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Special article - Review

SODIUM AND MENOPAUSE. DOPAMINE D1-RECEPTORS, NA$^+$-K$^+$-ATPASE AND CD4$^+$ IN PERIPHERAL BLOOD AS MARKERS OF RENAL INFLAMMATION

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Abstract

It has been observed that menopausal women have a greater sensitivity to sodium intake than women in fertile age. Furthermore, it is now known that the immune system activated by high sodium intake plays an essential role in the development of hypertension. Changes observed in components of the immune system in an animal model of menopause, show the key role of estrogens in the regulation of the immune response to a high salt load. Faced with the same challenge, intact rats have adequate renal sodium management, and the immune system is not activated, while hormone-deprived rats retain sodium and show a pro-inflammatory profile. Pharmacological treatment of hypertension reduces blood pressure, but the observed renal damage remains. Therefore, menopausal women should be especially advised to have an adequate sodium intake that does not pose a risk to their health.

Key words: hypertension, menopause, estrogen, high salt intake

Resumen

Sodio y menopausia. Receptores de dopamina D1, Na$^+$-K$^+$-ATPasa y CD4$^+$ en sangre periférica como marcadores de inflamación renal

Se ha observado que las mujeres menopaúsicas tienen una sensibilidad mayor al consumo de sodio que las mujeres en edad fértil. Por otra parte, actualmente se conoce que el sistema inmune, asociado a una ingesta elevada de sodio, desempeña un papel esencial en el desarrollo de hipertensión. Los cambios observados en componentes del sistema inmune en un modelo experimental de menopausia evidencian el papel clave de los estrógenos en la regulación de la respuesta inmune ante una carga elevada de sal. Ante el mismo desafío, las ratas intactas tienen un manejo renal adecuado del sodio y el sistema inmune no se activa, mientras que las ratas privadas de hormonas retienen sodio y muestran un perfil proinflamatorio. El tratamiento farmacológico de la hipertensión logra reducir la presión arterial, pero

se mantiene el daño renal observado. Las mujeres menopaúsicas deberían ser especialmente alertadas para tener un consumo adecuado de sodio que no represente un riesgo para su salud.

Palabras clave: hipertensión, menopausia, estrógeno, alto consumo de sal

KEY POINTS
Current knowledge

- High salt intake increases blood pressure in the general population. Menopausal women have a greater sensitivity to sodium.
- The immune system accompanies the development of hypertension with a proinflammatory response in tissues such as the kidney.
- Female sex hormones play a regulatory role that is affected by the decrease in hormone concentration.

Contribution to current knowledge

- In a model of menopause, we found that sodium-handling-related proteins increase in peripheral immune cells upon high sodium intake in hormone-deprived animals which is associated to activation of immune cells
- Successful treatment of hypertension does not change the activation profile of these cells or renal infiltration if high sodium intake continues

The association between high salt (NaCl) intake and high blood pressure (BP) is well known[1]. At menopause, this relationship is not different from that in the general population. However, two sex-related characteristics distinguish hypertension (HT) in menopause: the absence or very low levels of female sex hormones and a greater sensitivity of women to high sodium (HS) intake[2].

Therefore, given the fact of salt sensitivity in menopausal women[3] and that menopause in developed societies or developed communities begins at the average age of 52 years[4] when most women are still, and increasingly, quite active in society, it should be prudent and fair to consider those characteristics to prevent or treat HT in menopausal women based on this particular profile.

According to clinical and experimental studies, HS intake is detrimental to women's health. HS consumption is associated with a poor response to HT treatment[5]. And, in experimental models of HT or menopause, despite adequate BP-lowering treatment, kidney damage continues[6,7].

This work describes the experiences of different research groups and our own, on aspects related to the importance of female sex hormones in the regulation of blood pressure, sodium balance and interactions with the immune system, emphasizing an animal model of menopause with which we have been working in recent years.

Salt response in fertile age

HS is associated with the development of HT in many people, however, it was only in recent years that scientific studies, whether clinical or experimental, began to focus on the gender of the study population. In addition to sexual functions, sex hormones have receptors on different cells, which allow them to have a regulatory role in various systems, such as renal function and immune response.

Experimental studies show that the response of immune cells (peripheral blood mononuclear cells, PBMC) to HS (1% NaCl in drinking water) is different in intact female rats (IF) compared to sex hormone-deprived rats (HD), an experimental model of menopause[8]. The expression of dopamine D1- receptors (D1DR), Na^+-K^+-ATPase (NKA) and $CD4^+$ changes in an opposite manner in IF rats compared to HD rats. The function of these molecules in lymphocytes is related to activation and proliferation.

Anti-inflammatory vs. pro-inflammatory response upon high sodium intake

In IF rats, $CD4^+$ and NKA are downregulated in PBMCs after a HS intake. In addition, IF rats increase sodium excretion to match sodium intake and blood pressure remains normal throughout this process. Besides, the renal microscopic structure does not show any change from normal. This is a normal response in IF rats when challenged with a moderately high sodium in-

take. The amount of sodium intake resembles that often found in humans in the office setting. The downregulation phenotype of D1DR, NKA and $CD4^+$ expression in PBMC is associated with less active cells and, thus, an anti-inflammatory response when immune cells are challenged with HS. Other groups working on lymphocyte response to HS found a similar result. They observed an increase in the regulatory helper cells phenotype (Treg cell) and a decrease in effector T cells (cytotoxic or activated T cells)[9].

On the other hand, many groups working on salt-induced hypertension and immune response directly found a pro-inflammatory phenotype with the associated response, i.e. inflammation and infiltration of renal tissue by inflammatory cells. They use more aggressive models to induce hypertension like ANG II infusion plus high salt or DOCA-salt induced HT or rat strains genetically modified that develop hypertension[10]. Besides, the concentration of added NaCl is 4%. These models are particularly useful to study the hypertensive response at different levels of immune or renal response, but they apparently lack the possibility to observe an initial phase of anti-inflammatory cellular response as described when using less aggressive experimental designs.

The concept of hormesis

This type of behaviour in IF animals seems to fit with the concept of hormesis. This phenomenon has been described as an adaptive or preconditioning response. Living systems have the capacity to respond to stress, which is defined as a signal generated by any physical, chemical or biological factor (stressor), which in a living system initiates a series of events to counteract, adapt and survive. A previous exposure to a low dose of a toxic agent or a stressful condition regulates adaptive mechanisms that protect against subsequent exposures to similar toxic agents or stressful conditions. A successful response to low doses of stressors improves the overall homeodynamics of cells and organisms, but an incomplete or failed homeodynamic response leads to the damaging and harmful effects of stress[11]. Therefore, hormesis is a biological phenomenon where a small amount of a potentially harmful substance or stimulus can have beneficial effects on the organism, while high doses produce toxic or harmful effects.

In IF animals, there appears to be self-regulation of PBMC to avoid a response to the salt stimulus, that is, to avoid cell activation and proliferation, and thus preventing infiltration into key tissues such as the kidney. When the stimulus increases and the salt concentration is very high, another response profile is manifested, where activation of the immune system is inevitable, and can be evidenced by an increase in proinflammatory markers in the blood, as well as tissue infiltration that contributes to the worsening of organ dysfunction and aggravates the pathology.

In agreement with the results in animals, the work of Yi Buqing et al.[12] shows the response of the immune system of healthy people to different salt intakes for prolonged periods, finding a positive association between salt levels in the diet and the amount of monocytes and proinflammatory cytokines. By reducing salt intake, not only do proinflammatory cytokines decrease but also anti-inflammatory cytokines increase, showing a beneficial or protective response.

Salt response in sex hormone-deprived animals

In a menopausal model, HD rats show different behaviour of PBMC compared to IF rats. After 1% NaCl ingestion, PBMCs from HD rats show an activation phenotype.

D1DR, NKA and $CD4^+$ are overexpressed when HD rats take HS. This phenotype is compatible with inflammation since Dopamine, through D1DR stimulation, is known to induce an activation/effector pattern in immune cells[13], and NKA overexpression is associated with lymphocyte activation and proliferation[14]. High NKA hyperpolarizes lymphocyte plasma membrane which favors cell activity and motion. NKA overexpression is also observed in T cells in the tumor microenvironment[15].

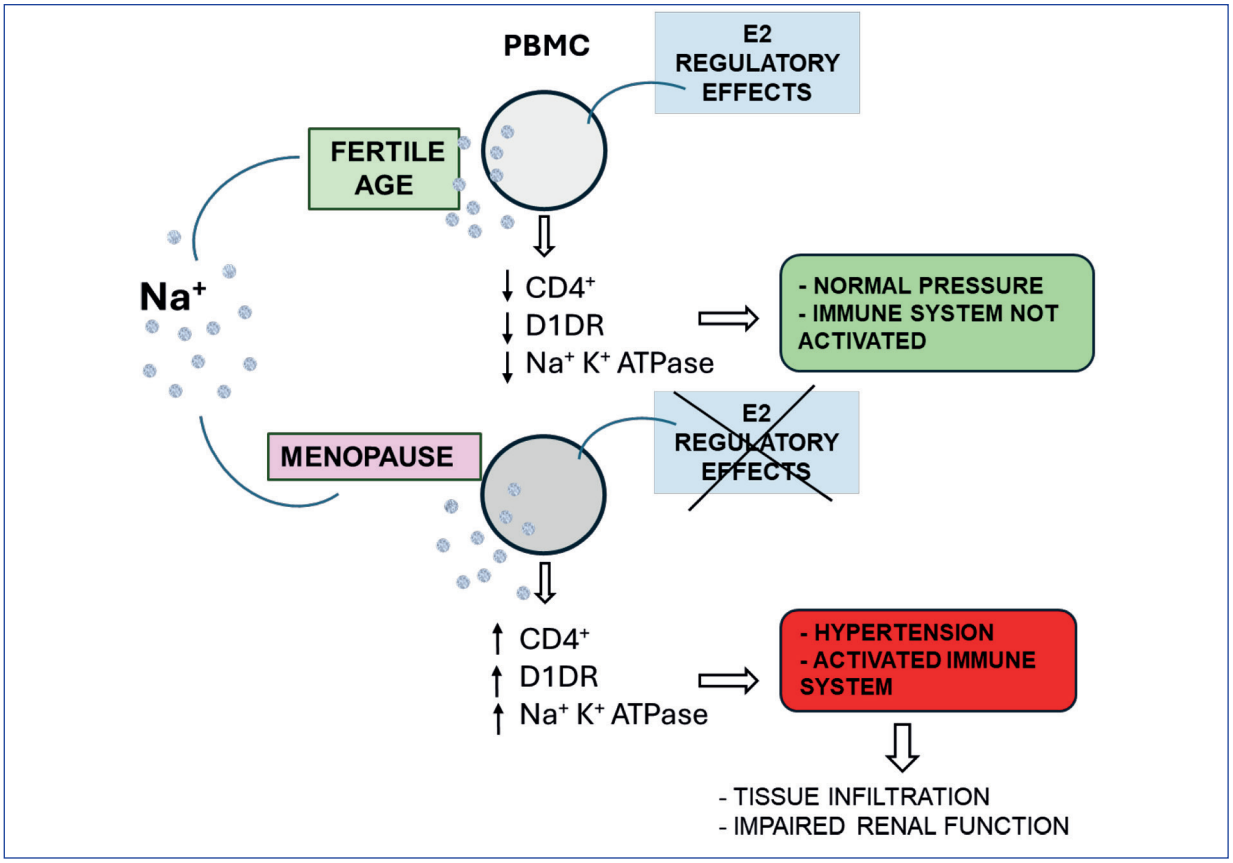
Thus, PBMC no longer show an anti-inflammatory phenotype, but a pro-inflammatory pattern with overexpression of D1DR and NKA and $CD4^+$ markers. In addition, the renal tissue presents a peritubular basolateral infiltration of CD45 cells. And BP increases to HT levels. Thus, the opposite of what was observed in IF rats

after HS intake (Figure 1). In HD context, when rats are exposed to sodium, even if it is not at very high doses, they lose the ability to regulate their response to this stressor, and the concept of hormesis can no longer be applied.

A possible explanation for changes in rats´ phenotype is given by Zielinski group. They describe a kind of dual response of T cells. When T cells detect that sodium is changing secondary to increased intake, the cells express an anti-inflammatory phenotype. But if in the tissue microenvironment T cells face a condition of increased inflammatory cytokines such as IL-1β or IFN-γ, then cells can switch to a pro-inflammatory phenotype[15]. Conditions of increased inflammatory cytokines can be infections, autoimmune diseases or excessive tissue sodium accumulation[16-19]. Thus, T cells can be challenged by an absorbed sodium or central plasma sodium and a more peripheral sodium, tissue sodium. Certainly, when T cells respond with an anti-inflammatory pattern, this type of profile accompanies effective sodium excretion and normal blood pressure. But pro-inflammatory changes, as observed in HD rats, have a pathophysiological response in which HT occurs. Cytokines promote an increase in sodium reabsorption in renal tubular cells[20].

In this sense, the absence of female hormones could play a permissive role and it could be the signal in menopause that allows the phenotype change. Estrogens maintain the integrity of the T cell response and the balance between effector T cells and Treg cells. Estrogens increase Treg cells[21,22]. On the other hand, in the kidney of HD rats, NKA is already overexpressed even under

Figure 1 | Response of peripheral blood mononuclear cells in fertile age and menopause to high sodium intake



The diagram shows the effects of sodium overload on peripheral blood mononuclear cells (PBMC) from fertile and menopausal rats. Rats in fertile age respond by decreasing the expression of dopamine D1 receptors (D1DR), Na^+-K^+-ATPase (NKA) and $CD4^+$ (anti-inflammatory response), and maintain normal blood pressure; while on the contrary, menopausal rats develop an inflammatory profile, increasing the expression of these proteins and raising their blood pressure. Lack of estrogen (E2) plus sodium overload in menopausal rats could be the cause of these differences

normal sodium intake, but HT does not develop until the challenge of HS intake[23,24]. An overexpression of NKA causes high electrolyte fluxes, as in activated PBMCs, and consequently HS can increase reactive oxygen species or activate the inflammasome[25]. Menopausal rats excrete less sodium than IF rats[23]. Therefore, at least the renal and immune systems are engaged in this model contributing to a systemic alteration to manage sodium balance. The result is an increase in blood pressure and renal damage.

High sodium or high blood pressure: which is responsible for immune cell activation and renal infiltration

To elucidate whether the changes observed in PBMCs of HD rats are in response to salt or increased blood pressure, HD rats under normal salt intake or HS were treated with drugs clinically used to lower BP in hypertension[7]. Despite a good BP response, with drugs given as HT prevention therapy or as HT treatment as commonly used in humans, the rest of the alterations remained unchanged. The activation of the PBMC phenotype and renal infiltration with CD45 cells was not modified by effective BP reduction. Therefore, HS intake rather than high BP seems to be the cause of immune system activation and renal infiltration. Another group of researchers working with a different model of HT and with different drugs to treat hypertension has shown similar results regarding renal inflammatory infiltration[6]. And, in male mice which did not develop HT, upon HS intake renal tissues were also infiltrated with inflammatory cells as in HT models[26]. Furthermore, in a clinical study with variables corrected by sex, age, medication, and BP they found that HS intake in women was the main cause of failure of HT treatment[5]. Therefore, it is clear that despite a good anti-HT treatment in menopausal women or in experimental animals which might be successful in lowering BP, the immune/inflammatory response in PBMC and renal tissue, will continue unless an associated reduction in sodium intake is achieved.

WHO recommends a daily sodium intake of no more than 5-6 g. In our society, the average amount is 11-12 g per day. Therefore, the scenario of absence of female sex hormones, menopause, plus HS intake creates a suitable microenvironment to develop immune activation associated with renal inflammation and hypertension. It seems prudent to consider the WHO recommendations to reduce sodium intake to avoid the harmful effects of HS on women's health. Menopausal women should be strongly advised to achieve a marked reduction in sodium consumption. And food processing companies should also be strongly suggested to reduce the amount of sodium in their products, since 75% of the daily sodium ingested comes from this type of food. Fresh foods do not have such high levels of sodium. A strong collaboration among health care systems, population, government and food companies is needed to reduce the daily amount of sodium intake, which is very harmful to health and in particular for postmenopausal women.

Conclusions

Based on our findings, we propose that in postmenopausal women the effect of excessive sodium intake associated with hypertension and renal inflammation can be followed using PBMC activation markers such as D1DR, NKA and $CD4^+$ to assess the degree of immune response and renal involvement. Any other potentially confounding inflammation should be ruled out. The proposal allows for the monitoring of a possible development of inflammation, together with hypertension, and to increase the accuracy to make indications to reduce both conditions and verify the response.

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Conflict of interest: None to declare

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