# Streszczenie w języku angielskim

**Title:** The impact of vasopressin and V1a receptor on cardiorespiratory response in normotensive and hypertensive rats.

**Introduction:** Vasopressin (AVP), also referred to as an antidiuretic hormone, in addition to its renal effects related to the maintenance of water-electrolyte balance, is an important neurohormone involved in adaptation to disturbances in body homeostasis. The arterial chemoreflex is a key cardiovascular reflex associated with the circulatory and respiratory response to hypoxia. The most important group of arterial chemoreceptors are located in the carotid bodies which are found in the bifurcation of the common carotid artery. Hypertension is one of the major risk factors associated with death from cardiovascular related causes. Most encountered is primary hypertension with a multifactorial and incompletely understood origin. Hypertension has been shown to be associated with an increased peripheral chemoreflex, as well as alteration in the regulation of the vasopressinergic system. A common animal model of primary hypertension used in preclinical studies are the SHR rats (*Spontaneously Hypertensive*) with genetically determined hypertension, for which normotensive Wistar-Kyoto (WKY) rats are the most used control.

**Aim:** The aim of the study was to assess what is the contribution of vasopressin, its type 1a receptor (V1a) and carotid body in cardiopulmonary regulation under conditions of primary hypertension. The specific objectives of the study included answering the following research questions: (1) Are cardiovascular and respiratory responses to pharmacological induction of the arterial chemoreflex enhanced in hypertensive SHR rats compared to normotensive WKY rats? (2) Are carotid bodies and tonic arterial chemoreflex activity involved in maintaining resting circulatory and respiratory parameters in SHR rats and WKY rats? (3) Do circulatory and respiratory responses to peripherally administered AVP differ between hypertensive SHR rats and normotensive WKY rats? (4) Do circulatory and respiratory responses to AVP administered to close to the carotid body differ between hypertensive SHR rats and normotensive WKY rats? (5) Are carotid bodies involved in the circulatory and respiratory responses to peripherally administered AVP in SHR rats and WKY rats? (6) Is the vasopressin V1a receptor involved in maintaining resting circulatory and respiratory parameters in SHR rats and WKY rats? (7) Do the circulatory and respiratory responses to peripheral and local administration of AVP into the carotid body depend on the V1a receptor in SHR rats and WKY rats? (8) Are V1a receptors present on chemosensitive (type I) cells of the carotid body in SHR rats and WKY rats?

**Materials and methods:** The experiments were conducted in accordance with national regulations and Council Directive 2010/63/EU of September 22, 2010 on the protection of animals used for scientific purposes, after approval by the 2nd Local Ethical Committee for Animal Experiments at the Warsaw University of Life Sciences [approval number WAW2/096/2019]. The study was conducted on adult male hypertensive SHR rats and control normotensive WKY rats, aged 12- 14 weeks. The following number of animals were used in the experiments: n = 36 SHR rats and n = 36 WKY rats. In the first part of the study, blood pressure measurements were made on the tail artery using a non-invasive method in the awake animals. In the second part of the experiments, cardiorespiratory variables were measured in urethane-anesthetized animals, in which haemodynamic parameters (mean arterial pressure - MABP, heart rate - HR, femoral artery flow - FABF) and respiratory parameters (minute ventilation - MV, respiratory rate - RR and end-expiratory carbon dioxide concentration - ETCO2) were recorded. For measurements, rats were implanted with arterial and femoral vein catheters for hemodynamic measurements and intravenous administration, respectively. A Doppler probe was also placed on the femoral artery to assess peripheral flow. A tracheostomy was then performed with the insertion of a tracheal tube and the attachment of a capnograph probe to record respiratory measurements. In some animals, catheter was implanted into external carotid artery to allow local administration of AVP to the carotid region. In selected rats, bilateral denervation of the carotid body or a sham procedure was also performed. The animals were divided into the following 6 experimental series: (1) trigger of arterial chemoreceptor reflex by intravenous administration of potassium cyanide (KCN) (30 μg/100 μL); (2) trigger of arterial chemoreceptor reflex by intravenous administration of KCN (30 μg/100 μL) after carotid body denervation; (3) intravenous administration of AVP (10 ng/100 μL) after sham carotid body denervation; (4) intravenous administration of AVP (10 ng/100 μL) after denervation of carotid body; (5) intravenous administration of the selective V1a receptor antagonist (d(CH2)51,Tyr(Me)2,Arg8) Vasopressin (5 μg/100 μL) and intravenous administration of AVP (10 ng/100 μL); (6) intra-arterial administration of AVP (4 ng/50 μL), before and after intravenous administration of the selective V1a receptor antagonist (d(CH2)51,Tyr(Me)2,Arg8) Vasopressin (5 μg/100 μL). After cardiorespiratory measurements, the animals were euthanized. Carotid artery bifurcations with carotid bodies were harvested from rats and V1a receptor expression in chemoreceptor cells was assessed using immunofluorescence staining with primary antibodies against the V1a receptor and antibodies against tyrosine hydroxylase (a type I chemoreceptor cell marker). The preparations were then imaged by confocal microscopy.

**Results:** Hypertensive SHR rats had significantly higher resting systolic, diastolic and mean arterial blood pressure. Also, in anesthetized hypertensive animals, the values of mean arterial pressure and minute ventilation were significantly higher than in normotensive WKY rats. Circulatory and respiratory responses to arterial chemoreflex were also significantly greater in hypertensive animals than in normotensive controls. Bilateral carotid body denervation did not significantly alter haemodynamic and respiratory parameters in SHR and WKY rats, and it was also associated with an inhibition of the response to pharmacological trigger of the peripheral chemoreflex in both groups. The pressor response to intravenous administration of AVP occurred in both strains but was significantly higher in SHR rats. Only in hypertensive rats there was a significant reduction in lung ventilation after vasopressin administration. Intravenous administration of a V1a receptor antagonist lowered blood pressure in both groups, and the decrease was significantly more marked in hypertensive rats. Blockade of the V1a receptor also led to an increase in lung ventilation, which was observed only in hypertensive animals. Bilateral carotid body denervation reduced the pressor response to vasopressin in normotensive WKY rats but had no significant effect on the vasopressin-dependent increase in pressure in SHR rats. In contrast, in hypertensive SHR rats, denervation of the carotid bodies abolished the inhibition of lung ventilation in response to vasopressin administration that was observed in animals without denervation. Administration of vasopressin to the carotid body region caused a slight increase in arterial pressure similar in both groups but led to a decrease in lung ventilation only in SHR rats. Blockade of the V1a receptor abolished the haemodynamic and respiratory responses to both intravenous and intraarterial administration of vasopressin. Immunofluorescence staining confirmed the presence of the V1a receptor for vasopressin on chemosensitive (type I) cells of the carotid bodies in both normotensive WKY and hypertensive SHR rats.

**Conclusions:**

1. The haemodynamic and respiratory responses to the arterial chemoreflex are increased in hypertensive SHR compared to WKY rats, confirming the increased sensitivity of the reflex under hypertensive conditions.
2. The arterial chemoreflex does not show tonic resting activity in anesthetized SHR and WKY rats.
3. The haemodynamic and respiratory responses to intravenous vasopressin are enhanced in hypertensive SHR compared to normotensive WKY rats, indicating increased activity of the vasopressinergic system under hypertensive conditions.
4. The pressor response to locally administered AVP into the carotid body region is similar in both hypertensive SHR rats and normotensive WKY rats, indicating that AVP- dependent pressor mechanisms are not enhanced at the carotid body level under hypertensive conditions.
5. The respiratory response to locally administered AVP into the carotid body region is present only in hypertensive SHR rats, indicating that AVP is involved in the regulation of the carotid body-dependent respiratory component under hypertensive conditions.
6. Bilateral carotid body denervation inhibits the pressor response to intravenously administered AVP only in normotensive WKY rats, indicating the involvement of the carotid body in cardiovascular regulation by AVP under normotensive conditions and strongly expressed pressor mechanisms independent of the carotid body in hypertensive animals.
7. Bilateral carotid body denervation inhibits the respiratory response to intravenously administered AVP in hypertensive SHR rats, indicating involvement of the carotid body in respiratory regulation by AVP under hypertensive conditions.
8. Cardiorespiratory responses to V1a receptor blockade are present only in hypertensive SHR rats, indicating the involvement of this receptor and AVP in maintaining resting values of haemodynamic and respiratory parameters under hypertensive conditions.
9. Circulatory and respiratory responses to AVP depend on the V1a receptor, as its  blockade prevented changes induced by intravenous (systemic) and intraarterial (local) administration of AVP.
10. The V1a receptors are found in chemosensitive (type I) cells in the carotid bodies of hypertensive SHR and normotensive WKY rats.

**Summary**: The results of the presented study provide new knowledge regarding the arterial chemoreflex, particularly for the peripheral chemoreceptors located in the carotid body, and the involvement of vasopressin in cardiorespiratory regulation in primary hypertension. The observed changes in circulatory and respiratory parameters after intravenous and local administration of vasopressin suggest that the hormone also affects cardiovascular and respiratory function through the carotid body, that this effect is more pronounced in hypertensive animals and depends on V1a receptors for vasopressin. These results indicate an increased involvement of the vasopressinergic system in cardiorespiratory regulation in hypertensive SHR rats, potentially providing a possibility of further research for new treatment methods for hypertension.