Relationship between serum or tissue ouabain and blood pressure in 1k1c hypertensive rats

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Abstract：Objective To evaluate the role of endogenous ouabain(EO)In the development of hypertension and the characteristics of EO secretion in 1k1c(one kidney, one clipped) hypertensive rats. **Methods** EO content of serum and tissues in 1k1c hypertensive rats and normal control Sprague-Dawley (SD) rats was detected by the method of enzyme linked immunosorbent assay (ELISA). The relationship between serum or tissue ouabain and blood pressure was analyzed in 1k1c hypertensive rats. **Results** The ouabain content of serum, heart, kidney, adrenal gland, pituitary and hypothalamus was significantly higher in 1k1c hypertensive rats then that in normal control SD rats (2.25, 2.63, 3.35,40.37,3.34,15.7 μg/kg tissue in 1k1c hypertensive rats vs 1.12, 1.79, 1.73, 27.54, 1.83, 10.10 μg/kg tissue in control SD rats, respectively. $P<0.05$ for all of these comparisons). The ouabain content of the adrenal gland and the hypothalamus was higher than that of other tissues or serum, both in 1k1c rats and in control SD rats. The EO content of serum, kidney and hypothalamus was significantly correlated with blood pressure in 1k1c hypertensive rats ($r=0.59,0.63,0.52$, respectively. $P<0.05$). The ouabain content of heart, liver, adrenal gland and pituitary was not correlated with blood pressure. **Conclusions** EO might play an important role in the development of hypertension in 1k1c hypertensive rats. The adrenal gland may be a major source of EO and the hypothalamus-pituitary-adrenal axis may be involved in the regulation of EO secretion.

Endogenous ouabain (EO), previously called endogenous sodium pump inhibitor or endogenous digitalis-like substance, is a hormone secreted from the adrenal cortex. Sodium, blood volume expansion, and angiotensin II could stimulate EO secretion. And EO has been shown to have many physiological and pathological roles. A great deal of evidence has shown that high level of EO might be related to hypertension.[1-4] The change in serum and tissue EO content and its significance in the development of hypertension in 1k1c (one kidney, one clipped) rats has not been studied before, although this is one of the most important hypertensive models. We evaluated the EO content in 1k1c rats with a highly accurate and specific enzyme linked immunosorbent assay (ELISA) method and analyzed the relationships between EO levels in serum and tissues and blood pressure in order to reveal the role of EO in the development of hypertension as well as the characteristics of EO secretion in 1k1c hypertensive rats.

METHODS

Establishment of the 1k1c hypertensive model

Twenty-two healthy adult male Sprague-Dawley (SD) rats (5-8 weeks old) weighing 180-210g were purchased from the Experimental Animal Center of Xi'an Medical University. They were given free access to tap water and standard rat chow. After one week, body weight and systolic

blood pressure were assessed to document normotension. They were divided randomly into two groups: 1k1c group (n=14) and control group (n=8). 1k1c hypertensive rats were established as follows:[5] the left renal artery was clipped with a 0.3mm diameter "U-shaped" silver clip and the opposite kidney was removed after a week. Indirect systolic blood pressure was recorded with the tail cuff method twice a week, using the Heart Rate & Blood Pressure Recorder for Rats (Model MRB-IIIA, Shanghai Hypertension Institute, Shanghai, China). The rats of both groups were killed 4 weeks after nephrectomy of the 1k1c group and serum, heart, liver, kidney, adrenal gland, hypothalamus and pituitary were collected.

Ouabain content of serum and tissues detected using an ELISA method

At the end of study, the rats were fasted overnight and killed by decapitation on the following morning. Trunk blood was collected and centrifuged immediately. Ouabain was extracted from the serum sample by mixing serum with an equal volume of 0.1% distilled trifluoroacetic acid for three hours at room temperature followed by centrifugation (3000g for 30 min) to pellet any insoluble material. The supernatant was passed through a C-18 disposable Bond Elute columns (Analytichem International, Harbor City, California, USA). Unbound material was washed off the column using several volumes of water. Ouabain was subsequently eluted with 25% acetonitrile. The elutes were dried with a vacuum centrifuge and the extracts dissolved using phosphate buffered saline (PBS0.01 mol/L, pH7.4).[6]

Tissues were removed, and excess fat and connective tissue were removed. They were then rinsed in PBS, lightly blotted, weighed and homogenized using a polytron in 10vol methanol containing 2 mmol/L ascorbic acid. The homogenates were separated by centrifugation at 1500g for 25min, and the supernatant was dried by vacuum centrifugation and reconstituted in water containing 0.1% trifluoroacetic acid. Following centrifugation, ouabain was extracted from the supernatant using a C-18 column as described above.

The serum and tissue ouabain content was detected according to the method of ELISA that we established previously.[7]In brief, enzyme immunoassay plates were coated with ovalbumin-ouabain by adding 100μl of 1μg/ml ovalbumin-ouabain in coating buffer (10 mmol/L, pH9.6 carbonate-buffered saline) to each well. Plates were treated by storage for 18-24 hours at 4℃ before use. Unbound ovalbumin-ouabain was removed by washing each well three times with 250 μl rinse solution (10 mmol/L, pH7.4PBS containing 0.05% Tween-20). Unoccupied protein binding sites were blocked by adding 200μl blocking buffer (10 mmol/L, pH7.4 PBS containing 2% bovine serum albumin) to each well for 1 hour at 37℃. Before use, plates were washed three times with 250μl rinse solution per well. Standard ouabain (Sigma Chemical Co., USA) was diluted to 0.39, 0.78, 1.56, 6.25, 12.5, 25 and 50 μg/L, with deionized water. 50μl of samples or standards were added to successive wells. 50μl rabbits anti-ouabain antiserum (dilution of 1:12000) was added to each well. Plates were incubated at 37℃ for 2 hours with continuous shaking followed by four rinses of 250μl rinse solution per well. Anti-ouabain antibodies remaining bound to the ovalbumin-ouabain were reacted with 100μl per well of a 1:1000 dilution of goat anti-rabbit IgG-peroxidase conjugate (Sino-American Biotechnology Company, China) by incubating the plates for an additional 30 minutes at 37℃ with continuous shaking. Unbound anti-rabbit IgG-peroxidase conjugate was washed away by rinsing as described above. The presence of peroxidase enzyme remaining in each well was determined by the addition of 100μl per well of Ophenylenediamine (OPD) substrate solution. After 15 minutes at room temperature, the substrate reaction was terminated by the addition of 50μl of 2 mol/L H_2SO_4. The absorbance

of each well was measured at 490 nm using the ELISA Recorder (Model DG-3022A, Huadong Electron Tube Factory, Shanghai, China). The concentration of EO in each sample was calculated from the absorbance according to the ouabain standard curve.

Statistical analysis

Values were expressed as $\bar{x} \pm s$. Student's *t* test was performed and correlation coefficients were calculated by MS Excel 5.0 statistical software.

RESULTS

Changes of blood pressure of 1k1c rats after operation

The systolic blood pressure began to increase 1 week after nephrectomy as compared with preoperation [130.5±13.5mmHg (1 mmHg=0.133 kPa) vs 104.3±12.3mmHg, P<0.01]. The systolic blood pressure increased significantly 4 weeks after nephrectomy (164.7±16.9mmHg vs 104.3±12.3mmHg, P<0.001). However, the systolic blood pressure of control SD rats did not change significantly during the period of this study (109.3±10.3mmHg vs 102.8±9.6mmHg, P>0.05).

EO content of serum and tissues in 1k1c hypertensive and control rats

The EO content of serum, heart, kidney, adrenal gland, pituitary and hypothalamus in 1k1c hypertensive rats was significantly higher than that in control SD rats (2.25, 2.63, 3.35, 40.37, 3.34, 15.7 μg/kg tissue in 1k1c hypertensive rats vs 1.12, 1.79, 1.73, 27.54, 1.83, 10.10 μg/kg tissue in control SD rats, respectively. P<0.05 for all comparisons). The EO content of adrenal gland and hypothalamus was higher than that of other tissues and serum, both in 1k1c rats and control SD rats (Fig.1).

图 1 略

Correlation between serum or tissues EO content and blood pressure of 1k1c rats

The EO content of serum, kidney and hypothalamus in 1k1c rats was significantly correlated with systolic blood pressure (r=0.59, 0.63, 0.52, respectively. P<0.05, Figs. 2-4), while the EO content of the heart, liver, adrenal gland and pituitary was not correlated with systolic blood pressure.

图 2 略

图 3 略

图 4 略

DISCUSSION

The results of our study showed that the EO content of serum and tissues in 1k1c hypertensive rate was significantly higher than that in control SD rats, possibly related to sodium and water retention, renin-angiotensin system activation or increased sympathetic nervous system activation or increased sympathetic nervous system activity,[5] all of which have been reported to be related to increased EO secretion. Much evidence has shown that blood volume expansion could cause EO secretion to increase significantly and higher EO levels in the body might play an important role in the mechanism of blood pressure increase in hypertensive rats with sodium water storage and blood volume expansion, such as "DOCA-salt" and "partially nephrectomized" hypertensive rats. [2,4] Recently, Laredo[6] found that angiotensin II could stimulate the secretion of endogenous ouabain from cultured bovine adrenocortical cells. This provides direct evidence for the hypothesis that angiotensin II is a powerful factor that stimulates EO secretion. Yamada and his colleagues[8] reported that central sympathetic nerve injury could decrease the EO content

of the hypothalamus and serum 90% and 70%, respectively, indication that higher sympathetic nervous system activity might be one of the causes of increasing EO secretion.

In this study, we found that the EO content of the adrenal gland was significantly higher than that of other tissues, suggesting that the adrenal gland might be a major source of EO. Interestingly, other groups observed that human or bovine cultured adrenocortical cells could secrete EO.[6] Recently, Schneider and his colleagues[9] successfully separated ouabain directly from bovine adrenal gland tissue and this direct evidence supports the notion that EO content of the hypothalamus and the adrenal gland is higher than other tissues, both in 1k1c rats and in control SD rats. From this,[4] we infer that the hypothalamus-pituitary-adrenal axis might be involved in the regulation of EO secretion, but we acknowledge that further evidence is needed.

We previously reported that ouabain antibody could decrease the blood pressure of 1k1c hypertensive rats in a dose-dependent manner.[10] In this study we found that the EO content in 1k1c rats was significantly higher than that in control SD rats and that EO levels in serum, hypothalamus and kidney were significantly correlated with systolic blood pressure. This suggests that higher EO levels might play an important role in the pathogenesis of hypertension and may participate in the development of hypertension. Ouabain may increase blood pressure as a result of the inhibition of activity of the sodium pump in heart muscle, the smooth muscle of vessels, and sympathetic nerves.[1-4] Inhibition of the sodium pump could increase intracellular Na^+ level and result in the development of hypertension in many ways:[1-4] more intracellular sodium may result in Na^+-Ca^{2+} change to a greater extent with subsequent increase of Ca^{2+} concentration in cells, thus directly causing vascular contraction; increasing the activity of sympathetic nervous system; sensitizing the resistant vasculature to other endogenous vasopressors and decreasing the reactive ability of the vasculature to some relaxation factors of the vessels; altering the effects of the “renin-angiotensin-aldosterone system”; enhancing collagen secretion and promoting hypertrophy and hyperplasia in arterial smooth muscle cells, thereby increasing the peripheral vascular resistance.

In summary, our results suggest that EO might play an import role in the development of hypertension in 1k1c hypertensive rats. The EO content of hypothalamus, pituitary, adrenal gland, kidney and heart in 1k1c rats was significantly higher than those in control SD rats, indicating that these tissues might be involved in the regulation of EO secretion or the mechanism of ouabain induced hypertension. Since the mechanism of blood pressure increase in 1k1c rats is similar to human hypertension, this study might be helpful in further exploring the role of EO in human hypertension.

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