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**Original Paper**

# Histone Deacetylase Activity and the Renin-Angiotensin-Aldosterone System: Key Elements in Cardiorenal Alterations Provoked by Chronic Malnutrition in Male Adult Rats

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**Key Words**
Chronic malnutrition • Renin-Angiotensin-Aldosterone System • Histone deacetylases • Arterial hypertension • Signaling pathways

**Abstract**
***Background/Aims:*** Chronic malnutrition (M) affects >1 billion people worldwide. Epidemiological data point to long-term renal and cardiovascular outcomes (e.g. arterial hypertension, cardiorenal syndromes). The renin-angiotensin-aldosterone system (RAAS) has been implicated in the physiopathology of these disturbances, but M-induced alterations in RAAS-modulated renal $Na^+$ handling and their cardiovascular repercussions are not known. Moreover, altered tissue-specific histone deacetylases (HDAC) results in arterial hypertension and the use of sodium Valproate (Val; a HDAC inhibitor) reduces blood pressure. However, there are no reports regarding the renal and cardiovascular effects of HDAC inhibition in M, or on the signaling pathways involved. The central aim of our study has been to investigate whether alterations in the HDAC/RAAS axis underpin alterations in active $Na^+$ transport in the kidney and heart, and affects blood pressure. ***Methods:*** Male rats aged 28 days were given either a control (C) or a multideficient diet (Regional Basic Diet, RBD), which mimics alimentary habits from developing countries. Subgroups received Losartan (Los), a blocker of type 1

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Angiotensin II receptors. When the rats reached 70 days, new subgroups received Val until they were 90 days of age. Homogenates and enriched plasma membrane fractions from renal *cortex corticis* and cardiomyocytes were obtained by differential centrifugation of the tissues. The activity of renal and cardiac deacetylases was assayed by measuring – after incubation with the membranes – the amount of deacetylated lysines in a substrate containing an acetylated lysine side chain. Protein kinases activities were measured following the incorporation of the γ-phosphoryl group of [γ-$^{32}$P]ATP into Ser/Thr residues of histone type III-S. The activity of $Na^+$-transporting ATPases (kidney and heart) was quantified by measuring the release of $P_i$ from ATP that was sensitive to ouabain (($Na^+$+$K^+$)ATPase), or sensitive to furosemide ($Na^+$-ATPase). Tail-cuff plethysmography was used to measure systolic blood pressure and heart rate. ***Results:*** M provoked HDAC downregulation, which was reversed by Los and Val, either alone or in combination, with selective upregulation of protein kinases C and A (PKC, PKA) in renal *cortex corticis*, but not in left ventricle cardiomyocytes. The 2 kinases were strongly inhibited by Los and Val in both organs. Malnourished rats developed elevated systolic arterial pressure (SAP) and heart rate (HR) at 70 days of age; Los and Val restored the control SAP, but not HR. Functional and the above biochemical alterations were associated with the deregulation of renal and cardiac $Na^+$-transporting ATPases. ($Na^+$+$K^+$)ATPase activities were downregulated in M rats in both organs, and were further inhibited by the pharmacological treatments in the renal *cortex corticis* (C and M groups) and the left ventricle (only in C rats). No additional effect was found in cardiac ($Na^+$+$K^+$)ATPase from M rats. Ouabain-resistant $Na^+$-ATPase was upregulated in renal *cortex corticis* and downregulated in cardiomyocytes, returning to C values after administration of Los and Val. ***Conclusion:*** The HDAC/RAAS axis appears to be a key regulator of $Na^+$-transporting ATPases in renal *cortex corticis* and cardiomyocytes *via* an appropriate balance of PKC and PKA activities. Modifications within the HDAC/RAAS axis provoked by chronic M – with repercussions in renal and cardiac $Na^+$ transport – underpin alterations in bodily $Na^+$ homeostasis that culminate with the onset of arterial hypertension and potential cardiorenal syndrome.



## Introduction

Malnutrition is a serious epidemiological reality in many countries worldwide, resulting in severe alterations in physical and mental development and reduced life expectancy. The risk of prevalent morbidities aggravated by malnutrition, such as kidney and cardiovascular diseases, are frequently associated with alterations in the renin-angiotensin-aldosterone system (RAAS) [1–3]. In the last few decades, several studies tried to elucidate the mechanisms by which malnutrition-induced systemic and local RAAS upregulation – in different windows of development – alters renal and cardiac $Na^+$ metabolism, leading to diseases such as arterial hypertension and cardiorenal syndrome [4–12]. We have demonstrated that renal and cardiac $Na^+$ handling is compromised by chronic multifactorial malnutrition due to upregulation of the $AT_1R \rightarrow$ PKC signaling pathway [8, 9], culminating in the onset of arterial hypertension [8]; the effects resulting from RAAS activation were blocked by Losartan (Los), an antagonist of the type 1 angiotensin II (Ang II) receptors ($AT_1R$), thus confirming the role of RAAS in the pathophysiology of malnutrition-induced renal and cardiovascular lesions.

$Na^+$ transport in kidney is central in bodily $Na^+$ homeostasis [13–15], and the cardiac counterpart is also central for electric potential generation and propagation, and therefore for cardiac contractility [16]. Due to their importance, altered regulation of active $Na^+$ transport, mainly in relation to RAAS and the kinases associated with its pathways, the central protein kinase C different isoforms (PKC) and the cyclic AMP-dependent protein kinase (PKA) [13, 17–22], is one of the most important steps towards the genesis of cardiorenal syndromes [23]. Two $Na^+$-transporting ATPases have been described in living organisms from mammals to parasites: the classical ouabain-sensitive ($Na^+$+$K^+$)ATPase [24, 25] and the ouabain-resistant furosemide-sensitive $Na^+$-ATPase [26, 27], which was purified and cloned a decade ago [28, 29].

Recently a novel functional crosstalk between RAAS and histone acetylations/deacetylations – which are post-translational processes that modify the structure and functions of chromatin [30–32] – has been described; this crosstalk seems to be important in the physiopathology of the cardiovascular system. Sustained administration of $Na^+$ Valproate (Val), considered to be an inhibitor of all histone deacetylase isoforms [33], reduced blood pressure in spontaneously hypertensive [34, 35] and obese [36] rats. Possibly the molecular mechanisms involve reduction of cardiac hypertrophy, inflammation, oxidative stress and renal fibrosis [34, 35]. However, any relationship between RAAS and acetylations/deacetylations remain unexplored, especially with respect to PKC- and PKA-linked signaling and $Na^+$-transporting ATPases during one of the outcomes of chronic malnutrition, namely arterial hypertension.

We therefore investigated the activity of histone deacetylases (HDAC) in hypertensive malnourished rats after blocking the $AT_1R$-associated pathway, and have followed the influence of RAAS and HDAC blockade: (i) in renal *cortex corticis* (where proximal tubules predominate [27] and reabsorption of ~75% of filtered $Na^+$ occurs) and left ventricle cardiomyocytes ($Na^+$+$K^+$)ATPase and $Na^+$-ATPase activities, and (ii) the onset of malnutrition-related arterial hypertension. We also addressed the issue of how the association of malnutrition and pharmacological interventions in RAAS and HDAC impacts PKC- and PKA-mediated signaling, which have also been described as modulators of different classes of histones and transcription [31, 32], beyond their influence on $Na^+$-transporting ATPases in the kidney and heart.

## Materials and Methods

*Materials*

Radioactive orthophosphoric acid ($H_3{}^{32}P\text{-}PO_4$) was obtained from the Institute for Nuclear and Energetic Research (São Paulo, Brazil). [$\gamma$-$^{32}$P]ATP was synthetized according to Maia et al. [37]. The HDAC activity measurement kit (K331) was purchased from BioVision (Milpitas, CA, USA). Ouabain (O3125), furosemide (F4381), histone type III-S (H5505), and $PKAi_{(5–24)}$ peptide (P7739) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Calphostin C (208725) was purchased from Calbiochem (San Diego, CA, USA). Losartan (Los) was purchased from Biosintética (Jurubatuba, Brazil) and $Na^+$-Valproate (Val) was purchased from Abbott (Chicago, IL, USA).

*Diets*

We used the multideficient diet called Regional Basic Diet (RBD). It was formulated by Teodósio et al. [38], based on epidemiological studies of the dietary habits of populations from different regions of Pernambuco State, Brazil, mimicking those diets widely used in vast regions of developing countries. RBD is low in protein (8% against 23% in the control diet, CTR), low in fat (1.7% against 4.5% in the CTR diet), and high in carbohydrates (78% against 56%). RBD is normocaloric compared to CTR diet (~359 kcal/100 g dry weight against ~357 kcal/100 g dry weight from CTR diet). The RBD ingredients are (in g%): manioc flour (*Manioc esculenta*) 65, beans (*Phaseolus vulgaris*) 18, sweet potatoes (*Ipomoea batatas*) 13, and jerked meat 4, with extremely low levels of vitamins [38]. The ingredients were cooked separately, dehydrated at 60°C, ground, mixed and supplemented with water to obtain small wet pieces similar to those of the standard chow diet, and finally dehydrated for 1 day at 60°C. The CTR diet (Neovia Nutrição e Saúde Animal, Descalvado, Brazil) follows the recommendations of the American Institute of Nutrition for rodents (AIN-93G) [39]. The $Na^+$ content determined by flame photometry was 0.3 g% (13 mequiv/100 g dry weight) in the CTR diet and 0.2 g% (9 mequiv/100 g dry weight) in RBD.

*Animals and experimental groups*

Female rats (n = 27) were bred and kept in the Vivarium of Neglected Diseases and Malnutrition at Carlos Chagas Filho Institute of Biophysics/Federal University of Rio de Janeiro, under veterinary supervision. At weaning, the male offspring aged 28 days were randomly separated into 4 groups that received over 42 days (6 weeks): (*i*) CTR diet (n = 33); (*ii*) CTR diet plus a daily dose of 30 mg/kg body mass of Los diluted in

drinking water (n = 21); (*iii*) RBD (n = 40); and (*iv*) RBD plus the same daily dose of Los (n = 33). Each group was distributed in cages hosting 4–5 rats, with a maximum of 2 rats from any one mother per group to avoid the litter effect. Food and filtered and autoclaved water were offered *ad libitum*. At 70 days of age (10 weeks), a selection of the animals from each group received Val diluted in the drinking water (100 mg/kg body mass for the following 20 days), constituting 4 new groups that received: (*v*) CTR diet plus Val (n = 15); (*vi*) CTR diet plus Los and Val (n = 11); (*vii*) RBD plus Val (n = 18); and (*viii*) RBD plus Los and Val (n = 19). All rats had their blood pressure and heart rate measured, after which we used them to isolate homogenates and plasma membranes from cardiomyocytes and proximal tubule cells, pooling membrane preparations from each experimental group for the *in vitro* experiments (n = 4–6). At 90 days of age (13 weeks) the animals were decapitated, and kidneys and hearts were immediately removed to obtain homogenates and enriched plasma membrane fractions from the external part of the renal cortex (*cortex corticis*) and left ventricle. The groups and the timeline of administrations are set out in Supplementary Fig. 1 (for all supplementary material see www.cellphysiolbiochem.com). Supplementary Table 1 gives the abbreviations of the 8 groups by which they can be identified throughout the manuscript. The dose of Los was chosen because it was demonstrated that 30 mg/kg (daily) reduces blood pressure and prevents renal injury in spontaneously hypertensive rats (SHR) [40]. It promotes renoprotection by acting on the intrarenal RAAS [41]. The dose of Val (100 mg/kg) during 3 weeks attenuates cardiac remodeling after infarction in rats [42].

*Isolation of homogenates and enriched plasma membrane fractions from renal cortex corticis tubule cells and cardiomyocytes*

The kidneys were immersed in a solution containing 10 mM Hepes-Tris (pH 7.4), 250 mM sucrose, 2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (a proteases inhibitor) and 0.15 mg/ml trypsin inhibitor type II-S (T1021; Sigma-Aldrich). Thin transverse renal slices were removed using a Stadie-Riggs microtome (Thomas Scientific, Swedesboro, NJ, USA) and the *cortex corticis*, which cell population corresponds almost entirely (95%) to proximal tubules [27], was dissected using ocular scissors. The hearts were immersed in a solution containing 1 mM Tris-imidazole (pH 7.6), 250 mM sucrose and 1 mM EDTA and left ventricles were isolated with small scissors.

*Cortex corticis* and left ventricles were homogenized at 4°C in the respective isotonic solutions described above (1 g tissue: 4 ml solution) using a Potter Elvejhem homogenizer fitted with a Teflon pestle (5 × 1 min at 1,700 rpm). Part of the homogenates was separated for HDAC activity assays, and the other to isolate membranes for determination of $Na^+$-transporting ATPases, PKA and PKC activities. The second part of the renal homogenate was centrifuged at 10,000 *g* for 15 min at 4°C (JA-20 rotor, Beckman Avanti J-E centrifuge; Beckman Coulter, Fullerton, CA, USA). The resulting supernatant was centrifuged at 15,000 *g* for 20 min at 4°C (JA-20 rotor, Beckman Avanti J-E centrifuge) and the recovered supernatant at 35,000 *g* for 44 min at 4°C (70 Ti rotor, Beckman Optimal L-90K ultracentrifuge). The second part of the ventricular homogenate was centrifuged at 1,650 *g* for 15 min at 4°C (JA-20 rotor, Beckman Avanti J-E centrifuge) and the resulting supernatant was centrifuged at 115,000 *g* for 60 min at 4°C (70 Ti rotor, Beckman Optimal L-90K ultracentrifuge). The pellets recovered after the final centrifugation were resuspended in 250 mM sucrose at ~15 mg/ml final concentration of protein, aliquoted and stored at -80°C. Protein concentration was determined by the Folin-phenol method [43] using bovine serum albumin as the standard.

*Activity of histone deacetylases (HDAC)*

The assays followed the manufacturer's instructions (kit K331). Briefly, the HDAC substrate (5 µl containing 50 nmol) containing an acetylated lysine side chain was incubated with homogenates from the renal cortex or left ventricle (85 µl containing 0.2 mg protein) for 1 h at 37°C in a 96-well microtiter plate. Further treatment with a lysine developer (10 µl) produced a chromophore, which was analyzed after 30 min at 405 nm using an ELISA plate reader. The results were expressed as mmol deacetylated lysine/mg. HeLa nuclear extracts (10 µl containing 50 µg) were used as the positive control. Assays supplemented with the HDAC inhibitor Trichostatin A (2 µl containing 2 nmol) served as the negative control.

*Cyclic AMP-dependent protein kinase (PKA) and protein kinase C (PKC) activities*

Cyclic AMP-dependent protein kinase (PKA) and calphostin C-sensitive protein kinase C (PKC) activities were measured in the renal and cardiac membrane preparations as previously described [8, 10, 44, 45]. Activities were quantified by measuring the incorporation of the γ-phosphoryl group of the [γ-$^{32}$P]ATP

into Ser/Thr residues of histone type III-S (a mixture of histones H1, H2a, H2b, H3 and H4) in the absence or presence of 10 nM $PKAi_{(5-24)}$ (PKA inhibitor) or 10 nM calphostin C (PKC inhibitor) in a medium (0.1 ml) containing 20 mM Hepes-Tris (pH 7.0), 4 mM $MgCl_2$, 12 mM NaF, 1.5 mg/ml histone and 0.7 mg/ml of membrane protein. The reaction was started by adding 10 mM [$\gamma$-$^{32}$P]ATP (specific activity: 1 µCi/nmol) and stopped by adding 0.1 ml of 40% trichloroacetic acid (TCA) (w/v). After stirring, 0.1 ml of the suspension was filtered through a Millipore filter (0.45 mm pore size) and washed with 8 ml of an ice-cold 20% (w/v) solution of TCA and 9 ml of 0.1 M phosphate buffer (19.5% of 0.2 M $NaH_2PO_4$ and 30.5% of 0.2 M $Na_2HPO_4$, pH 7.0). Each determination was carried out in triplicate. Radioactivity was quantified in a liquid scintillation counter (Packard Tri-Carb 2100TR, PerkinElmer, Waltham, MA, USA).

*Activity of ouabain-sensitive ($Na^+$+$K^+$)ATPase and ouabain-resistant furosemide-sensitive $Na^+$-ATPase*

Ouabain-sensitive ($Na^+$+$K^+$)ATPase and ouabain-resistant furosemide-sensitive $Na^+$-ATPase activities were measured in renal and cardiac membranes as previously described [7, 8, 10, 46]. ($Na^+$+$K^+$)ATPase activity was determined by measuring inorganic phosphate ($P_i$) release from ATP (Sigma-Aldrich, A2383) in the absence or presence of ouabain. Renal and cardiac membrane preparations (0.025 mg/ml final concentration) were preincubated or not with 2 mM ouabain (10 min at 37°C) in a medium containing 50 mM Bis-Tris-Propane (pH 7.4), 0.2 mM EDTA, 5 mM $MgCl_2$ and 120 mM NaCl. The reaction was started by adding 24 mM KCl and 5 mM ATP (final concentrations), and stopped 10 min later by adding 0.5 ml of a suspension of activated charcoal in 0.1 M HCl. The suspension was centrifuged (13,300 *g* for 10 min) and 0.5 ml of the resulting supernatant was mixed with 0.5 ml of the colorimetric reagent (0.2 N $H_2SO_4$, 10 mM ammonium molybdate and 0.3 M $FeSO_4$). After 20 min, released $P_i$ was quantified spectrophotometrically at 660 nm. ($Na^+$+$K^+$)ATPase activity was calculated by the difference between the activities in the absence and presence of ouabain.

The ouabain-resistant furosemide-sensitive $Na^+$-ATPase was assayed in the absence or presence of furosemide. The renal and cardiac membranes (0.05 mg/ml final concentration) were pre-incubated in the absence or presence of 2 mM furosemide (10 min at 37°C) in a medium containing 2 mM ouabain, 20 mM Hepes-Tris (pH 7.0), 10 mM $MgCl_2$ and 120 mM NaCl. The reaction (10 min) was started by adding 5 mM ATP (final concentration in a volume of 0.5 ml), and stopped 10 min later by adding 0.5 ml of activated charcoal suspension in 0.1 M HCl. The final procedures were the same assays described for the ($Na^+$+$K^+$) ATPase activity. $Na^+$-ATPase activity was calculated by the difference between the activities in the absence and presence of furosemide.

*Systolic arterial pressure and heart rate measurements*

Before Val administration (week 10, 70 days of age) and at the end of rats breeding period (week 13, 90 days of age), systolic arterial pressure (SAP) and heart rate (HR) were measured by pletismography [47] with the use of Insight V3.0 equipment (Insight, Ribeirão Preto, Brazil). At the day before the measurements, rats from different litters were acclimated for 10–15 min in a chamber at 30–32°C. For the recordings, the rats were kept in the same conditions, and when the rats had stopped moving the measurements were made. Recordings were obtained 3 to 5 consecutive times. Measurements were validated after 10 min stability of the heart rate.

*Statistical analysis*

Data are presented as mean ± SEM of independent experiments. Differences were assessed by unpaired Student's *t*-test or two-way ANOVA followed by Bonferroni's test, as indicated in the corresponding figure legends and Supplementary Table S2. Differences were considered significant at $p < 0.05$. Statistical analysis and graphs drawing were carried out with GraphPad Prism 6 software (version 6.01, GraphPad Software, Inc., San Diego, CA, USA).

## Results

*General data*

Supplementary Fig. 2A shows the body mass evolution of the 8 experimental groups. At 9 weeks after weaning (90 days of age), the body mass of chronically malnourished rats was always lower than that of normonourished rats, the difference increasing with time, being 70% lower at the end of the study. Panel 2B shows more clearly that the pharmacological treatments, single or in combination, did not modify the body mass in normonourished or malnourished rats.

Reduced body mass impacted renal and cardiac masses, which are also reduced in chronically malnourished rats regardless of any pharmacological treatments (Supplementary Table 2). Diets and drugs, however, had selective effects when the indexes (organ mass/body mass ratio) were considered. (*i*) The renal index was unaffected by chronic malnutrition, in contrast with the significant increase in cardiac index. (*ii*) Los treatment – alone or in combination with Val – increased the renal index of malnourished rats compared to the normonourished group. (*iii*) Los treatment – alone or in combination with Val – decreased the cardiac index in malnourished rats, but was still significantly higher than that of the control. (*iv*) Val did not affect the cardiac index of malnourished rats, and, therefore it was higher compared to normonourished animals.

*Histone deacetylase (HDAC) activity in renal cortex corticis and left ventricle from normonourished and malnourished rats*

Fig. 1 gives the activities of HDAC in kidney cortex and left ventricle homogenates, showing the effects of malnutrition and the responses to treatment with Los, Val and the 2 drugs in combination. Malnutrition inhibited HDAC activity by 30% in the kidney cortex and by 80% in the left ventricle (Fig. 1A, B), and the 3 treatments prevented inhibition (Fig. 1C–H): the HDAC values in the groups ML, MV and MLV increased to the levels found – or close to – those of normonourished C groups from renal and cardiac tissues (2.0±0.1 and 2.6±0.1 mmol deacetylated lysine/mg, respectively). Deserve special mention the effects of the pharmacological treatments in the normonourished C rats, as well as the similarities and differences in comparing kidney and heart (compare *left* section of the panels corresponding to each treatment). Treatment with Los (Fig. 1C, D), *i.e.* blocking of the Ang II → $AT_1R$ axis, increased HDAC activity in the same proportion in both organs from C rats; this increase corresponds to that responsible for overcoming any inhibition provoked in the M group. Administration of Val for 20 days to normonourished C rats (Fig. 1E, F), *i.e.* when chromatin of the nucleus of proximal tubule cells and cardiomyocytes was modified, decreased cardiac HDAC activity by 55% without influencing renal HDAC. The combination Los/Val (Fig. 1G, H) reproduced in both organs the trends seen with Val alone.

*Protein kinase C (PKC) and cAMP-dependent protein kinase (PKA) activities and effects of Losartan and Valproate in renal cortex corticis and cardiomyocytes: PKC/PKA imbalance in malnourished rats*

As stated above, PKC and PKA are key elements in the signaling pathways coupled to type 1 and type 2 Ang II receptors ($AT_1R$ and $AT_2R$) that regulate $Na^+$-transporting ATPases in the kidney and the heart [13, 19, 48, 49]. Moreover, kinases-mediated phosphorylations are known to be important processes in modulating the structure and function of chromatin [32]. These lines of evidence led us to investigate the influence of multifactorial malnutrition on the activities of PKC (key in the $AT_1R$ pathway) and PKA (that participates in cardiovascular and renal effects linked to $AT_2R$) from renal *cortex corticis* and cardiomyocytes, and how Los and Val modulate possible effects of the malnutrition.

PKC activities were of similar magnitude in renal proximal tubule cells and left ventricular cardiomyocytes from normonourished rats, being modified by malnutrition in contrasting manner, depending on the organ. Whereas PKC hugely increased by more than 350% in the kidney, it was unmodified in the heart (Fig. 2A, B). The enzyme of renal origin was slightly

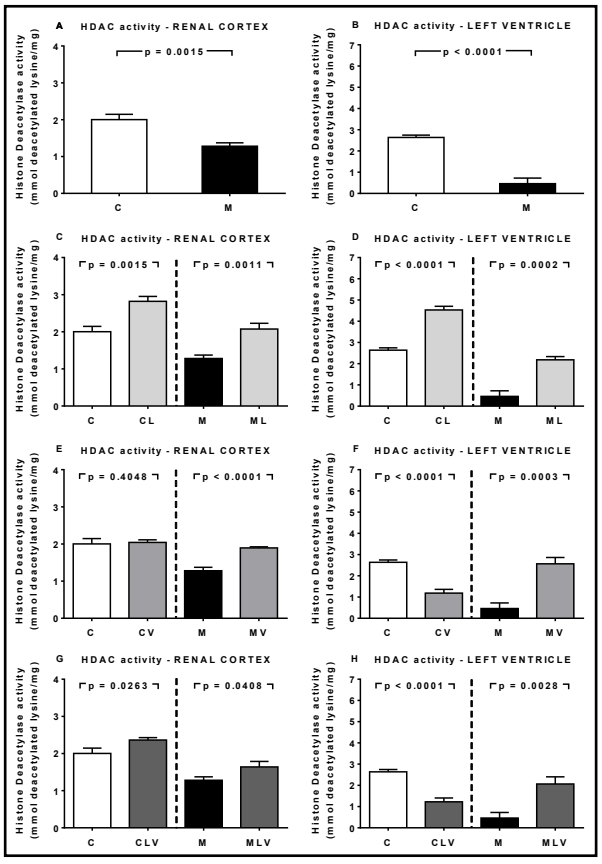


**Fig. 1.** Histone deacetylase (HDAC) activity in renal *cortex corticis* tubule cells (A, C, E, G) and cardiomyocytes (B, D, F, H) from rats aged 90 days was downregulated in malnourished rats, and restored by Losartan and Valproate. Panels A and B show HDAC activity in homogenates from renal *cortex corticis* and left ventricle cardiomyocytes, respectively. Empty bars: normonourished rats (C); black bars: malnourished (M) rats. In the rest of the panels, vertical dashed lines separate normonourished and malnourished groups. The *left* sections of all the panels correspond to normonourished rats; the *right* sections correspond to malnourished rats. Abbreviations of the groups are indicated on the *abscissae*, according to the nutritional status and the timeline of pharmacological interventions with Losartan, Valproate or combination of both drugs, as described in the text and Supplementary Table 1. The data are mean ± SEM (n = 5–6). Differences were assessed by Student's *t*-test. The p values are indicated within the panels.

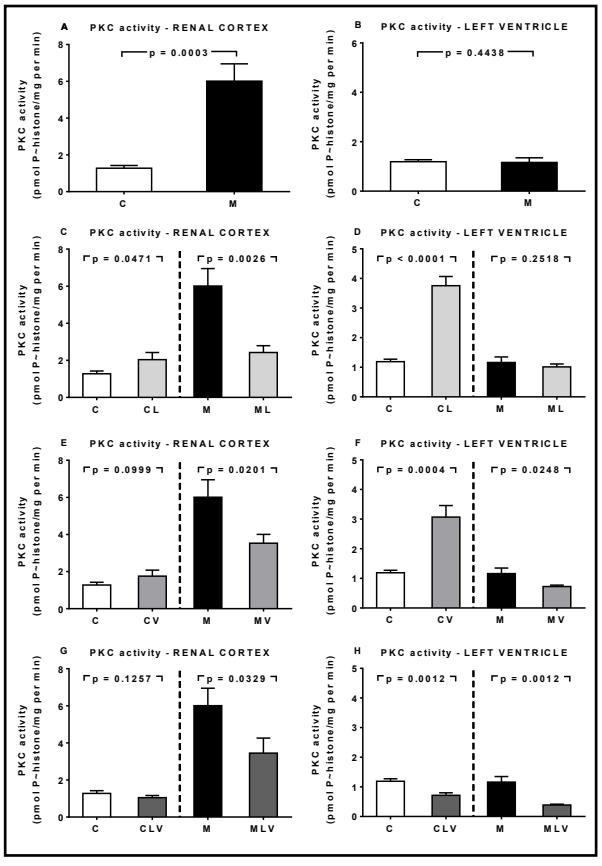


**Fig. 2.** Tissue-specific modifications of PKC induced by chronic malnutrition: different responses to Losartan and Valproate treatment in kidney and heart. Panels A and B show PKC activity in basolateral membranes from renal *cortex corticis* tubules and plasma membranes from left ventricle cardiomyocytes, respectively. Empty bars: normonourished rats (C); black bars: malnourished rats (M). In the rest of the panels, vertical dashed lines separate normonourished and malnourished groups in each organ; the *left* sections correspond to normonourished rats; the *right* sections correspond to malnourished rats. Abbreviations of the groups are indicated on the *abscissae*, according to the nutritional status and the timeline of pharmacological interventions with Losartan, Valproate or a combination of both drugs, as described in the text and in Supplementary Table 1. The data are mean ± SEM (n = 6). Differences were assessed by Student's *t*-test. The p values are indicated within the panels.

stimulated by Los in C rats, without being modified with Val or Los plus Val administration (Fig. 2C, E, G, *left* section of the panels). With malnourished animals, upregulation seen in Fig. 2A decreased by 50% in the groups that received Los, Val or their combination (Fig. 2C, E, G, *right* section of the panels). Regarding cardiac PKC, Los and Val increased its activity in normonourished rats by >200 and 150%, respectively, whereas the combination of the 2 drugs resulted in a 30% inhibition (Fig. 2D, F, H, *left* section of the panels). Activity of the cardiac enzyme remained unmodified in the M rats receiving Los (Fig. 2D), and was inhibited by Val (30%) (Fig. 2F), but much more (60%) when the drugs were given together (Fig. 2H) (*right* section of the panels).

Fig. 3A, B shows that malnutrition *per se* upregulated the renal PKA by >200% without effect on the cardiac enzyme. The results with the drugs are summarized as follows: (*i*) PKA from renal *cortex corticis* of normonourished rats increased by 150% with Los and 300% with Val, and only by 60% when the drugs were combined (Fig. 3C, E, G, *left* section of the panels). (*ii*) Renal PKA of the malnourished group decreased 60% in rats that received Val; only a non-significant tendency to inhibition occurred with Los, alone or in combination with Val (Fig. 3C, E, G, *right* section of the panels). (*iii*) Cardiomyocyte PKA of the normonourished group increased with the Los or Val treatments, even though to a lesser extent than found with the renal enzyme; combination of the drugs resulted in a pronounced inhibition of 70% (Fig. 3D, F, H, *left* section of the panels). (*iv*) Finally, in the case of the malnourished groups, the 3 pharmacological treatments downregulated enzyme activity from the heart, *i.e.* accentuating the tendency seen in kidney, and furthermore this inhibition was greater in rats given Val (Fig. 3D, F, H, *right* section of the panels).

Since PKC and PKA have opposite effects on the cardiovascular and renal systems [20–22], we analyzed the ratio between the activities of the 2 kinases to see whether they vary as the result of malnutrition, and as a consequence of the pharmacological treatments. Chronic malnutrition *per se* increased the PKC/PKA ratio in kidney, but not in the heart (Fig. 4A, B) as the result of the remarkable upregulation of renal *cortex corticis* PKC over PKA and the lack of modification of enzyme activities in cardiomyocytes (compare Fig. 2A, B with Fig. 3A, B). That the type of organ, as well as its nutritional status and pharmacological interventions influence the PKC/PKA ratio – and therefore its functional crosstalk – is summarized as follows: (*i*) Los did not influence the ratio, which is depressed by >60% in normonourished rats treated with Val, and by 30% when the drugs were given in combination (Fig. 4C, E, G, *left* section of the panels). (*ii*) In malnourished rats, Los decreased the ratio by a fraction similar to the increase caused by Val, together with the lack of modification by combination of the drugs (Fig. 4C, E, G, *right* section of the panels). (*iii*) In cardiomyocytes of normonourished animals, the ratio approximately doubled with the 3 treatments (Fig. 4D, F, H, *left* section of the panels). (*iv*) Los and Val augmented the ratio in malnourished rats, an effect cancelled out by combination of the drugs (Fig. 4D, F, H, *right* section of the panels).

*Effects of malnutrition on $Na^+$-transporting ATPases, and responses to the treatments with Losartan and Valproate*

Fig. 5A, B confirms our previous observations that chronic malnutrition downregulates ($Na^+$+$K^+$)ATPase activity in the proximal tubule cells and cardiomyocytes [8] by 60 and 35%, respectively. Los and Val alone or in combination also reduced to a great extent the ($Na^+$+$K^+$)ATPase activity in both tissues from normonourished rats (Fig. 5C–H, *left* section of the panels). The profile changed in the case of malnourished animals: a further decrease – significant, though smaller compared with that in normonourished controls – was seen only in renal *cortex corticis*, but not in cardiomyocytes (Fig. 5C–H, *right* section of the panels).

The opposite effects were induced by malnutrition on ouabain-resistant furosemide-sensitive $Na^+$-ATPase activity, and also different effects were found with pharmacological treatments (Fig. 6). Malnutrition *per se* increased its activity in kidney by 85% and reduced it by 25% in the heart (Fig. 6A, B), and the organs and drugs specificities can be summarized as follows: (*i*) Los, Val and their combination decreased renal $Na^+$-ATPase in C rats (Fig. 6C, E, G, *left* section of the panels); (*ii*) Los and Los plus Val, but not Val alone, reduced the renal

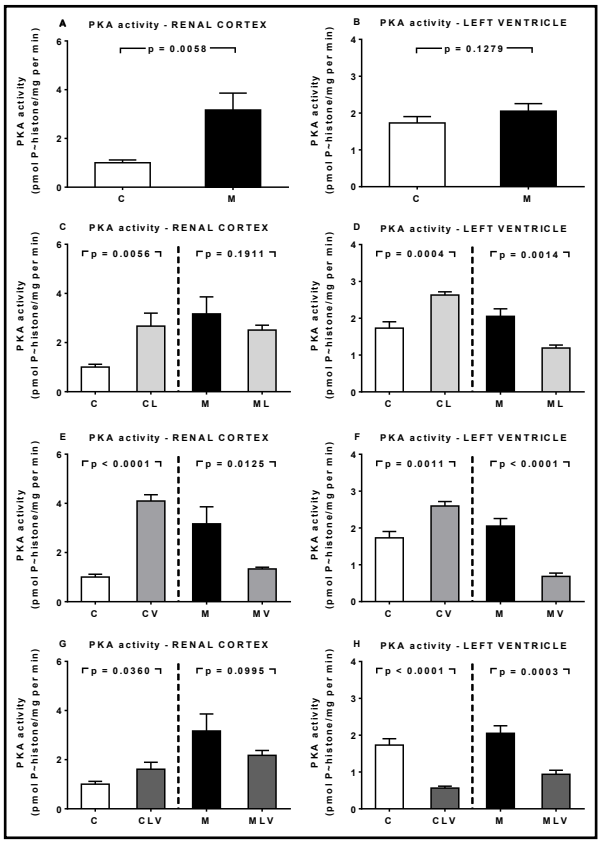


**Fig. 3.** Tissue-specific modifications of PKA caused by chronic malnutrition: different responses to Losartan and Valproate treatment in kidney and heart. Panels A and B show PKA activity in basolateral membranes from renal *cortex corticis* tubules and plasma membranes from left ventricle cardiomyocytes, respectively. Empty bars: normonourished rats (C); black bars: malnourished rats (M). Vertical dashed lines separate normonourished and malnourished groups in the rest of the panels. The *left* sections correspond to normonourished rats; the *right* sections correspond to malnourished rats. Abbreviations of the groups are indicated on the *abscissae*, according to the nutritional status and the timeline of pharmacological interventions with Losartan, Valproate or a combination of both drugs, as described in the text and in Supplementary Table 1. The data are mean ± SEM (n = 6). Differences were assessed by Student's *t*-test. The p values are indicated within the panels.

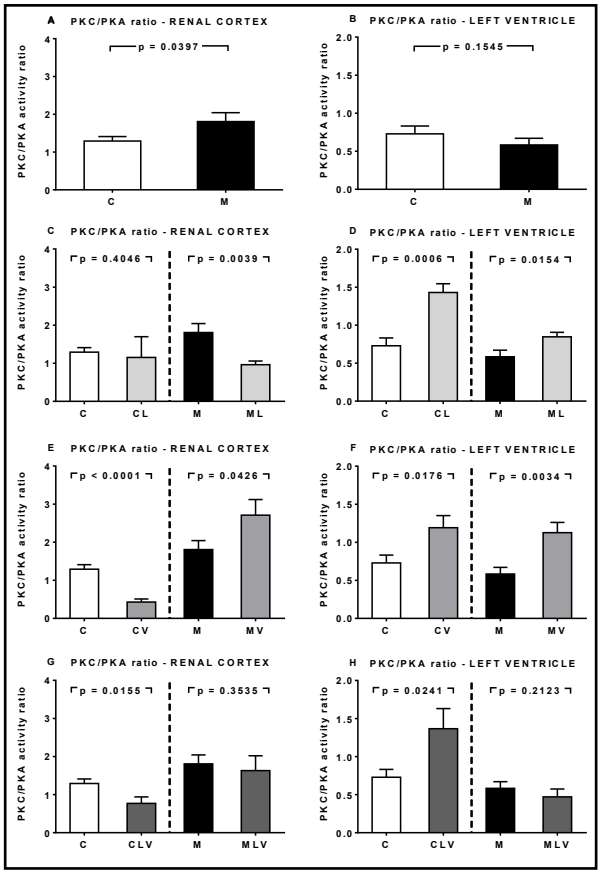


**Fig. 4.** Different profiles of the PKC/PKA ratio in kidney and heart result from nutritional status and pharmacological treatments with Losartan and Valproate. Groups are those presented in Figs. 2 and 3, as indicated on the *abscissae*. The ratios were calculated using the same membrane preparation for PKC and PKA assays. Differences between mean values were assessed by Student's *t*-test. The p values are indicated within the panels.

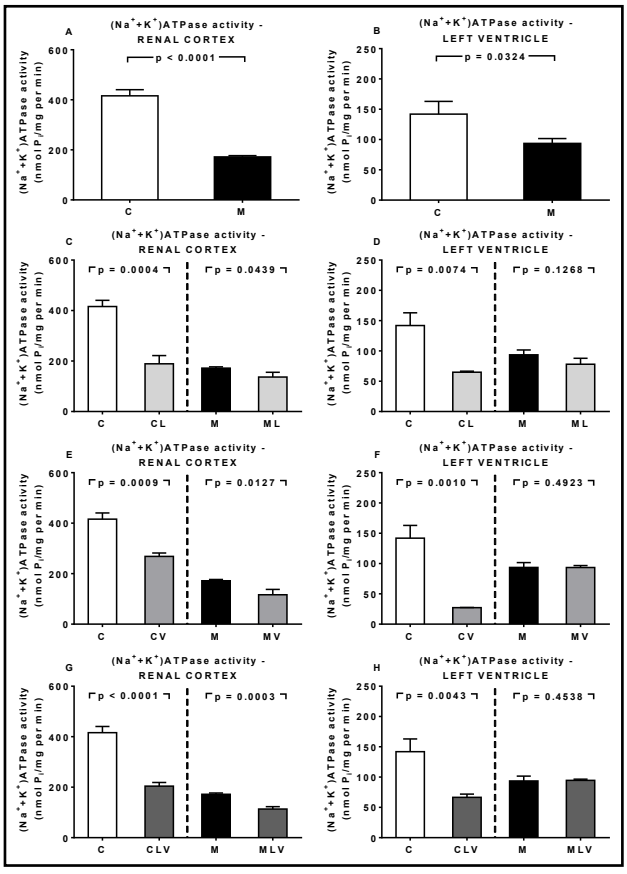


**Fig. 5.** $(Na^+ + K^+)$ATPase activity from proximal tubule cells and cardiomyocytes are similarly downregulated in chronically malnourished rats, but respond differently to pharmacological treatments. Panels A and B show $(Na^+ + K^+)$ATPase activity in basolateral membranes from renal *cortex corticis* tubules and plasma membranes from left ventricle cardiomyocytes, respectively. Empty bars: normonourished rats (C); black bars: malnourished rats (M). Vertical dashed lines separate normonourished and malnourished groups in the rest of the panels. The *left* sections correspond to normonourished rats; the *right* sections correspond to malnourished rats. Abbreviations of the groups are indicated on the *abscissae*, according to the nutritional status and the timeline of pharmacological interventions with Losartan, Valproate or a combination of both drugs, as described in the text and in Supplementary Table 1. The data are mean ± SEM (n = 4–5). Differences were assessed by Student's *t*-test. The p values are indicated within the panels.

activity in malnourished rats (Fig. 6C, E, G, *right* section of the panels) and when the drugs were administered in combination, the activity in group MLV (110±3 nmol $P_i$/mg per min) returned to C values (106±3 nmol $P_i$/mg per min; t = 0.95, p = 0.1828) (Fig. 6G). Regarding cardiac $Na^+$-ATPase from C rats, Los inhibited activity by approximately the same proportion (25%), alone or in combination with Val, but the latter *per se* was ineffective (Fig. 6D, F, H, *left* section of the panels). Regarding the cardiac enzyme from malnourished animals, the drugs had no effect when given alone, but upregulated activity by 25% in the group MLV when given in combination (Fig. 6D, F, H, *right* section of the panels); the net result was the recovery of the activity (224±19 nmol $P_i$/mg per min) to those of normonourished rats (210±12 nmol $P_i$/mg per min; t = 0.62, p = 0.2752) (MLV *vs* C groups, Fig. 6H).

*Blood pressure and heart rate in 70 and 90-day-old rats*

Young rats aged 70 days malnourished since weaning had moderately higher SAP: 139 *vs* 124 mmHg in the normonourished control (Fig. 7A), and elevated HR (384 *vs* 341 bpm) (Fig. 7B). Los, given during the same time, strongly decreased SAP from M rats to values below the control levels, without any effect on HR (Fig. 7C and Fig. 7D, respectively, *right* section of the panels). Blocking the Ang II → $AT_1R$ → PKC axis with Los had no effects either on SAP or HR in normonourished rats (Fig. 7C, D, *left* section of the panels).

Twenty days later the same profile persisted regarding SAP and HR in control and malnourished rats, and on the effect of Los administration (Fig. 8A–D). Treatment with Val alone during this period caused a moderate, though significant, decrease in the elevated SAP of malnourished rats without affecting that of C group (Fig. 8E) and the HR from the same groups (Fig. 8F). Combination of Los and Val (Fig. 8H) gave the same results as those obtained with Los alone.

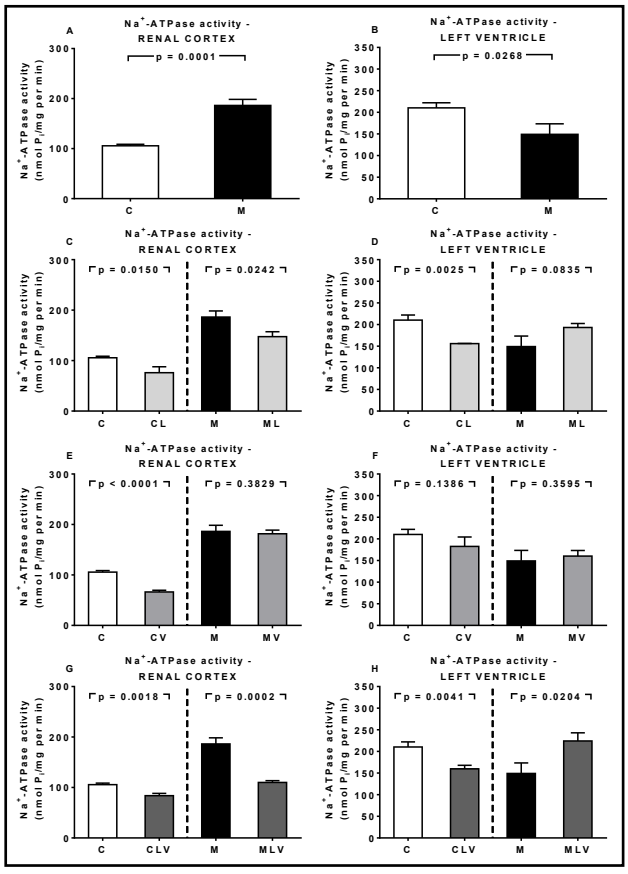


**Fig. 6.** Upregulation of ouabain-resistant $Na^+$-ATPase in proximal tubule cells and downregulation in cardiomyocytes: treatment with Losartan (alone or in combination) predominates over treatment with Valproate, with opposite profiles depending on the tissue. Panels A and B show $Na^+$-ATPase activity in basolateral membranes from renal *cortex corticis* tubules and plasma membranes from left ventricle cardiomyocytes, respectively. Empty bars: normonourished rats (C); black bars: malnourished rats (M). Vertical dashed lines separate normonourished and malnourished groups in the rest of the panels. The *left* sections correspond to normonourished rats; the *right* sections correspond to malnourished rats. Abbreviations of the groups are indicated on the *abscissae*, according to the nutritional status and the timeline of pharmacological interventions with Losartan, Valproate or a combination of both drugs, as described in the text and in Supplementary Table 1. The data are mean ± SEM (n = 4–5). $Na^+$-ATPase and ($Na^+$+$K^+$) ATPase were assayed in the same membrane preparation. Differences were assessed by Student's *t*-test. The p values are indicated within the panels.

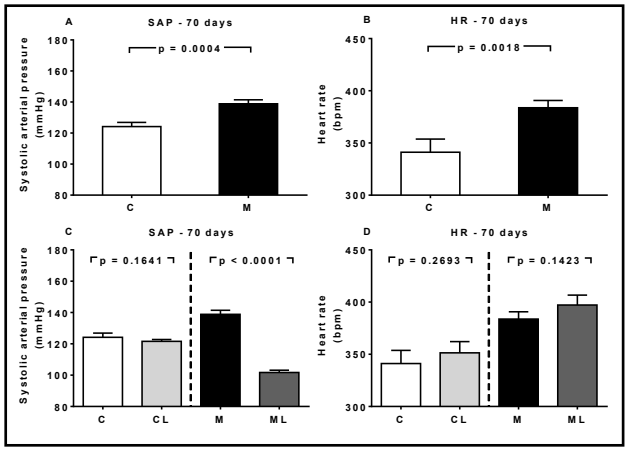


**Fig. 7.** Malnutrition increases systolic arterial pressure and accelerates heart rate in rats aged 70 days: Losartan cancels the effects on systolic arterial pressure in malnourished rats, without affecting the heart rate. (A and C) Systolic arterial pressure (SAP). (B and D) Heart rate (HR). Empty bars, normonourished control rats (C); black bars, malnourished rats. Vertical dashed lines separate normonourished and malnourished groups in panels C and D. The *left* sections correspond to normonourished rats; the *right* sections correspond to malnourished rats. Abbreviations of the groups are indicated on the *abscissae*, according to the nutritional status and the timeline of pharmacological interventions with Losartan, Valproate or a combination of both drugs, as described in the text and in Supplementary Table 1. The data are mean ± SEM (n = 10–20). Differences were assessed by Student's *t*-test. The p values are indicated within the panels.

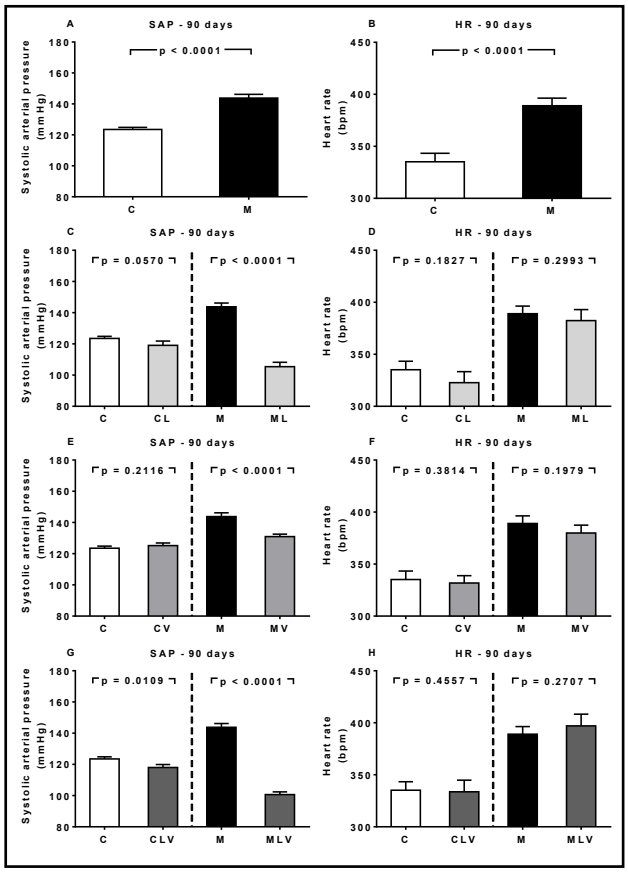


**Fig. 8.** At 90 days of age the higher systolic pressure and the accelerated heart rate persist at the same levels: Losartan and Valproate decrease systolic arterial pressure, but not heart rate. (A) Systolic arterial pressure (SAP). (B) Heart rate (HR). Empty bars, normonourished control rats (C); black bars, malnourished rats (M). Pharmacological treatments: panels C, E and G (SAP); panels D, F and H (HR). Vertical dashed lines separate normonourished and malnourished groups. The *left* sections correspond to normonourished rats; the *right* sections correspond to malnourished rats. Abbreviations of the groups are indicated on the *abscissae*, according to the nutritional status and the timeline of pharmacological interventions with Losartan, Valproate or a combination of both drugs, as described in the text and in Supplementary Table 1. The data are mean ± SEM (n = 10–20). Differences were assessed by Student's *t*-test. The p values are indicated within the panels.

## Discussion

The main, and new results, described in this study can be grouped as follows. First, malnutrition-induced downregulation of renal *cortex corticis* and left ventricle HDAC, which is totally reversed by blockade of the $AT_1$R-associated pathway and by the administration of Val, the inhibitor of all HDAC isoforms, indicate a crosstalk between RAAS and HDAC at the level of the cardiorenal axis. Second, these molecular observations are associated with the upregulation of PKC and PKA in the kidney, but not in heart, evidence that the hypothesized crosstalk involves kinase-mediated phosphorylation of a tissue-dependent manner. Third, since the malnutrition upregulated kinases in kidney and the unmodified kinases activities in heart are strongly inhibited by Los and Val, the machinery responsible for their regulation is probably a target of RAAS and HDAC. Fourth, malnutrition-induced alterations of $Na^+$-transporting ATPases, as well as the diverse responses to Los and Val (which will be discussed below), points to opposite and tissue-specific modifications that could underpin the onset of arterial hypertension and its prevention by blockade of RAAS together with restoration of HDAC activity in both organs.

*What are and what could be meant by the modifications of renal and cardiac indexes?*

Supplementary Fig. 2 clearly shows the strongly negative influence of the poor quality of the RBD on the development of rats. Reduced body mass is accompanied by reduced kidney and heart mass, and Supplementary Table 2 indicates that malnutrition affected the 2 organs in an opposite manner: whereas the renal index remained unmodified, the significant relative increase in heart mass with respect to body mass points to ongoing processes of cardiac hypertrophy and heart failure that are key elements of structural remodeling [50, 51] associated to electrical remodeling [8, 52]. The chronic administration of Los (alone or in

combination with Val) – but not of Val alone – decreased the cardiac index value; on the basis of previous reports [53], this could be due to impairment of the $AT_1R$-associated signaling pathway. On the contrary, Los (again alone or combined with Val) augmented the kidney index in malnourished rats, an increase that could be ascribed to changes in the structure and composition of tubulointerstitium and vasodilation of post-glomerular vessels, a RAAS effect that has been recently described [54]. The observations that Val did not alter malnutrition-associated modifications, and that any influence of pharmacological treatment in normonourished animals was not encountered indicate that malnutrition negatively impacts renal and left ventricular structures through a pathway in which RAAS, but probably not HDAC, plays a central role.

*Crosstalk between HDAC and RAAS in renal cortex corticis cells and cardiomyocytes*

The strong inhibition of renal HDAC and an almost crippled left ventricle HDAC demonstrate – as far as we are aware – that multifactorial malnutrition has, as the key molecular basis of its transcriptional effects, the shifting of renal cortical and left ventricular chromatin towards a more open configuration [33, 55]. The data in Fig. 1 at first sight point to a paradox (except for cardiac HDAC in C rats): when the rats were given Val during 3 weeks, alone or in combination with Los, HDAC activity increased to (or close to) the C levels. However, this apparently paradoxical result might be indicative that acetylation of other proteins, including protein kinases [30], besides the histones that were assayed for histone deacetylase activity in the experiments described in Fig. 1, co-regulates HDAC from kidney and heart.

The existence of a crosstalk between the RAAS and HDAC becomes also evident from the experiments shown in Fig. 1. When Fig. 1C and Fig. 1D are compared, it is possible to quantitatively demonstrate that the recovery of HDAC activities by Los in proximal tubule cells and cardiomyocytes in malnourished rats is the same as the augmentation seen in both controls. This combination of effects is suggestive of some influence on the Ang II → $AT_1R$ axis favoring a less compact histone configuration [33] that recovers the alterations induced by malnutrition. There are few examples that one can discuss as a consequence of functional crosstalk between RAAS and HDAC that support to the view we present: HDAC, for example, controls genes responsible for the expression of several components of RAAS that are essential for the development of kidney and urinary tract [56].

Deserving special comparison and discussion, the data depicted in Fig. 1 show the contrasting effect of Val treatment on HDAC activity and the strong differences seen in renal *cortex corticis* and left ventricle in normonourished and malnourished rats, demonstrating tissue-specific selectivity of the drug and, therefore of its targets, as well as the nutritional status modifying the response to Val in each organ. It may be that the unexpected increased HDAC activity in the presence of Val (an inhibitor of HDAC), which was observed in renal *cortex corticis* and left ventricle from malnourished rats, is a result of the decreased PKC and PKA activities. The experiments in Fig. 1 also indicate that, regardless of the nutritional status, the interactions between Los and Val – and therefore between their targets – are different in renal *cortex corticis* tubules and the left ventricle. In renal *cortex corticis*, they seem to act through the same mechanism, with Val predominating and partially competing with Los because HDAC activity is significantly lower in the CLV than in the CL group and in the MLV than in the ML group. In heart, the mechanism of Los and Val actions seems to be exerted through different and independent pathways towards the same effector, and the effect of Val always predominates.

*Are PKC- and PKA-mediated phosphorylations involved in the crosstalk between HDAC and RAAS in renal cortex corticis and cardiomyocytes?*

That nearly all types of histones become phosphorylated in specific Ser and Thr residues in steps of the cell cycle such as transcriptional regulation, DNA replication and DNA repair, was explained a decade ago [57]. Several kinases have been implicated in the wide spectrum of phosphorylations that can modify the activity of HDAC [31, 32]. In this study, we focused

on 2 protein kinases central in RAAS signaling, PKC and PKA [13, 17–22], which were modified (Figs. 2–4). The general picture of the effects of Los and Val on both renal and cardiac kinases from chronically malnourished rats was – though quantitatively different – the mirror image of that encountered with HDAC (compare with Fig. 1), *i.e.* whereas HDAC activities increased with pharmacological interventions, the opposite was true with both kinases. In terms of the proposed functional relationships of the kinases with HDAC, the inhibition by Val of the upregulated renal and of the unmodified cardiac PKC and PKA activities deserves special attention. This inhibition suggests that increased acetylations and decreased phosphorylations can be associated events in transcriptional processes in both renal *cortex corticis* tubule cells and cardiomyocytes altered by chronic malnutrition.

Clearly, this reasoning cannot be limited to the independent analyses of the PKC and PKA activities, but one must also consider their ratio (Fig. 4), especially in terms of RAAS because PKC is central within the $AT_1R$ signaling pathway, whereas PKA is a key component in the signaling route starting in $AT_2R$ [22, 49, 58, 59]. Since the absolute values of the 2 kinase activities are different, depending on the pharmacological treatment, their ratio can vary, allowing for example perceiving that Val stimulated PKC to a greater extent in heart (from normonourished and malnourished rats) and in renal *cortex corticis* of the malnourished group. These data lead us to propose that the PKC and PKA phosphorylation sites in the histones of renal *cortex corticis* and cardiomyocytes are different and differentially modified by malnutrition. Moreover, it may be that local factors participate in the tissue selectivity of the HDAC inhibitor; the local RAAS being a potential candidate.

*$Na^+$-transporting ATPases are differentially modulated in kidney and heart by blockade of $AT_1R$ and inhibition of HDAC*

We have previously described the downregulation of $(Na^+ + K^+)$ATPase and the upregulation of the $K^+$-independent, ouabain-resistant $Na^+$-ATPase, in renal tubular cells and cardiomyocytes of malnourished rats, through mechanisms involving altered RAAS [7–10, 60]. With respect to the $(Na^+ + K^+)$ATPase (Fig. 5), the remarkable inhibition in renal *cortex corticis* and cardiomyocytes by Los in normonourished rats confirms the physiological importance of the Ang II → $AT_1R$ signaling axis in regulating the $(Na^+ + K^+)$ATPase-mediated transepithelial $Na^+$ transport [8, 13, 49], and the $(Na^+ + K^+)$ATPase-mediated modulation of contractility and electrical activity [61, 62]. $(Na^+ + K^+)$ATPase was also the final target of a Val-sensitive pathway, which causes inhibition of the pump in the kidney and heart from normonourished rats in the absence or presence of Los, indicating that the drugs shift the pump to a comparable state of lower catalytic activity through non-competing, different pathways. The data further support the idea that acetylations and RAAS, the latter possibly *via* kinase-mediated regulatory phosphorylations [22, 32], are coordinated in the physiological processes at the level of the cardiorenal axis.

The profile of $(Na^+ + K^+)$ATPase activities and the pharmacological interventions were changed in malnourished rats. Besides downregulation due to malnutrition itself, there is a difference between what happened in renal *cortex corticis* tubules and the cardiomyocytes: whereas Los and Val potentiated slightly the malnutrition-induced downregulation in kidney, no difference was seen in the heart, suggesting that alterations of the pump caused by chronic administration of the multideficient diet also selectively modified tissue-specific non-histone proteins [30, 63, 64]. Some of the processes regulated by acetylation of non-histone proteins are mediated by ERK and phosphatidylinositol phosphate kinases, which are involved in the regulation of $Na^+$-transporting ATPases in malnourished rats [8, 9, 65].

The ouabain-resistant $Na^+$-ATPase, considered responsible for fine-tuning of active $Na^+$ transport [9, 66, 67], showed that malnutrition *per se* provoked opposite effects depending on the organ and pharmacological interventions (Fig. 6), again indicative of organ selectivity of the same pathways with respect to the different $Na^+$-transporting ATPases. The profiles are completely different from those encountered for $(Na^+ + K^+)$ATPase. This probably mirrors the differences existing in their primary structure and organization of their ion-binding domains [28, 29]. Despite similarities in their conserved catalytic domains [29], there

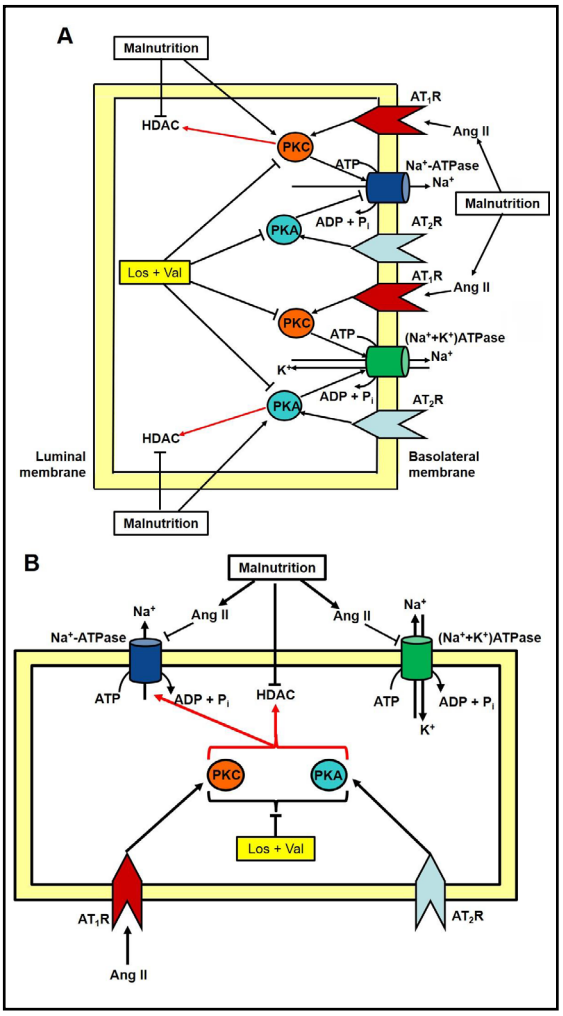


**Fig. 9.** Suggested mechanisms of tissue-specific malnutrition-induced changes and the responses to Losartan and Valproate at the level of the HDAC/RAAS axis in *cortex corticis* tubules and cardiomyocytes. (A) *Cortex corticis* tubule. Malnutrition, which increases intrarenal Ang II [9], induces downregulation of HDAC activity (black hammers) and upregulation of PKC and PKA (black arrows). Downregulation of HDAC is totally reversed by blockade of the Ang II → $AT_1$R-associated pathway (Losartan) and by the administration of Valproate (inhibitor of HDAC) (black hammers). Losartan and Valproate restore HDAC, possibly by inhibition of PKC and PKA (red arrows). Unbalance between PKC and PKA culminates with malnutrition-induced downregulation of $(Na^+ + K^+)$ATPase and upregulation of $Na^+$-ATPase. (B) Cardiomyocyte. Malnutrition stimulation of the Ang II → $AT_1$R-associated signaling [8] (upper black arrows) results in downregulation of both $Na^+$-transporting ATPases activities (upper black hammers). Losartan and Valproate recover the control HDAC and $Na^+$-ATPase activities (red arrows), likely due to the inhibition of PKC and PKA activity (lower black hammer), and restores RAAS signaling. The drugs, showing that $(Na^+ + K^+)$ATPase is not influenced by the crosstalk between RAAS and HDAC in the heart, do not modify the activity of this pump. Even though PKA is central in $AT_2$R-associated signaling, we do not have, at present, evidence regarding the participation of $AT_2$R in the crosstalk between HDAC and RAAS in kidney or heart.

are differences in putative phosphorylatable sites [9], which probably control catalysis in physiological and pathological conditions and confer some functional dissimilarity to each other. Malnutrition-associated upregulation of renal enzyme and downregulation of the cardiac one is a difference that is now suggestive of tissue-specific regulatory mechanisms of the pump, probably linked to RAAS because regulation of ACE, the enzyme responsible for Ang II formation, involves acetylation of histones [68]. We propose that a combination of different acetylations and phosphorylations [32] is responsible for the inhibition by Los and Val (except for left ventricle) of the $Na^+$-ATPase from the normonourished rats. The same mechanism may underlie the lack of Val effects in the renal *cortex corticis* from the malnourished rats and the recovery of the control levels in both tissues.

*Systolic arterial blood pressure and heart rate increased in malnourished rats: only blood pressure being diminished by pharmacological treatments*

We have previously demonstrated the onset of arterial hypertension in chronically and prenatally malnourished rats [8, 10]. In these rats we have now found that there is an early (70 days of age) dissociation between an elevated blood pressure and an accelerated heart rate, which has been described as evidence of impaired integration of autonomic function in SHR [69]. As far our knowledge reach, this is the first observation demonstrating that malnutrition impacts the mechanisms regulated by carotid body innervation, the peripheral nervous structure responsible for the modulation of hemodynamic responses in physiological

and pathological conditions [70, 71]. It is remarkable in terms of the underlying mechanisms that Los and Val, alone or in combination, decreased the elevated systolic pressure without any effect on heart rate, and that the drugs act only on the undernourished rats. It may be: (i) that the drugs influence the mechanisms of arterial pressure regulation when a tissular pro-hypertensive microenvironment exists, as demonstrated in SHR [49]; and (ii) that the anti-hypertensive influence of Val, potentiated by Los, is due to inhibition of RAAS within the HDAC/RAAS axis (which was evidenced in Fig. 8 at 90 days of age), as recently encountered in overweight mice [36]. The lack of effects of Los and Val on HR suggests that, in contrast to SAP, the alterations that malnutrition provoked in the carotid body innervation are not associated to RAAS or HDAC, but possibly to the autonomous nervous system.

## Conclusion

The simultaneous deregulation of HDAC and RAAS (HDAC/RAAS axis), including those of PKC and PKA, underlie modifications that chronic malnutrition provokes in $Na^+$-transporting ATPases from proximal tubule cells and cardiomyocytes. Therefore, simultaneous alterations of the massive $Na^+$ reabsorption and cardiac ion transport remodeling configure a type V cardiorenal syndrome [72]. The reciprocal influence of Los and Val on the central components of the signaling network coupled to Ang II receptors (the PKC/PKA couple) supports our conclusion that deacetylations are physiological and molecular processes that modulate RAAS-mediated responses in the kidney and the heart. Although Los and Val have different pharmacological actions, they could have complementary effects – though by different molecular mechanisms – in the crosslink between HDAC and RAAS, as demonstrated in this study. We propose that combined therapy using both drugs opens up new vistas in the treatment of arterial hypertension and cardiorenal syndromes. Fig. 9 summarizes the suggested mechanisms of malnutrition-induced changes and the responses to Los and Val at the level of the HDAC/RAAS axis in kidney and heart.

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*Author Contributions*

HM-F and AV conceived, designed and supervised the research, and obtained funding. HM-F, LBJ, ACSB, SA-B, DSA-B, AP-A, BSNF, DS-P and GC-S acquired the data. HM-F, LBJ, ACSB, SA-B, DSA-B, AP-A, BSNF, DS-P, GC-S and AV analyzed and interpreted the data, and performed the statistical analyses. HM-F and AV wrote the manuscript. All authors read and approved the final form of the manuscript. English correction by BioMedES (UK) is also acknowledged.

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*Statement of Ethics*

All experimental procedures were approved by the Committee for Ethics in Animal Experimentation of Federal University of Rio de Janeiro (protocols 007/16 and 012/19), and were carried out in accordance with the Committee's guidelines, which follow the Uniform Requirements for Manuscripts Submitted to Biomedical Journals.

## Disclosure Statement

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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