CHANGES IN ARTERIAL PRESSURE AFTER EXPOSURE TO INCREASING ANGIOTENSIN CONVERTING ENZYME ACTIVITY AND NORMALIZATION OF ACTIVITY WITH DIHYDROQUERCETIN IN MALE WISTAR RATS

Abstract:

Changes in blood pressure and heart rate were studied after exposures that increase the activity of angiotensin-converting enzyme: ionizing radiation, an NO-synthase inhibitor (L-NAME) and dexamethasone. We also determined the effect of dihydroquercetin and the angiotensin-converting enzyme inhibitor enalapril on the activity of this enzyme, blood pressure and heart rate during these exposures. Male Wistar rats were irradiated with X-rays at a dose of 2.5 Gy. Angiotensin-converting enzyme activity in aortic segments was determined by hydrolysis of Hip-His-Leu. BP and heart rate were recorded non-invasively using a tail cuff on a PowerLab 8/35 computer unit. With an increase in the activity of angiotensin-converting enzyme after irradiation, blood pressure and heart rate did not change. With prolonged action (7 days) of an NO synthase inhibitor and dexamethasone, an increase in enzyme activity was accompanied by an increase in blood pressure, and in the case of an inhibitor

NO-synthase - a decrease in heart rate. Dihydroquercetin normalized the activity of the enzyme and reduced blood pressure, but not to the normal level. Enalapril normalized blood pressure, increased against the background of consumption of an NO-synthase inhibitor solution, while the activity of angiotensin-converting enzyme decreased by more than 2 times from the norm. Key words: aorta, angiotensin-converting enzyme, arterial pressure, dihydroquercetin. Blood pressure is regulated by several systems: baroreceptors in the vessels; renin-angiotensin system, which regulates vascular tone, salt balance and blood volume; adrenergic system that regulates the frequency and degree of heart contractions and vascular tone; vasoreactive factors produced by vessels that cause both relaxation (NO) and vascular constriction (endothelin-1 and ROS). The main factor in the regulation of blood pressure in the renin-angiotensin system and essential in the system of vasoreactive factors is the activity of angiotensin-converting enzyme (ACE). ACE converts angiotensin I to vasopressor angiotensin II, destroys the vasodilator bradykinin. In addition, an increase in ACE activity activates NADH oxidase, which leads to an increase in ROS in the vessels. Previously, it was shown that when rats were exposed to the NO synthase inhibitor, the stress hormone dexamethasone, and ionizing radiation, ACE activity in the aorta increased, while the flavonoid dihydroquercetin (DHA) normalized it. The purpose of this work is to investigate the change in blood pressure and heart rate under these influences, as well as during the normalization of ACE DHA activity. RESEARCH METHOD. We used male Wistar rats (n=32) weighing 300-320 g at the age of 9-10 weeks (animal collection of the Institute of Theoretical and Experimental Biophysics, Pushchino). Rats were kept in cages with free access to water and standard rat chow (control group). In the experiments, ethical standards for working with laboratory animals were followed. The experimental groups consumed standard dry food and solutions of L-NAME (1 mg/ml; "Sigma"), DHA (1 and 3 ÿg/ml; "Sigma") or enalapril maleate (0.1 mg/ml; "Sigma"). Rats drank 100±5 ml of water or solutions per day per 1 kg of body weight, except for

L-NAME solution + enalapril: 85 ml/day per 1 kg of weight. With this consumption of solutions, rats received 100 mg of L-NAME, 100 and 300 ÿg of DHA per 1 kg of body weight per day; in the case of a solution of L-NAME and enalapril - 85 mg of L-NAME and 8.5 mg of enalapril per 1 kg of body weight per day. Rats were irradiated at room temperature with X-rays at a dose of 2.5 Gy at a dose rate of 1 Gy/min at the RUT-250-15-1 Institute of Cell Biophysics, Russian Academy of Sciences (20 mA, filters: 1 mm Al, 1 mm Cu). Dexamethasone ("KRKA") was administered subcutaneously once a day at a dose of 30 ÿg/kg. During the study, systolic blood pressure and heart rate were recorded in animals by a non-invasive method using a tail cuff on a Power Lab 8/35 computer unit (AD Instruments Pty Ltd). From 3 measurements, the average values of blood pressure and heart rate were determined. BP and heart rate were recorded 1 day before the start of the experiments to adapt the animals to the measurement procedure, immediately before exposure to irradiation or the agents mentioned above and 2.4 and 24 hours after irradiation or 1 week after the administration of dexamethasone and consumption of the test solutions. A significant individual variability of heart rate (from 350 to 430 beats/min) and blood pressure (from 90 to 120 mm Hg) was found, so the average values of these indicators in the formed groups varied: heart rate — from 355 to 400 beats/min, blood pressure — from 98 to 116 mmHg Art. depending on which animals fell into groups (table). The mean blood pressure in the groups differed, but was the same in the same group at the first and second measurements (table). These data indicate that BP is a stable characteristic of a group of animals, and when calculating the effects of exposure, it is necessary to use the average BP in this group, and not the average BP for all groups. The mean heart rate also varied in the groups, but, unlike blood pressure, it increased by an average of 3-4% at the first measurement compared to the second (table). Apparently, in some susceptible animals (65%), first-time manipulations cause an increase in heart rate. Given the heterogeneity of groups of animals in terms of heart rate and blood pressure, the effects of exposure were determined in relation to their average values in the group, determined immediately before the experiment (i.e., not in the first preliminary measurement). At the end of exposure, heart rate and blood pressure were measured, after which the chest was opened under ether anesthesia in rats, heparin (500 units) was injected into the heart to prevent blood clotting, and adjacent fat was removed from the aorta. Then the aorta was dissected at the base of the arch and a branch of the renal artery, washed with cold (4°C) Hank's solution with HEPES pH 7.4, and placed in the same solution. The time from the beginning of the operation to the removal of the aorta took about 3 minutes. The aorta was cut into segments 4-5 mm long, starting from the point where it becomes parallel to the spine. The aortic segments were numbered from 1 to 8, starting with the aortic segment closest to the arch. Parts of the aorta were cut lengthwise and tied with a synthetic thread to a plastic pipette tip with the endothelium outward. After measuring the ACE activity, the aortic segments were removed and the linear dimensions were measured with an accuracy of 0.1 mm to determine the area. Additionally used 6 rats to determine the activity of ACE in the aorta of control rats. ACE activity was determined by hydrolysis of Hip-His-Leu (Sigma) according to a modified method. Isolated aortic segments were placed in Hank's solution with HEPES pH 7.4 (450 ÿl) and incubated for 10 min at 37°C with shaking (25 Hz, 1 mm amplitude) for adaptation before adding the ACE substrate. The reaction was started by adding 10 mM Hip-His-Leu (50 ÿl). After 30 min incubation at 37°C, the reaction was stopped by adding 1000 µl

0.1 N NaOH. After stirring the reaction mixture, the aortic segments were removed from the solution and their sizes were determined. The remaining solution (200 ÿl) was incubated with 50 ÿl of o-phthalodialdehyde (20 mg/ml; Sigma) for 30 min at 37°C. The reaction was stopped by adding 2 ml of 0.8 N HCI. Samples were centrifuged for 8 min at 3000 g, 2°C, and fluorescence was measured using an MF44 fluorometer (Perkin-Elmer) at excitation and emission wavelengths of 360 and 500 nm, respectively. To determine ACE activity, a standard curve of fluorescence versus His-Leu concentration was used. ACE activity was expressed in pmol Hip-His-Leu, hydrolyzed in 1 min per 1 mm2 of the inner surface of the aorta. Statistical analysis was carried out in the program "Origin" using ANOVA. Results are presented as mean ± standard error of the mean. Differences were considered statistically significant at $p<0.05$. RESULTS OF THE RESEARCH. Data on the relative change in heart rate and blood pressure are shown in Figure 1. Previously, it was shown that ACE activity significantly increased (by 1.5 times) 2 hours after irradiation, and then gradually decreased and reached an almost control level after 2 days. Heart rate and blood pressure did not change at the same time (Fig. 1). Obviously, a significant, but short-term increase in ACE activity does not lead to changes in heart rate and blood pressure due to the compensatory reaction of baroreceptors in the vessels and the adrenergic system of blood pressure regulation. With prolonged action (7 days) of factors that increase ACE - dexamethasone and an NO synthase inhibitor - blood pressure also increased (Fig. 2.3). ACE activity in the aorta of control rats in these experiments was 20.8±0.9 pmol/min/mm2. Heart rate slightly and insignificantly decreased under the action of dexamethasone (Fig. 2), but significantly decreased (by 13%) under the action of L-NAME (Fig. 3). DHA at a dose of 100 µg/kg reduced ACE activity under the action of L-NAME almost to normal (Fig. 3), and DHA at a dose of 300 µg/kg normalized ACE activity under both treatment options (Fig. 2,3). At the same time, BP decreased, but not to the control level (Fig. 2.3), and heart rate remained reduced on the background of both doses of DHA (Fig. 3). Normalization of blood pressure and an increase in heart rate occurred only under the action of the ACE inhibitor enapapril, when ACE activity decreased by more than 2 times from the norm (Fig. 3). Heart rate is normally determined by the balance of activity between sympathetic and parasympathetic (vagus nerve) regulation of the heart: an increase in sympathetic activity - tachycardia, parasympathetic - bradycardia. With an increase in blood pressure in the aorta or carotid artery caused by the consumption of

L-NAME, pressoreceptors are irritated. The excitement that has arisen in them comes to the central nervous system and increases the excitability of the center of the vagus nerves, as a result of which the number of inhibitory impulses passing through them increases, which leads to a slowdown and weakening of heart contractions. Hypertension induced by an NO synthase inhibitor and dexamethasone is mainly due to the same factors: NO deficiency and an increase in the concentration of angiotensin II. The latter is the result of an increase in ACE activity (Fig. 2, 3). NO deficiency occurs for two reasons. Firstly, due to a decrease in the rate of its formation due to the suppression of the activity of all L-NAME NO synthases and only the inducible one by dexamethasone. Secondly, due to an increase in the rate of NO degradation due to an increase in ROS actively interacting with it, the number of which increases with ACE activation. It is known that NO has both a direct vasodilating effect on blood vessels and an indirect effect through the suppression of

activity of the sympathetic nervous system. A decrease in the concentration of NO in the body under the action of an NO synthase inhibitor and dexamethasone causes hypertension due to a decrease in the direct and mediated through the sympathetic innervation of the vessels of the vasodilatory effect of NO. In addition, an increase in ACE activity leads to an increase in vasopressor angiotensin II. DHA reduces the activity of ACE (decrease in angiotensin II) and normalizes the amount of ROS in the vessels, the latter leads to a decrease in NO degradation. As a result, the concentration of NO increases and blood pressure decreases, but does not normalize. Apparently, DHA does not restore the concentration of NO to normal. The ACE inhibitor enalapril normalizes blood pressure (Fig. 3). In addition to the obvious effect of ACE inhibitors (decrease in angiotensin II, increase in the concentration of the vasodilator bradykinin), they also

increase the concentration of kinins in the body and induce the expression of endothelial NO-synthase. Both effects contribute to lowering blood pressure. However, ACE activity under the action of enalapril decreases by more than 2 times compared with the norm (Fig. 3), which causes a corresponding decrease in the concentration of angiotensin II. Since angiotensin II is involved not only in the regulation of blood pressure, but also in many other processes in the body, such a sharp deviation in the concentration of angiotensin II from
norms can adversely affect these processes and manifest itself in various negative side effects of ACE inhibitors. Thus, with a short-term (within 1 day) increase in ACE activity in the aorta under the influence of ionizing radiation, blood pressure and heart rate do not change. With prolonged (1 week) exposure to agents (L-NAME, dexamethasone), an increase in ACE activity is accompanied by an increase in blood pressure. DHA at doses of 100 and 300 mcg/kg reduces blood pressure, increased by the action of these agents.

Authors:
Korystova A.F.
Kublik L.N.
Levitman M.Kh.
Shaposhnikova V.V.
Kim Yu.A. Slashcheva
G.A. Lobanov A.V.
Tukhovskaya E.A.
Korystov Yu.N.

Edition: Bulletin of Experimental Biology and Medicine **Year of publication:** 2018 **Volume:** 5 pages.

Additional information: 2018.-N 7.-S.36-40. Bible 13 titles **Views:** 4