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SIMPOSIO 11.
REGULACIÓN CENTRAL Y PERIFÉRICA DEL BALANCE DE AGUA Y SALES

HOMEOSTATIC CONTROL OF HYDROMINERAL BALANCE: THE ROLE OF CARBON MONOXIDE AND NITRIC OXIDE IN HYPEROSMOLALITY-INDUCED ATRIAL NATRIURETIC PEPTIDE RELEASE BY HYPOTHALAMUS *IN VITRO*

[1]JOSE ANTUNES-RODRIGUES, [1]DAYANE APARECIDA GOMES, [1]WAGNER LUIZ REIS, [1]RENATO RIZO VENTURA, [1]ALEXANDRE GIUSTI-PAIVA, [1]LUCILA LEICO KAGOHARA ELIAS, [2]LAURA VIVAS AND [1]FERNANDO QUEIROZ CUNHA

[1]Department of Physiology and Pharmacology, School of Medicine of Rib. Preto, SP, Brazil;
[2] Instituto de Investigación Médica Mercedes and Martyn Ferreira, 5000 Córdoba, Argentina.

The atrial natriuretic peptide (ANP) immunoreactive neurons are localized to the region extending from the paraventricular nucleus (PVN) rostraly to the subfornical organ (SFO) and ventrally to the organum vasculosum lamina terminalis (OVLT), and their axons also project to the median eminence (ME) and neurohypophysis (NH). ANP neurons are activated by acute and chronic changes in body fluid homeostasis. After volume load or volume depletion there is an increase or a reduction in the ANP concentration in the ME and NH, respectively. Acute extracellular volume expansion (EVE) induced by intra-atrial injection of hypertonic, isotonic saline or 5% glucose determined a rapid increase in plasma ANP concentration, which was blocked by lesions of the AV3V, ME or NH. Recent experiments have indicated that nitric oxide (NO) controls the physiological release of hypothalamic peptides and classical neurotransmitters. The enzyme NO synthase (NOS) has been detected in several brain areas, such as the magnocellular neurons of the SON, PVN, NH, SFO, MNPO and OVLT where it may operate as a neuromodulator.

Carbon monoxide (CO) is also produced by neurons and modulates synaptic activity. CO is generated by heme oxygenase (HO), the enzyme that cleaves heme and produces biliverdin, CO, and iron. Heme oxygenase is present in rat hypothalamus, and both isoenzymes HO1 and HO2 localized in the PVN and SON. We have speculated that NO may be involved in the regulation of ANP secretion in the CNS. In the present study we investigated, *in vitro*, the ANP release and NOS activity from basal hypothalamus when incubated in a medium with graded NaCl concentration. We also evaluated the effect of different NO donors on basal and hyperosmolality-induced ANP release and the interaction between endogenous CO and the NO system as a modulator of the hypothalamic response to osmotic stimulation. The increase in the medium osmolality (NaCl, 340 mOsm/Kg H_2O) induced an elevated ANP release, which was associated with a decrease in NOS activity ($p<0.001$), nitric oxide (NO) production and nitrate ($p<0.001$) release into the medium. The NO donors sodium nitroprusside (300 µM), S-nitroso-N-acetylpenicillamine (300 µM) and 3-morpholinosydnonimine chloride (300 µM) promoted a significant decrease in ANP release in response to hyperosmolality ($p<0.001$). ANP release observed in the present study did not result from injury to the BH caused by the increase in medium osmolality nor a toxic effect of the NO donors as demonstrated by the ANP release after incubation with KCl (56 mM). Furthermore, hyperosmolality or NO donors did not increase the lactic dehydrogenase (LDH) content in the medium. The heme oxygenase inhibitor, zinc deuteroporphyrin 2,4-bis glycol, prevented the hyper osmotic-induced ANP release and reduction of NOS activity. In conclusion these results suggest that NO, the production of which is dependent on CO, modulates the osmolality-induced ANP release by BH fragments.

SISTEMA DOPAMINÉRGICO RENAL Y REGULACIÓN DE LA EXCRECIÓN DE SODIO

FERNANDO R. IBARRA

Doctor de la Universidad de Buenos Aires,
Médico Investigador del Laboratorio de Riñón del Instituto de Investigaciones Médicas «A. Lanari», UBA.

La dopamina (DA) de origen renal produce diuresis y natriuresis y es una hormona clave en el control del volumen extracelular y la presión sanguínea. Otras fuentes alternativas de DA como la plasmática o la neural, para tales efectos tienen escasa o nula importancia. La DA urinaria es sintetizada en los túbulos proximales del nefrón. Allí, la L-dopa filtrada en el glomérulo es decarboxilada por la enzima decarboxilasa de aminoácidos aromáticos (AADC). También en el riñón, la DA es degradada por monoamino oxidasa (MAO) a ácido dihidroxifenilacético (DOPAC) y por catecol-*O*-metiltransferasa (COMT) a 3-metoxitiramina. La acción diurética y natriurética de DA se observará a partir de la estimulación de receptores específicos, sobre todo subtipos D_1, D_2 y D_3, sin embargo, el incremento de DA urinaria no siempre se ve acompañado por un aumento de natriuresis. Este efecto dependerá del estado de expansión del volumen extracelular o del contenido de Na^+ de la dieta, lo que a su vez modificará la disponibilidad de DA renal, alterando la síntesis o degradación de la amina. Así, la dieta hipersódica produce un aumento de la síntesis y una disminución de la degradación de la amina, y en la dieta hiposódica se observa lo contrario. Los cambios de sodio en la dieta podrían también alterar el transporte tubular del precursor L-dopa. La inhibición de las enzimas MAO y COMT no siempre se acompaña de un aumento de la DA urinaria y de la natriuresis. En este sentido, sigue siendo discutida la importancia relativa de cada una en el metabolismo y disponibilidad renal de DA. Además, también participan en la degradación de otras sustancias como noradrenalina y serotonina cuyos efectos renales son contrarios a los de DA. A nivel tubular, la DA inhibe el transporte transepitelial de Na^+ disminuyendo la actividad de los principales transportadores del ión a lo largo del nefrón. Uno de los transportadores que ha sido más estudiado acerca de su regulación por DA es la Na^+,K^+-ATPasa (NKA). La bomba de Na^+, como otros transportadores, es regulada por mecanismos de fosforilación/defosforilación. La fosforilación por PKA o PKC en sitios específicos de la subunidad catalítica alfa-1 produce una inhibición de la actividad de la NKA. La DA, luego de unirse a sus receptores, estimula por intermedio de diferentes mensajeros la activación de PKA o PKC y modifica el estado de fosforilación de la NKA, causando una disminución de la actividad de la bomba en los diferentes segmentos del nefrón. Si bien el efecto final de la DA sobre la actividad de la NKA es inhibitorio, el cambio en el estado de fosforilación de la bomba variará según se estudien segmentos proximales o asas de Henle. El Na^+ intracelular podría jugar un rol importante en estos cambios, así como las modificaciones en la fosforilación inducidas por sustancias con efectos opuestos a la DA como las alfa adrenérgicas.

Así el sistema Dopaminérgico renal es un importante y complejo regulador de la excreción hidroelectrolítica.

CEREBRAL NETWORK UNDERLYING SODIUM BALANCE REGULATION: ROLE OF THE OXITOCINERGIC AND SEROTONINERGIC SYSTEMS

L. VIVAS[1], A. GODINO[1], L. MARGATHO[2], L. FRANCHINI[1] AND J. ANTUNES RODRIGUES[2]

[1]Instituto de Investigación Médica M. y M. Ferreyra, Córdoba, Argentina. [2]Dpto de Fisiología, Facultad de Medicina, Riberao Preto, San Pablo, Brasil.

Forebrain and hindbrain excitatory and inhibitory systems work together in the process of restoring sodium balance. Our recent morphofunctional studies provide evidence indicating that specific neuronal groups of the lamina terminalis, hindbrain and particular subdivisions of the paraventricular nucleus of the hypothalamus (PVN), supraoptic nucleus (SON) and Extended Amygdala (ExA) complex are activated after induced sodium consumption. In these experiments Fos immunoreactivity (ir) was used to map the neuronal groups activated after sodium ingestion induced by peritoneal dialysis (PD) in rats. Oxytocin (OT) and serotonin (5HT)-ir in combination with Fos-

ir was also analyzed to evaluate whether the OT neurons of the PVN and 5HT cells within the raphé system (RS) might be involved in the inhibitory circuit sub-serving sodium homeostasis. Sodium ingestion stimulated by PD produced Fos-ir within defined cells groups of the nucleus of the solitary tract (NTS), lateral parabrachial nucleus (LPBN), the lamina terminalis nuclei and the central ExA division. Also, particular parvocellular and magnocellular OT subdivisions of the PVN and SON were double labeling after PD-induced sodium consumption. Within different nuclei of the RS the number of Fos-ir cells was increased after spontaneous and induced sodium ingestion, and the double-labeled cells (Fos-5HT-ir) of peritoneal dialyzed animals without access to the 2% NaCl solution was significantly lower compared with the animals in near normal sodium balance, or in the process of restoring sodium balance by consuming NaCl. Our results are consistent with the idea that there is a tonic inhibition of sodium appetite by serotonergic cells of the RS. Such an inhibitory influence would probably be reduced in the state of sodium deficiency and increased when the animals ingest NaCl and restore body sodium balance. This system is therefore likely to prevent excess NaCl and/or water consumption and reduce the possibility of excess extracellular volume expansion. Additionally, in light of these findings and previous studies demonstrating that 5HT agonists and antagonists injected into the LPBN attenuate or enhance induced sodium ingestion respectively, it seems reasonable to postulate the presence of 5HT pathways with cells bodies in the RS that project to the LPBN as well as forebrain structures to release 5HT and exert a modulation of NaCl intake. Our recent experiments to explore this working hypothesis indicate the existence of direct connections between the LPBN and neuronal groups of the RS, the lamina terminalis nuclei, ExA, PVN and NTS, activated after induced ingestion. In summary, our results reveal OT and 5HT neuronal population aggregates along the brain activated after sodium ingestion, suggesting they may constitute a cerebral circuit involved in the reestablishment of sodium balance regulating sodium intake and excretion. Supported by ANPCyT, CONICET and CnPQ.