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Vasopressin V1a receptors are present in the carotid body and contribute to the control of breathing in male Sprague-Dawley rats

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ABSTRACT

Vasopressin (AVP) maintains body homeostasis by regulating water balance, cardiovascular system and stress response. AVP inhibits breathing through central vasopressin 1a receptors (V1aRs). Chemoreceptors within carotid bodies (CBs) detect chemical and hormonal signals in the bloodstream and provide sensory input to respiratory and cardiovascular centers of the brainstem. In the study we investigated if CBs contain V1aRs and how the receptors are involved in the regulation of ventilation by AVP.

We first immunostained CBs for V1aRs and tyrosine hydroxylase, a marker of chemoreceptor type I (glomus) cells. In urethane-anesthetized adult Sprague-Dawley male rats, we then measured hemodynamic and respiratory responses to systemic (intravenous) or local (carotid artery) administration of AVP prior and after systemic blockade of V1aRs. Immunostaining of CBs showed colocalization of V1aRs and tyrosine hydroxylase within glomus cells. Systemic administration of AVP increased mean arterial blood pressure (MABP) and decreased respiratory rate (RR) and minute ventilation (MV). Local administration of AVP increased MV and RR without significant changes in MABP or heart rate. Pretreatment with V1aR antagonist abolished responses to local and intravenous AVP administration.

Our findings show that chemosensory cells within CBs express V1aRs and that local stimulation of the CB with AVP increases ventilation, which is contrary to systemic effects of AVP manifested by decreased ventilation. The responses are mediated by V1aRs, as blockade of the receptors prevents changes in ventilation. We hypothesize that excitatory effects of AVP within the CB provide a counterbalancing mechanism for the inhibitory effects of systemically acting AVP on the respiration.

1. Introduction

Vasopressin (AVP) plays a fundamental role in maintaining body homeostasis by regulating water balance, cardiovascular system and stress response [1–3]. The plasma concentration of AVP depends on its synthesis in the hypothalamic paraventricular and supraoptic nuclei with subsequent release from the pituitary gland [1]. The most typical stimulus for release of AVP from the pituitary gland is increased plasma osmolarity. However, high levels of plasma AVP are present under conditions of homeostatic disturbances, such as hypovolemia, hypotonia, myocardial infarction, heart failure, brain trauma, hypoxemia or severe pneumonia [1]. All these disturbances are often accompanied by tachypnea and increased ventilation [4–9].

Chemoreceptors of the carotid bodies (CBs) detect numerous chemical and hormonal signals in the arterial bloodstream to the central nervous system (CNS) and provide the key sensory input to the respiratory and cardiovascular centers of the brainstem [10]. They are stimulated by low oxygen pressure, increased acidity, high carbon dioxide pressure, hypoperfusion and hypoglycemia [11]. Furthermore, there are numerous transmitters and mediators, which affect and modulate their activity, including dopamine, norepinephrine, acetylcholine, adenosine, ATP, angiotensin II (Ang II), proinflammatory cytokines, progesterone and glucocorticoids [12–15]. Activation of the CBs triggers arterial chemoreflex, which consists of cardiovascular and respiratory effects leading to sympathoexcitation, increase in ventilation and arousal [11,16]. Such coordinated response helps in adjusting

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the cardiovascular system to disturbances in body homeostasis and providing adequate blood flow and oxygen supply to the CNS.

It is well established both experimentally and clinically that hypovolemia and hypotension stimulate breathing and may lead to hyperventilation [6,8,17,18]. On the other hand, hypovolemia and hypotension are among the strongest stimuli for AVP release [1]. In this line, low oxygen levels lead to the increase in hypophyseal blood flow and release of AVP, which is dependent on peripheral chemoreceptors [19–22]. Furthermore, AVP released under hypoxic conditions prevents fall in the blood pressure due to hypoxic vasodilation by acting on vascular vasopressin 1a receptors (V1aRs) [23].

Accumulating body of evidence suggests that centrally acting AVP has inhibitory effects on breathing, which are mediated by V1aRs in the ventral lateral medulla of the brainstem [24] and in the area postrema (AP) [25]. Moreover, peripherally administered AVP was also shown to inhibit ventilation in rats [17,23], and the effect was abolished by pretreatment with V1aR antagonist [23]. However, there is also evidence that vasopressinergic neurons originating in the hypothalamic paraventricular nucleus project to the pre-Bozinger complex and the rostral ventrolateral medulla, where they have excitatory effects on phrenic nerve and sympathetic activity, which are mediated by V1aRs [26,27]. AVP, peripherally released or administered intravenously, may affect respiratory centers of the brain by interacting with the circumventricular organs, such as the AP, which are devoid of the blood-brain barrier and allow for cross-talk between the CNS and peripheral blood [25,28]. Alternatively, it may act on the CBs, which provide the key sensory input to the respiratory centers of the brain. However, to the best of our knowledge, the effect of AVP on CBs activity has not been determined yet. Therefore, the present study was aimed to clarify if V1aRs are present in the CB and to evaluate the effects of direct vasopressin application to CB on respiration and blood pressure.

2. Methods

2.1. Animals

The experiments were performed on normotensive adult Sprague-Dawley male rats, weighing between 300 and 380 g. The rats were obtained from the breeding facility at the Medical University of Warsaw. Before the experiments, animals were housed 3 per cage with the 12:12 h light/dark cycle and with access to a standard rat pellet diet and tap water *ad libitum*. All surgical procedures and recordings were performed under terminal anesthesia with urethane (1.5 g/kg intraperitoneally, Sigma-Aldrich, Europe).

The study was carried out according to domestic regulations and the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. The experimental protocol was approved by the Local Ethics Committee for Animal Experimentation at the Medical University of Warsaw.

2.2. Immunostaining

Rats were transcardially perfused with 200 mL of ice-cold heparinized PBS solution (50 IU/100 mL), followed by 200 mL of ice-cold 4% PFA solution. The carotid bifurcations were carefully dissected and placed in 4% PFA overnight. This was followed by immersion of tissue samples in increasing concentrations of sucrose solutions (10–20–30 weight/volume percentage) for cryopreservation. The samples were stored at −20 °C till sectioning. The sections were cut on a cryostat (Leica CM1850, Leica, Germany) at 20 μm and placed on gelatin coated slides for immunostaining. To confirm localization of V1aRs in the chemoreceptor cells of the CB, sections were co-stained using either primary rabbit polyclonal antibody against intracellular C-terminus part of V1aR (1:200; Cat # AVR-010, Alomone Labs, Jerusalem, Israel) together with mouse primary antibody against tyrosine hydroxylase (1:200; Cat # MAB318, Merck Millipore, Darmstadt, Germany), or extracellular N-terminus part of V1aR (1:200; Cat # AVP1A12-A, Alpha Diagnostic International Inc., San Antonio, TX, USA) together with the mouse primary antibody against tyrosine hydroxylase. This was followed by incubation with the goat anti-rabbit secondary antibody (1:200; Cat # A-11034, Alexa Fluor 488, Invitrogen, Thermo Fisher Scientific, Waltham, MA USA) and the goat anti-mouse secondary antibody (1:200; Cat # A-11030, Alexa Fluor 546, Invitrogen, Thermo Fisher Scientific, Waltham, MA USA). Cell nuclei were stained with Hoechst 33342 (1 μg/ml; Sigma-Aldrich, Europe). To confirm that non-specific binding of secondary antibodies to the tissue sections was absent, negative controls were done along the above staining protocol except for omitting the primary antibodies during the first incubation. In addition, absorption test was done for the rabbit antibody against intracellular C-terminus part of V1aR. For this purpose, before the staining the antibody was incubated overnight with a specific peptide fragment of V1aR (0.8 μg/μl peptide per 0.85 μg/μl antibody in 1:200 dilution). The peptide was provided by the Alomone Labs. All the incubations with primary and secondary antibodies were done at 4 °C overnight. Sections were covered with Fluorescence Mounting Medium (Dako Denmark A/S, Glostrup, Denmark). After staining, the samples were visualized with a laser scanning confocal microscope (Zeiss LSM 710, Carl Zeiss, Oberkochen, Germany) with ZEN Black software.

2.3. Surgical procedures

2.3.1. Venous and arterial catheters

For recording of mean arterial blood pressure (MABP) and heart rate (HR) intravascular polyurethane catheter (Cat # BB520-25 and BB520-40; Scientific Commodities, Inc., Lake Havasu City, AZ, USA) was implanted into the aorta below the branching of the renal arteries via the femoral artery. Another polyurethane catheter for intravenous infusions was inserted into the vena cava inferior via the femoral vein. Catheters were filled with heparinized sterile saline (100 IU/ml; WZF Polfa S.A., Poland). The procedure is described in details elsewhere [29].

2.3.2. Intracarotid catheter

In a subset of animals, a skin incision was made in the sagittal plane from the mandibula to the sternum and the common carotid artery (CCA) and the carotid bifurcation on the left side were exposed. The external carotid artery (ECA) was carefully isolated and ligated with a suture, 2 mm distal from the carotid bifurcation. The CCA was closed at the cardiac side and then cannulated with a fine polyurethane catheter connected to the microsyringe. In order to prevent back-flow of blood into the catheter's lumen, the catheter was closed with blunt occluder till the intracarotid infusion started. The catheter was filled with AVP and its volume was pre-determined at 50 μL. Applied procedure prevented the flow of investigated substances into the cerebral circulation and prolonged their local effect at the CB (Fig. 1A).

2.3.3. Tracheal tube

After implantation of catheters, the trachea was exposed rostrally from the sternum and a 1.5 mm cut was performed with a sharp blade. A polyethylene tubing (internal diameter – 2 mm; external diameter – 2.5 mm; length – 15 mm) was inserted through the cut and secured to the trachea with a suture. Then, the tracheal tube was tightly connected to the flow head dedicated to small laboratory animals (RX237B, Biopac Systems, Goleta, CA, USA) for recording of the airflow. The length of the extratracheal part of the tube and the flow head amounted to ca. 30 mm, which was comparable to the anatomical length of the upper airways, thus any increase in the respiratory dead space was prevented.

2.4. Hemodynamic and ventilatory measurements

The arterial catheter was connected to the blood pressure transducer and the tracheal tube was attached to the flow head and