms that link proximal tubular reabsorption with increased levels of BP and plasma Na concentration, however, remain elusive. Humoral inhibitors of the vascular Na pump have been implicated as a link between volume retention and increased arterial tone [24,25]. Inhibition of the vascular sodium pump promotes calcium influx via the Na–Ca exchanger [26] and recent studies [4,26,27] show that the vascular Na–Ca exchanger and the vascular α -2 Na pump are key mediators of the increase in vascular tone in salt and ouabain-dependent hypertension.

Although inhibition of the vascular Na pump by ouabain or endogenous ouabain can raise BP, the net effects of these humoral factors on the renal tubular Na pump appear to be more complex. For example, high concentrations of ouabain inhibit the Na–K pump, whereas lower nanomolar concentrations augment the Na pump abundance in the tubular basolateral membrane and increase the pump activity [28–30]. At low concentrations, endogenous ouabain and ouabain, therefore, appear to stimulate sodium retention, whereas at higher concentrations, the direct inhibitory actions of ouabain may augment sodium excretion. This biphasic effect of ouabain has been demonstrated in cultured renal tubular cells [31] and can be detected in isolated membranes [12,32]. Therefore, it is difficult to predict in the whole organism whether a given concentration may exhibit an inhibitory or stimulatory effect on the Na pump in any given tissue. Moreover, the effect of a given concentration of ouabain on the Na pump may be modulated by a variety of factors, some of which may be relevant to the present results. For example, the ouabain inhibitory potency of Na–K ATPase varies according to the α -isoform, extracellular K, lipid composition of the cell membrane, intracellular Na and ROS, cytoskeleton proteins status, NOS and/or ANP levels, among others. Therefore, the same concentration may exhibit opposite effects according to the modification of the modulatory factors mentioned above. We suggest that modification of one or more modulatory factors may explain the switch from endogenous ouabain-stimulated Na retention in the basal state to the endogenous ouabain-facilitated Na excretion following saline infusion in the high endogenous ouabain subgroup.

In conclusion, under basal conditions, our findings are in agreement with that of earlier studies [8–10,12,26,27,30], showing that both ouabain and Trp adducin may activate the renal Na–K pump in cultured renal tubular cells and increase BP and tubular reabsorption at the whole organism level. After a saline load, carriers of the Trp adducin continued to reabsorb more Na at any given level of BP compared with their wild adducin counterparts. Conversely, in the high endogenous ouabain subgroup, the tubular reabsorption capacity for any given level of BP is decreased compared with the low endogenous ouabain subgroup.

Saline infusion unmasked a pressor interaction between endogenous ouabain and ADD1 Trp allele on BP as shown in Fig. 2. The mechanism of this interaction is not clear but may involve augmented sensitivity of the vascular sodium pump that may be most significant in the high endogenous ouabain patients. Clearly, the different BP responses to the saline load in patients with wild adducin compared with those with mutated adducin could not be explained by variations in the renal Na handling alone.

The patients in this study had a relatively recent onset of hypertension, were not previously treated, and may be especially suitable to unravel the pathophysiological and molecular mechanisms underlying their hypertension without the interference of previous antihypertensive therapies or secondary vascular changes that occur in the later stages of hypertension. Moreover, these patients were under a controlled Na diet to reduce the ordinarily wide variability of Na intake.

The striking similarity between the changes in proximal tubular reabsorption and endogenous ouabain in humans and those produced in isolated cells by ouabain and the mutated ADD1 strengthens the notion that these two molecules trigger a similar mechanism within the kidney (i.e., activation of the basolateral Na–K pump).

Nevertheless, we recognize the following limitations: the lithium clearance data indicate an increased proximal tubular reabsorption triggered by high endogenous ouabain or the mutated ADD1. The basic assumption for renal clearance is the steady state of all factors involved that, in our study, is disturbed by the saline infusion. This deviation from the steady state should apply equally to all subgroups, but there are no means to determine whether this goal was met.

A residual if remote possibility is that human endogenous ouabain is an isomer of ouabain. Extensive studies have been performed to validate the RIA for endogenous ouabain in plasma [17,33] and the immunoassay we used does not distinguish endogenous ouabain from ouabain. Moreover, among 21 patients, a positive correlation ($P<0.001$) has been observed between plasma endogenous ouabain measured by both HPLC mass spectrometry and RIA (P. Manunta, M. Ferrandi, J.M. Hamlyn, unpublished observation). Therefore, measurements made with our RIA are not likely to be compromised by other materials (e.g., digoxin, marinobufagenin) that may be in the circulation and are structurally distinct from ouabain.

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Abbreviations

ABPM ambulatory blood pressure monitoring

ADD1	alpha adducing
BMI	body mass index
DBP	diastolic blood pressure
EO	endogenous ouabain
FELi	fractional excretion of lithium
FENa	Fractional excretion of sodium
MBP	mean blood pressure
PRA	plasma rennin activity
Press/Nat	pressure natriuresis relationship
RIA	radioimmunoassay
UK	urinary K excretion
UNa	urinary Na excretion

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Fig. 1.

Baseline values of various factors. (a) Baseline values of diastolic blood pressure (DBP), plasma Na, and FELi in patients according to their plasma endogenous ouabain level quartiles. (b) Baseline values DBP, plasma Na, and FELi in patients according to their ADD1 genotype. The number of patients for each subgroup is indicated in bold. The patients with the highest levels of plasma endogenous ouabain had higher DBP, plasma Na, and proximal tubular reabsorption (detected as the decrease in FELi) compared with the low endogenous ouabain subgroups. Patients with the ADD1 Trp allele had higher plasma Na and proximal tubular reabsorption than the ADD1 Gly patients. Statistical significance for trend was achieved for all the parameters, except for the DBP between Trp and Gly ADD1 carriers where the trend was not significant ($P = 0.09$).

Fig. 2.

Interaction between ADD1 genotypes and circulating endogenous ouabain in hypertensive patients after an acute Na load. For this comparison, patients were subdivided into two subgroups according to the level of endogenous ouabain below or above the median level of 236 pmol/l. The changes (Δ) in diastolic blood pressure (DBP) and renal Na handling [as fractional lithium (FELi, %) or sodium (FENa) excretion] are shown. Patients with low endogenous ouabain carrying the wild-type ADD1 Gly genotype (open bar) or at least one ADD1 Trp variant (closed bar) vs. those carrying either high plasma endogenous ouabain with ADD1 Gly (dashed bar) or ADD1 Trp (closed bar) are reported.

Table 1

Characteristics of the 155 hypertensive patients grouped by their ADD1 genotype and quartiles of endogenous ouabain

	(a) ADD1			(b) Endogenous ouabain				
	Gly/Gly	Gly/Trp + Trp/Trp	P	<157	157–236	236–337	>337	P
Sex (men/women)	83/13	45/11	NS	31/8	36/4	29/8	35/4	NS
Age (years)	51.5 ± 0.90	48.8 ± 1.43	NS	50.8 ± 1.42	50.0 ± 1.23	49.1 ± 2.27	51.5 ± 1.23	NS
BMI (kg/m ²)	25.9 ± 0.32	25.2 ± 0.43	NS	25.1 ± 0.62	26.2 ± 0.43	25.0 ± 0.48	26.4 ± 0.48	NS
SBP (mmHg)	142.4 ± 2.04	144.2 ± 2.01	NS	141.3 ± 2.04	141.0 ± 2.01	143.1 ± 1.42	146.8 ± 2.27	NS
DBP (mmHg)	93.8 ± 1.33	94.4 ± 1.49	NS	91.2 ± 1.21	93.8 ± 1.26	92.4 ± 1.49	99.3 ± 1.35	>0.001
Creat. Cl (ml/min per 1.73 m ²)	115.2 ± 4.04	114.9 ± 5.54	NS	112.0 ± 7.43	118.0 ± 5.69	114.3 ± 7.67	114.2 ± 5.97	NS
UNa (mEq/24 h)	145.3 ± 7.52	143.6 ± 7.20	NS	152.7 ± 9.30	151.4 ± 9.76	143.4 ± 11.9	128.3 ± 11.6	NS
UK (mEq/24 h)	58.2 ± 2.67	53.6 ± 2.29	NS	58.9 ± 3.60	55.2 ± 2.80	60.4 ± 5.10	51.2 ± 3.09	NS
PRA [ng/(ml h)]	1.26 ± 0.11	0.90 ± 0.19	0.049	1.04 ± 0.14	1.29 ± 0.19	1.33 ± 0.24	0.89 ± 0.10	NS
Plasma aldosterone (ng/dl)	132.4 ± 6.47	119.5 ± 12.6	NS	122.6 ± 5.47	109.5 ± 11.6	142.6 ± 6.47	113.3 ± 10.6	NS
Plasma endogenous ouabain (pmol/l)	297.3 ± 19.4	272.5 ± 22.7	NS	132.5 ± 3.00	193.0 ± 3.80	279.9 ± 4.54	547.2 ± 27.5	–

Values are arithmetic mean ± SEM. The *P* values for between-group differences were adjusted for multiple comparisons by Tukey's test (means).

Creat. Cl, creatinine clearance; DBP, diastolic blood pressure; *P*, plasma value; PRA, plasma renin activity; SBP, systolic blood pressure; *U*, urinary value.

Table 2

Impact of an acute Na load: changes (Δ) from baseline

	(a) ADD1 polymorphism		(b) Endogenous ouabain		
	Gly/Gly (n = 76)	Gly/Trp + Trp/Trp (n = 48)	P	<236 (pmol/l) (n = 64)	>236 (pmol/l) (n = 60)
Δ SBP (mmHg)	4.81 $\pm$ 1.06 ^a	7.73 $\pm$ 1.26 ^a	0.084	6.28 $\pm$ 1.04 ^a	5.27 $\pm$ 1.28 ^a
Δ DBP (mmHg)	1.64 $\pm$ 0.66 ^a	4.69 $\pm$ 0.76 ^a	0.004	3.03 $\pm$ 0.67 ^a	2.39 $\pm$ 0.79 ^a
Δ MBP (mmHg)	2.66 $\pm$ 0.66	5.73 $\pm$ 0.80	0.004	4.18 $\pm$ 0.70	3.35 $\pm$ 0.79
UNa exc. rate [mEq/(l min)]	399.9 $\pm$ 16	433.0 $\pm$ 26.1	NS	383.6 $\pm$ 29	463.4 $\pm$ 39.5
Slope press/Nat [mmHg/(mEq min)]	0.009 $\pm$ 0.003	0.017 $\pm$ 0.002	0.044	1.33 $\pm$ 0.17	1.92 $\pm$ 0.20
Δ FENa (%)	1.75 $\pm$ 0.21	1.61 $\pm$ 0.20	NS	0.016 $\pm$ 0.003	0.008 $\pm$ 0.002
Δ FELi (%)	16.6 $\pm$ 4.07	10.4 $\pm$ 2.21	NS	15.7 $\pm$ 5.33	13.5 $\pm$ 1.87
Slope MBP/ULi [mmHg/(μ Eq min)]	0.78 $\pm$ 0.18	1.62 $\pm$ 0.26	0.009	1.34 $\pm$ 0.22	0.76 $\pm$ 0.20

Values are arithmetic mean $\pm$ SEM. The *P* values for between-group differences were adjusted for multiple comparisons by Tukey's test (means).

DBP, diastolic blood pressure; FELi, fractional excretion of Li; FENa, fractional excretion of Na; MBP, mean blood pressure; SBP, systolic blood pressure; ULi, urinary Li excretion rate; UNa exc. rate, urinary excretion rate.

^aRepeated measure within-subject *P* < 0.001.