Original article

MOLECULAR METABOLISM

Mechanisms underlying prorenin actions on hypothalamic neurons implicated in cardiometabolic control

Soledad Pitra [1], Yumei Feng [2], Javier E. Stern [1,*]

ABSTRACT

Background: Hypertension and obesity are highly interrelated diseases, being critical components of the metabolic syndrome. Despite the growing prevalence of this syndrome in the world population, efficient therapies are still missing. Thus, identification of novel targets and therapies are warranted. An enhanced activity of the hypothalamic renin-angiotensin system (RAS), including the recently discovered prorenin (PR) and its receptor (PRR), has been implicated as a common mechanism underlying aberrant sympatho-humoral activation that contributes to both metabolic and cardiovascular dysregulation in the metabolic syndrome. Still, the identification of precise neuronal targets, cellular mechanisms and signaling pathways underlying PR/PRR actions in cardiovascular- and metabolic related hypothalamic nuclei remain unknown.
Methods and results: Using a multidisciplinary approach including patch-clamp electrophysiology, live calcium imaging and immunohistochemistry, we aimed to elucidate cellular mechanisms underlying PR/PRR actions within the hypothalamic supraoptic (SON) and paraventricular nucleus (PVN), key brain areas previously involved in cardiometabolic regulation. We show for the first time that PRR is expressed in magnocellular neurosecretory cells (MNCs), and to a lesser extent, in presympathetic PVN neurons (PVN_{PS}). Moreover, we show that while PRR activation efficiently stimulates the firing activity of both MNCs and PVN_{PS} neurons, these effects involved AngII-independent and AngII-dependent mechanisms, respectively. In both cases however, PR excitatory effects involved an increase in intracellular Ca^{2+} levels and a Ca^{2+}-dependent inhibition of a voltage-gated K^{+} current.
Conclusions: We identified novel neuronal targets and cellular mechanisms underlying PR/PRR actions in critical hypothalamic neurons involved in cardiometabolic regulation. This fundamental mechanistic information regarding central PR/PRR actions is essential for the development of novel RAS-based therapeutic targets for the treatment of cardiometabolic disorders in obesity and hypertension.

Keywords Prorenin receptor; Sympathetic; PVN; SON; Angiotensin; Potassium

1. INTRODUCTION

It is well-established that the brain renin-angiotensin system plays a critical role in hydromineral and cardiovascular regulation, and that an elevated central RAS contributes to cardiovascular diseases, particularly neurogenic hypertension [1–3]. In addition to its canonical role in cardiovascular function, the RAS has recently emerged as a critical mediator of the hypothalamic control of body weight and metabolic functions [4,5]. An increase RAS activity, resulting in elevated levels of AngII and AngII type 1a receptors (AT1a) in the brain, has been reported both in obese humans and animal models [4,6–8]. Moreover, increasing central RAS activity, either genetically or pharmacologically, decreased body weight by inhibiting food intake and elevating energy expenditure [9–12].
Hypertension and obesity are highly interrelated diseases, being critical components of the metabolic syndrome. Diet-induced obesity in humans and rodents is associated with increased prevalence of hypertension [13–15], and several studies implicate an altered central RAS in obesity-induced hypertension [16–18]. Furthermore, neurogenic forms of hypertension caused by an elevated brain RAS activity display several metabolic disturbances [5]. Importantly, a mechanistic interaction between energy balance-related signals (e.g., leptin) and the central RAS in the functional regulation of sympathetic nerve activity has been reported both in health and disease conditions [19,20]. Finally, AngII is a pro-inflammatory factor [21], and inflammation within the hypothalamus has been associated both with hypertension and obesity [16,22–25]. Collectively, these results suggest that an altered brain RAS activity could be a common mechanism underlying both cardiovascular and energy balance changes in the metabolic syndrome.
Neurons within the paraventricular nucleus of the hypothalamus (PVN) are well suited to mediate the effects of the central RAS on cardiovascular and metabolic regulation. Parvocellular presympathetic neurons, via projections to the brainstem and spinal cord [26], influence

[1]Department of Physiology, Medical College of Georgia, Augusta University, United States [2]Departments of Pharmacology, Physiology and Cell Biology, Center for Cardiovascular Research, University of Nevada School of Medicine, United States

*Corresponding author. Department of Physiology, Medical College of Georgia, Augusta University, 1120 15th Street, CA-3137, Augusta, GA 30912, United States.
E-mail: jstern@augusta.edu (J.E. Stern).

Received July 13, 2016 • Revision received July 26, 2016 • Accepted July 27, 2016 • Available online 4 August 2016

http://dx.doi.org/10.1016/j.molmet.2016.07.010

food intake, energy expenditure and blood pressure [27–29]. Magnocellular neurosecretory cells (MNCs) that project to the posterior pituitary (also present in the supraoptic nucleus, SON) [26], release oxytocin and vasopressin onto the circulation. These hormones, in addition to their well-established effects on fluid and electrolyte homeostasis, also influence metabolic function and food intake [30–34]. All the components of the RAS needed for the local generation of angiotensin peptides are present within the SON and PVN [35], including two of the most novel players, namely prorenin (PR) and its receptor (PRR) [36–38]. Binding of PR to PRR stimulates the catalytic activity of the receptor, converting angiotensinogen (AGT) to angiotensin I and II [36,38]. In addition, interaction of PR with PRR initiates intracellular signaling pathways, including mitogen-activated protein kinases (MAPK) and extracellular signal-regulated kinases 1 and 2 (ERK1/2) [39–41]. Thus, the PR/PRR complex can mediate both AngII-dependent and independent effects.

An altered RAS activity within the SON/PVN leads to aberrant sympatho-humoral outflows characteristic of both hypertension and the metabolic syndrome. For example, an increased PRR expression was reported in the SON and PVN in hypertensive mice and rats [42,43], while brain-targeted PRR knockdown decreased blood pressure, sympathetic tone and plasma VP levels in these rodents [38,42]. Importantly, both AngII-dependent [44] and independent [43], mechanisms where shown to mediate effects of PRR in these nuclei. Furthermore, deletion of AT1a receptors in the PVN of high-fat diet obese mice increased food intake, decreased energy expenditure and decreased systolic blood pressure [45], supporting a key role for AT1a receptors in the PVN in the regulation of cardiometabolic function during obesity. While PR and PRR in adipose tissue have been recently implicated in the development of obesity and obesity-induced hypertension [46,47], their contribution to hypothalamic control metabolic function, both in health and disease is much less understood.

Despite all this evidence, the identification of precise neuronal targets, cellular mechanisms and signaling pathways underlying PR-mediated sympatho-humoral activation within cardiovascular and metabolic related brain centers remain unknown. This fundamental mechanistic information regarding central PR/PRR actions is critical before this signaling unit can become an efficient and novel therapeutic target for the treatment of cardiometabolic disorders.

The degree of sympathetic and hormonal outputs from the SON/PVN is dependent on neuronal activity in these nuclei, which is in turn determined by the combined actions of intrinsic (ion channels) and extrinsic (neurotransmitters) factors. Thus, it is reasonably to speculate that PR/PRR actions within the SON/PVN to stimulate sympatho-humoral activation are mediated by increasing membrane excitability and evoking firing activity in these neuronal populations. Surprisingly however, there have been no studies in the literature thus far that investigated this, nor explored the precise underlying cellular mechanisms by which PR mediates neurohumoral activation within the brain. Here, we used a multidisciplinary experimental approach including patch-clamp electrophysiology, live confocal calcium imaging and immunohistochemistry to elucidate cellular mechanisms underlying PR/PRR actions within the SON/PVN.

2. METHODS

2.1. Ethical approval

All procedures were performed in agreement with guidelines of the Augusta University Institutional Animal Care and Use Committee and were approved by the committee. Male heterozygous transgenic eGFP-VP Wistar rats (4–6 weeks old) were used [48]. Rats were housed in rooms with constant temperature of 22–24 °C and under a controlled light/dark cycle (12 h: 12 h), with normal rat chow and drinking water ad libitum.

2.2. Retrograde tracing

To identify presympathetic PVN neurons for patch-clamp or immunohistochemistry, rhodamine-labeled microspheres (Lumaflor) or cholera toxin B (CTB; 1%; List Biological Laboratories, 400 nl), respectively, were microinjected into the RVLM, a major brainstem sympathetic center. Coordinates used starting from bregma: 12 mm caudal along the lamina, 2 mm medial lateral, and 8 mm ventral as previously described [49]. The location of the tracer was verified histologically. Animals were used for electrophysiological or immunohistochemical studies 3–4 days after surgery.

2.3. Slice preparation

Hypothalamic brain slices were prepared according to methods previously described [50,51]. Briefly, rats were anesthetized with pentobarbital (50 mg/kg ip); brains dissected out and hypothalamic coronal slices (240 μm) containing the SON/PVN were cut in an oxygenated ice-cold artificial cerebrospinal fluid (aCSF), containing in mM: 119 NaCl, 2.5 KCl, 1 $MgSO_4$, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 20 D-glucose, 0.4 ascorbic acid, 2 $CaCl_2$, and 2 pyruvic acid; pH 7.3; 295 mOsm. When indicated a 0 Ca^{2+} ACSF (in which Ca^{2+} was replaced by Mg^{2+}, and EGTA 2 mM was added) was used. Slices were placed in a holding chamber containing aCSF and kept at room temperature until used.

2.4. Electrophysiology

Hypothalamic slices were transferred to a recording chamber and superfused with continuously bubbled (95% O_2–5% CO_2) aCSF (30 °C–32 °C) at a flow rate of ~3.0 ml/min. Thin-walled (1.5-mm OD, 1.17-mm ID) borosilicate glass (G150TF-3; Warner Instruments, Sarasota, FL) was used to pull patch pipettes (3–5 MΩ) on a horizontal micropipette puller (P-97; Sutter Instruments, Novato, CA). The internal solution contained the following (in mM): 135 potassium gluconate, 0.2 EGTA, 10 HEPES, 10 KCl, 0.9 $MgCl_2$, 4 Mg^{2+}ATP, 0.3 Na^{+}GTP and 20 phosphocreatine (Na^{+}); pH was adjusted to 7.2–7.3 with KOH. When indicated, a Cs+ -based internal solution (in mM: 135 Cs MS, 0.2 EGTA, 10 HEPES, 10 TEACl, 0.9 MgCl) was used. Recordings were obtained from fluorescently labeled PVN_{PS} neurons and from eGFP-VP neurons with an Axopatch 200B amplifier (Axon Instruments, Foster City, CA), using a combination of fluorescence illumination and infrared differential interference contrast (DIC) videomicroscopy. Recordings of eGFP-VP neurons in the PVN were restricted to the core of the lateral magnocellular (LM) subnuclei, which contains only magnocellular VP neurons [26] and were further characterized as magnocellular neurons electrophysiologically, based on the presence of a transient outward rectification (not shown), a membrane property expressed in magnocellular but not parvocellular SON/PVN neurons. The voltage output was digitized at 16-bit resolution, 10 kHz and was filtered at 2 kHz (Digidata 1440A; Axon Instruments). In voltage-clamp mode, ramp-evoked currents were leaked-substracted. Data were discarded if the series resistance was not stable throughout the entire recording (>20% change) [50,51]. Mouse prorenin (2.5 nM, Anaspec) was pressure applied through a picospritzer pipette (5 s). Focal application of ACSF, used as a control for a potential mechanical effect on the recorded neuron, failed to evoke a change in any of the parameters measured (not shown). All drugs, with the exception of Losartan (LKT Laboratories) and the PRR antagonist PRO20 (generated in the laboratory of Dr. Feng, UNR) [52], were purchased from Sigma–Aldrich. Mean firing activity and membrane potential values were calculated

