Effects of 1-Deamino-1-Monocarb-Arginine-Vasotocin on Sodium Ion and Water Excretion by Rat Kidneys

T. A. Kanashkina, E. I. Shakhmatova, and Yu. V. Natochin

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Injection of 1-deamino-1-monocarb-arginine-vasotocin (0.05 μg/100 g body weight) to alert rats caused increased natriuresis, diuresis, and reabsorption of osmotically free water. Injection of furosemide (0.5 mg/100 g body weight) increased Na^+ excretion and did not increase reabsorption of osmotically free water. 1-Deamino-1-monocarb-arginine-vasotocin in molar conversion increased natriuresis by almost 6×10^4 times more effectively than furosemide.

Key Words: *rat; kidney; 1-deamino-1-monocarb-arginine-vasotocin; natriuresis; furosemide*

We previously showed in rat experiments that arginine-vasotocin (AVT; neurohypophyseal hormone in the majority of vertebrates) is characterized by high natriuretic activity [1], while arginine-vasopressin (AVP) increases Na^+ reabsorption in the thick ascending limb of Henle's loop. Pronounced natriuretic activity of AVT analog 1-deamino-AVT was demonstrated in experiments on rats [2]. It was interesting to synthesize another AVT analog and to study its effects on renal function. The regulation of Na^+ reabsorption remains a key problem in renal physiology and clinical practice concerning treatment of edemas and hypo- and hypernatremia. The need in the synthesis of new, more active AVT analogs is explained by the fact that this natural neurohypophyseal hormone injected to rats produced unique effects on the water-salt metabolism: it increased Na^+ salt excretion and simultaneously increased water reabsorption in the kidney [1], while other natriuretic hormones (for example, atriopeptide) stimulated natriuresis, but this was paralleled by appreciable loss of water [7,9]. AVT analogs can modulate ion and water transport in the kidney; presumably, they more actively promote excretion of Na^+ and retention of osmotically free water in the body. The aim of this work was to synthesize 1-deamino-1-monocarb-AVT (1d-1mc-AVT) and to study its effects on physiological activity of the kidney in rats.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 150-200 g. Non-narcotized rats received water (5 ml/100 g) intragastrically through a rubber tube; 1d-1mc-AVT, dissolved in 0.9% NaCl to a concentration of 0.05 nM (0.1 ml/100 g) or furosemide (FS; Hoechst; 0.1 ml; 0.5 mg/100 g) were injected intramuscularly. The animals were placed into penal cages with wire floor, the urine was collected into a tube through a funnel. The volume of urine and urinary concentrations of creatinine, Na^+, K^+, and Mg^{2+} ions and osmolality were measured. Blood for analysis was collected from the carotid artery under light ether narcosis.

Osmolality of the serum and urine was measured by the cryoscopic method on an MT-4 milliosometer (Burevestnik), creatinine concentration was measured by the method of Popper on a UNICO-

Laboratory of Kidney Physiology, I. M. Setchenov Institute of Evolutional Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg. ***Address for correspondence:*** natochin@iephb.ru. Yu. V. Natochin

2100 spectrophotometer with a flow cuvette, Mg^{2+} was measured in air-acetylene flame on a Hitachi-508 atomic absorption spectrophotometer. Serum concentrations of Na^+ and K^+ were measured on an AVL 9140 electrolyte analyzer, urinary concentrations of these ions were measured in air-propane flame on a Corning-410 flame photometer.

The synthesis of 1d-1mc-AVT was carried out at the Peptide Synthesis Company by automated solid-phase method using Fmoc technology on Applied Biosystems synthesizer (model 431A) by the standard program for single condensation of Fmoc amino acids [4].

The data were compared and the significance of differences was evaluated using Student's *t* test.

RESULTS

Intramuscular injection of 1d-1mc-AVT (1 ml of 5×10^{-11} M solution/100 g) caused a drastic increase in renal excretion of Na^+ ions; excretion of K^+ and Mg^{2+} ions increased slightly (Fig. 1). The maximum reaction was observed 35-40 min after injection, while after 2.0-2.5 h renal function virtually normalized. This dose corresponded to AVT dose causing natriuresis in rats [1]. Study of the relationship between the dose of injected 1d-1mc-AVT and excretion of Na^+ ions by rat kidneys showed that natriuresis was maximum after this dose (Fig. 2). Further increase in the dose of the injected nonapeptide was difficult because of a sharp increase in its antidiuretic effect and oliguria lasting for many hours.

Physiological analysis of 1d-1mc-AVT effect on the kidneys showed that this AVT analog sharply increased osmotic concentration of the urine and increased saluresis, particularly natriuresis (Fig. 3). Measurement of creatinine and Na^+ clearance showed that excreted Na^+ fraction reflecting reduction of tubular reabsorption increased from 0.5±0.1% (initial level) to 12.7±1.0% at the peak of the effect. Injection of 1d-1mc-AVT increased excretion of osmotically active substances by the kidneys because of reduction of their tubular reabsorption. In the kidneys, 1d-1mc-AVT stimulated excretion of ions, diuresis, and reabsorption of osmotically free water (Fig. 1). A peculiar physiological paradox was observed: ion excretion increased, diuresis increased at the peak of the effect from 1.4±0.2 to 48.3±6.8 μl/(min×100 g), and in parallel reabsorption of osmotically free water in the renal collection tubules also significantly increased (Fig. 3).

In order to evaluate the natriuretic effect of 1d-1mc-AVT, we compared it to the effect of FS, one of the most effective modern natriuretics. FS was injected intramuscularly in a high dose, in order to attain the maximum effect. Bearing in mind that humans with body weight of 70 kg are usually prescribed 40-80 mg FS [4], the rats received 0.5 mg/100 g. The increase of natriuresis after 1d-1mc-AVT and FS was comparable (Fig. 3), while the total excretion of ions during the experiment was higher after injection of 1d-1mc-AVT due to longer duration of its effect. A principally important physiological difference in the renal reaction is increased reabsorption of osmotically free water after injection of 1d-1mc-AVT and the absence of this effect after FS injection (Fig. 3).

Extremely high natriuretic efficiency of 1d-1mc-AVT becomes obvious in comparison with FS and

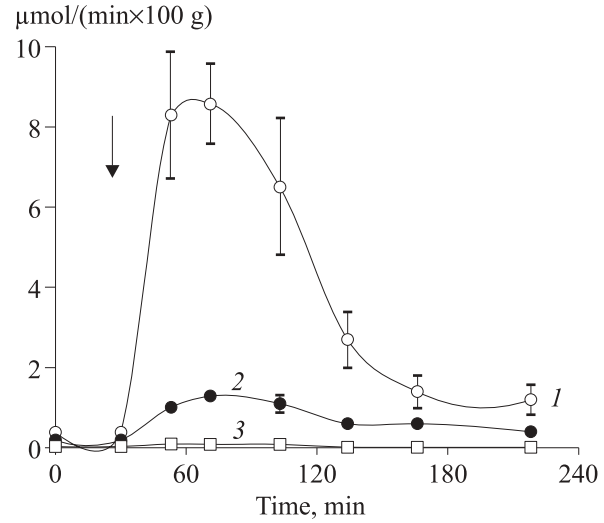

Fig. 1. Excretion of Na^+ (*1*), K^+ (*2*), and Mg^{2+} ions (*3*) by rat kidneys after injection of 1d-1mc-AVT following water load. Arrow shows intragastric administration of 5 ml water/100 g and intramuscular injection of 0.05 nM 1d-1mc-AVT.

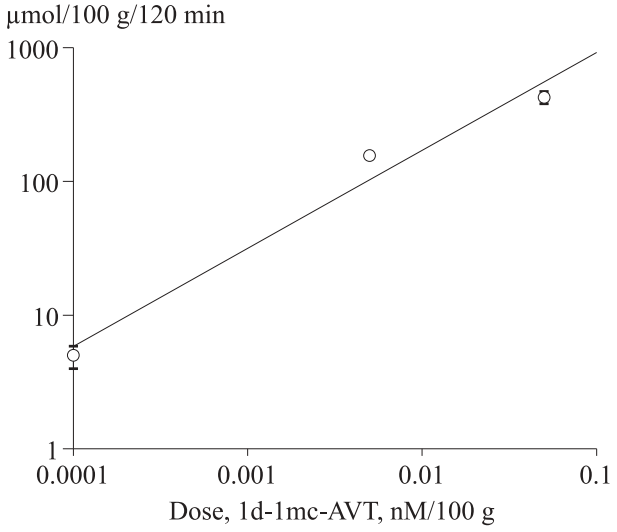

Fig. 2. Relationship between injected dose of 1d-1mc-AVT after water load and Na^+ excretion by rat kidneys.

conversion of the volume of excreted Na^+/mol injected peptide or FS (M^x_{Na}). We suggest the following equation for this conversion:

$$M^x_{Na}=[U_{Na}V]/[T_x],$$

where $U_{Na}V$ is the volume of Na^+ excreted by the kidneys during the effect of substance X (T_X is the injected dose of this substance). This substance is partially destroyed and excreted by different organs, but its part acting in the kidneys causes an increase in Na^+ excretion. The response of the kidneys to injection of the test agents was 141 Na^+ ions per one FS molecule and 8,220,000 Na^+ ions per 1d-1mc-AVT molecule. Hence, the peptide is almost 6×10^4 times more effective than FS. The effect of the peptide is long lasting (Fig. 3), and therefore, its summary effect is still more pronounced.

The effects of both substances at the cellular level in the kidneys differ qualitatively. Furosemide is filtered in the glomeruli, secreted by cells of the proximal segment of the nephron, reaches the lumen of the thick ascending limb of Henle's loop, and blocks Na^+,K^+,$2Cl^-$-cotransporter in the luminal membrane, whereas 1d-1mc-AVT modulates V_1- and V_2-receptors of the cell basolateral membranes and modifies cell functions through the corresponding systems of second messengers. The difference in the locus and mechanism of action determines significant differences in the intensity of the natriuretic effect. We previously developed a method for estimating FS efficiency by the ratio of FS and Na^+ excretion with the urine [3]. However, this approach can be used only when the substance acts from the side of the tubule lumen. In this system, when one physiologically active substance acts on outer surface of the membrane from the extracellular space (peptide) and the other acts on the outer surface of the luminal membrane from the tubule lumen, only the above estimations can be used.

Thus, our findings indicate that the kidneys excrete more Na^+ ions with the urine after injection of 1d-1mc-AVT than after loop diuretics. Presumably, this is caused by the effect of the peptide hormone on unknown elements of the renal function regulatory system. Blood concentration of AVP under condition of high activity of the pituitary can reach $1.2\text{-}2.5\times10^{-11}$ M [5,6,8]; in our experiments the rats intramuscularly received 0.1 ml of 5×10^{-11} M 1d-1mc-AVT solution. The hormone did not get into the blood at once; it was gradually absorbed, partially excreted by the kidney, and destroyed.

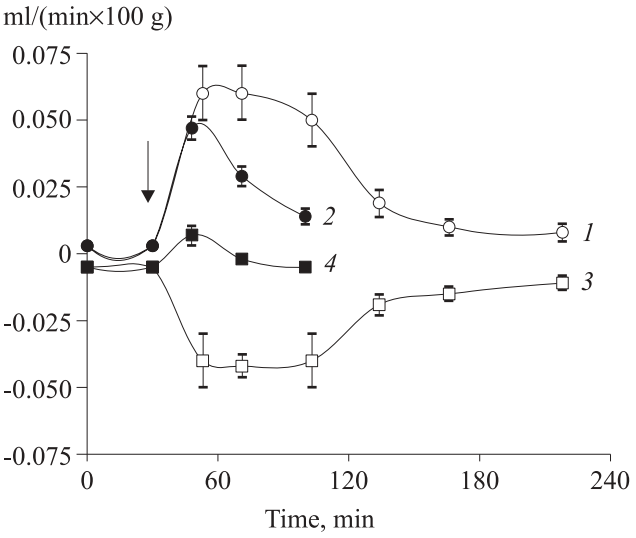

Fig. 3. Clearance of Na^+ (*1*, *2*) and osmotically free water (*3*, *4*) in rats after injection of 1d-1mc-AVT (*1*, *3*) and FS (*2*, *4*). Arrow shows intragastric administration of 5 ml water/100 g and intramuscular injection of 0.05 nM 1d-1mc-AVT or 1.5 μM FS.

This indicates similarity of plasma concentrations of the injected analog and endogenous AVP hormone, which emphasizes physiological significance of the described effect.

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