PRIMATOLOGY

Analysis of Antidiuretic Effect of Arginine-Vasotocin and Its Analogs in Primates

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Intramuscular injection of synthetic analogs of arginine-vasotocin to monkeys after 3% water loading caused antidiuretic reaction, manifesting by reabsorption of osmotically free water and paralleled by significant changes in excretion of sodium or potassium ions. It is shown that synthetic analogs can be more effective and selective than natural agonists.

Key Words: *arginine-vasotocin analogs; antidiuretic effect; primates; parenteral treatment*

Arginine-vasotocin (AVT) is one of the earliest hormones belonging to the vasopressin-oxytocin group, secreted by the neurohypophysis. It is the main hormone of osmotic regulation for all vertebrates, except mammals [13]. In mammals, the leading role in osmoregulation is played by arginine-vasopressin (AVP). Recently, numerous reports appeared that the effects AVT and AVP are mediated via common receptors V1a, V1b, V2 [9,12,14,16] activating G-protein system, which attests to close relationships between the two low-molecular-weight hormones at the molecular level in the target cell, including the polar epithelial cell expressing ionic channels and exchangers [11].

According to the data on the mechanism of vasotocin effect on asymmetrical epithelial cells in renal tubules and intestine of amphibians, fishes, and birds, AVT can be involved in the opening of sodium channels of the apical membrane, thus stimulating sodium transport through these cells [7, 10,15]. The increase in intracellular calcium concentration in the frog urinary bladder cells indicated the involvement of secondary messengers in vasotocin-induced transport of water [2]. It was shown that AVT increased water reabsorption and osmotic concentration of the urine in rats (similarly as vasopressin), while in a higher concentration this hormone exhibited a pronounced natriuretic effect [1]. Antidiuretic hormone/AVT receptors activating Na^+ transporter in the presence of PI_3 kinase were detected in cultured main epithelial cells of collector tubules of the mouse kidney cortical layer [9]. The peculiarities of antidiuretic and ion-regulatory effects of vasotocin and their relationship with targeted modifications in the structure of AVT molecule were heretofore not studied in primates.

MATERIALS AND METHODS

The study was carried out on non-narcotized male *Macaca mulatta* aged 3-4 years (4-6 kg). Before the experiment the animals were placed into primatological chairs without limitation in water and food. Several days before testing the antidiuretic reactions of animals was studied under conditions of suppressed endogenous vasopressin secretion [3]

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during water loading (30 ml water/kg). Arginine-vasotocin and its analogs D-arginine-vasotocin (D-AVT), 1-deamino-arginine-vasotocin (1-d-AVT), and 1-deamino-D-arginine-vasotocin (1-d-D-AVT) were tested. Desmopressin, a synthetic analog of vasopressin, served as the reference drug (its physiological effects were described previously [4,6]). Desmopressin was injected intramuscularly in a dose of 0.6 μg. AVT preparations were injected intramuscularly in a dose of 0.5 μg/kg directly after 3% water loading. The experiments were carried out no more often than once in 4 days in order to rule out the aftereffects of modified basal level of water-salt metabolism in animals. After water loading the duration of urine fraction release in spontaneous urination, its volume, creatinine, Na^+ and K^+ ion concentrations and osmolality were evaluated. Venous blood samples from the ulnar vein were collected before and 4 h after water loading. The following serum and urinary values were evaluated: concentrations of creatinine (by Jaffe's reaction using a Vital Diagnostic kit on a Clima 15 analyzer; Ral), electrolytes (by ion-selective electrodes on an Easy LyteQS analyzer; Medica), osmolality (by hygroscopic method on a Varpo™ analyzer; Wescor).

The synthesis of AVT analogs was carried out by the Peptide Synthesis Company [8]. Vasotocin from Sigma and desmopressin from Ferring were used. The data were statistically processed using Student's *t* test by the ANOVA software.

RESULTS

Oral water loading caused an increase in urination. Diuresis peaked 60-120 min after loading. It was functionally determined by changed osmotic permeability of the collecting tubules, as a result of which reabsorption of osmotically free water decreased and its excretion increased. On average, 87±17% water taken was excreted over 4 h.

Intramuscular injection of AVT in a dose of 0.05 nmol/100 g did not significantly change this parameter (88.5±10.3% administered water was excreted over 4 h), which indicates a weak antidiuretic effect of AVT. On the other hand, intramuscular injection of desmopressin after water loading led to a reduction in the rate of urination: only 20.8±1.0% water load volume was excreted over 4 h.

1-d-AVT and 1-d-D-AVT injected in the same dose as AVT more effectively delayed excretion of water loading than AVT and desmopressin. Only 7.8±3.0% administered fluid was excreted within 4 h after injection of 1-d-AVT and $^1/_4$ of its entire volume within 8 h. After injection of 1-d-D-AVT not a single portion of the urine was discharged during the first 4 h and about 25% of administered volume within 8 h.

Clearance of osmotically free water during 4 h of water loading test standardized per kg body weight was 23.8±5.9 ml/kg (Fig. 1). This meant that virtually all water excreted by the kidneys was osmotically free (about 30 ml/kg liquid was discharged within 4 h).

A similar conclusion can be made for injected D-AVT (in this series of experiments fluid excretion within 4 h was 24±7 ml/kg). 1-d-AVT caused an increase in the reabsorption of osmotically free water, and the value of osmotically free water clearance was negative. This phenomenon was less pronounced after injection of 1-d-D-AVT (Fig. 1). On the other hand, a significant reduction of kaliuresis was observed after injections of 1-d-AVT and 1-d-D-AVT and of natriuresis after 1-d-AVT (Table 1).

Physiological effect of desmopressin in monkeys differed from that in rats. Intramuscular injection of 0.02 μg to rats after 5% water loading was highly effective: the animals discharged not a single portion of the urine within 2 h, and just about 4.5% loading during 4 h [6]. The kidneys of monkeys after water loading and desmopressin excreted 20.9±0.6% additional water volume within 4 h. No changes in natri- and kaliuresis were observed in monkeys injected with desmopressin (*vs.* animals receiving water loading alone; Table 1). However, the pronounced increase in free water reabsorption (C_{H_2O} value became negative; Table 1) indicated the development of very intense antidiuretic reaction after injection of nanopeptide: no osmotically free water was excreted with the urine (osmotically free

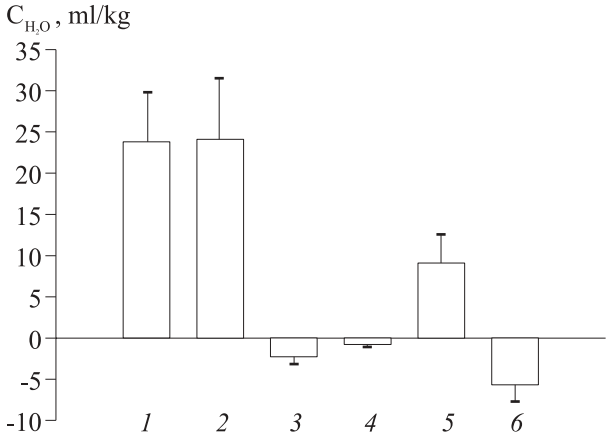

Fig. 1. Clearance of osmotically free water over 4 h after the start of the test. *1*) water loading (*n*=11); *2*) water loading+D-AVT (*n*=4); *3*) water loading+1-d-AVT (*n*=4); *4*) water loading+1-d-D-AVT (*n*=4); *5*) water loading+vasotocin (*n*=4); *6*) water loading+desmopressin (*n*=4).

TABLE 1. Renal Function Values in Monkeys 4 h before 3% Water Loading and Injection of AVT Analogs and Desmopressin (*M*±*m*)

Parameter	Water loading	D-AVT	1-d-AVT	1-d-D-AVT	AVT	Desmo-pressin
V, ml/kg	29.9±6.0	28.5±8.4	3.3±0.7**	1.6±0.2**	16.3±4.9	6.4±0.2**
C_{H2O}, ml/kg	23.8±5.9	24.1±7.5	-2.3±0.9**	-0.8±0.3**	9.1±3.6	-5.7±2.1**
$U_{Na}V^+$, μmol/kg	521±94	449±147	114±47**	48±16***	452±92	539±127
U_KV^+, μmol/kg	187±29	106±35	168±61	76±18**	357±47*	213±9

Note. $^+$Release of Na^+ and K^+, respectively, with urine, standardized per kg. *p<0.05, **p<0.01, ***p<0.001 compared to the corresponding parameter after water loading.

water was retained in the body). Hence, after intramuscular injection of desmopressin the kidneys of monkeys retained more water in the body than was introduced intragastrically.

Hence, the dose of desmopressin used in the study seemed to be adequate for this study of its antidiuretic effect on primates (the dose was estimated in proportion to animal body weight, based on the mean dose for humans).

It was previously shown in rat experiments that intramuscular injection of AVT in a dose of 0.05 nmol/100 g led to pronounced antidiuretic and natriuretic effects [1]. In experiments on monkeys AVT analogs exhibited antidiuretic, but not natriuretic effects. Clearance of osmotically free water acquired a negative value, while the most pronounced antidiuretic effect in monkeys was observed after injections of 1-d-AVT and 1-d-D-AVT (Table 1). Similar reactions to the same dose of AVT analogs (by effects on water reabsorption) were observed in monkeys. A significant decrease in sodium release after injection of 1-d-AVT and 1-d-D-AVT was noted (Table 1). Excretion of potassium ions by the kidney dropped in monkeys after injection of 1-d-D-AVT, but not after injections of other analogs (Table 1).

AVT and its analogs caused no changes in cardiovascular activity in monkeys [5]. In humans desmopressin exhibits a specific antidiuretic effect after water loading [3,4] without modifying cardiovascular activity.

Hence, the use of synthetic AVT analogs (in a dose comparable to that of the natural agonist) caused an increase in the reabsorption of osmotically free water and was paralleled by significant changes in sodium or potassium ion release. It was shown that synthetic analogs can be more effective and selective than natural agonists. This prompts creation and use of appropriate dosage forms for clinical practice for the maintenance and directed correction of water-electrolyte metabolism.

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