Delayed Nootropic Effects of Arginine Vasopressin after Early Postnatal Chronic Administration to Albino Rat Pups

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Intranasal administration of arginine vasopressin (10 μg/kg) to albino rat pups had a strong nootropic effect during training with positive and negative reinforcement. This effect was different in animals of various age groups: training with positive reinforcement was improved in "adolescent" rats and pubertal animals, while during training with negative reinforcement, the nootropic effect of the peptide was more prolonged and persisted also in adult animals.

Key Words: *regulatory peptides; arginine vasopressin; training with positive and negative reinforcement*

Regulatory peptides are the most intensively studied class of physiologically active substances. They produce various direct and indirect effects on many physiological processes. One of these neuroactive peptides is arginine vasopressin (AVP). In the central nervous system, AVP modulates a variety of behavioral reactions, response to stress factors, and social behavior [2]. AVP has a strong positive effect on acquisition of conditioned responses and memory-related functions. AVP in very low doses (1 μg/kg) accelerates the acquisition and delays the extinction of acquired skills, prevents the development of retrograde amnesia, and improves memory retrieval. The consequences of chronic neonatal treatment with AVP and AVP-like peptides are associated with delayed effects on animal behavior.

Here we studied the delayed nootropic effects of AVP after early postnatal chronic administration to albino rat pups.

MATERIALS AND METHODS

Experiments were performed on male and female outbred albino rats. The pups of each litter were divided into 2 groups (control and treatment). AVP in a dose of 10 μg/kg (1 μl/10 g body weight) was administered intranasally to animals of the treatment group on days 8-14 of life. Control rats received an equivalent volume of distilled water (solvent).

The effect of this peptide on behavioral parameters was studied by methods with positive and negative reinforcement during the following periods of life:
— days 35-46, prepubertal period;
— days 49-60, period of puberty; and
— days 63-74, adult (sexually mature) animals.

Training with positive reinforcement was performed in a complex food maze (CFM) during the acquisition of food-seeking behavior. Training with negative reinforcement was based on active avoidance (AA) conditioning with nociceptive stimulation. In experiments with CFM, the animals were deprived of food for 24 h. The rats were adapted to maze conditions for 30 min. White-bread pieces were regularly distributed in various compartments of the maze to reduce the exploratory response, decrease anxiety, and form primary association between the experimental conditions and food reinforcement. Over the next 4 days, these animals were repeatedly (5 times) put in the maze. The duration of each session did not exceed 3 min. White-bread pieces were used as positive reinforcement. On

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the day of training, the animals received food once daily (immediately after the experiment). The skill was tested 1 week after the last training session.

During AA conditioning a 4-day scheme was used. Each animal was subjected to 10 combinations of conditioned and unconditioned signals over 4 days of training. The bell sound (3 sec) and electrostimulation served as conditioned and unconditioned stimuli, respectively. The voltage was selected individually for each animal (35-80 V). The jump on a shelf was considered as the conditioned response. During the test session, each animal was placed in a chamber and adapted to experimental conditions for 25 sec. The conditioned stimulus was delivered in the follow-up period. The interval between conditioned stimulation and unconditioned reinforcement was 2 sec. The interval between combinations of conditioned stimulation and unconditioned reinforcement was selected randomly (15-30 sec). The skill was tested 1 week after the last training.

The results were analyzed by standard statistical methods. The means, standard deviations, and standard errors of means were calculated. The samples were compared by parametric (Student's test) and nonparametric tests (χ^2 test). Statistical tests and evaluation of data distribution were performed by means of Statistica software. Graphical data in figures are presented as the mean value and standard error. Intergroup differences were significant at $p<0.05$.

RESULTS

The following results were obtained after training with prepubertal animals. Vertical activity of treated animals in CFM tended to increase compared to controls on day 1 of training. This manifested in increased number of rearing postures and time of grooming and attested to a slight increase in orientation-and-exploratory activity and emotional strain in AVP-treated rats.

Animals of the treatment group demonstrated better performance compared to controls (Fig. 1): more than 4 reactions performed 8 rats in the treatment group *vs.* 2 rats in the control group. Moreover, the latency of exit from compartment 1 was slightly shorter in treated animals.

During AA conditioning, the number of short-latency AA reactions in treated rats was higher than in control animals on day 3 of training, which suggests that treated rats cannot differentiate unconditioned and conditioned stimuli during this period. It results in a decrease in the number of executed reactions. Skill testing showed that the number of executed reactions in peptide-treated rats was slightly higher than in control animals. Hence, treated rats exhibit better AA retention compared to controls. Peptide-treated rats are characterized by an "additional learning" during the period between termination of training and start of the test session. The number of executed reactions on day 11 was higher than on day 4 of training. These differences were not observed in animals of the control group.

Our results indicate that administration of AVP at the early stage of training improves the result of training with positive and negative reinforcement.

In pubertal rats, early postnatal chronic administration of AVP had another delayed effect. On day 4 of training in CFM, the reaction time in treated rats was longer than in controls. They exhibited increased number of rearing postures and errors. Skill testing showed that the time of leaving the start compartment of a maze was longer in treated animals compared to controls. Neonatal administration of AVP had little effect on training with positive reinforcement (days 49-60 of life), but increased the orientation and exploratory response of animals.

During AA conditioning, the number of executed reactions in rats of the treatment group tended to increase compared to control specimens (days 2 and 3 of training, Fig. 2). AVP-treated rats were characterized by greater number of intersignal reactions on day 2 of training (compared to the control). Therefore, peptide-treated animals knew the location of the shelf during this period, but the conditioned response to stimulation (bell sound) was not formed in these rats. Skill testing revealed a significant decrease in the number of executed reactions (compared to the 4th day of training) in control animals, but not in rats of the treatment group.

These data indicate that neonatal administration of AVP has a delayed nootropic effect on pubertal animals during training with positive reinforcement.

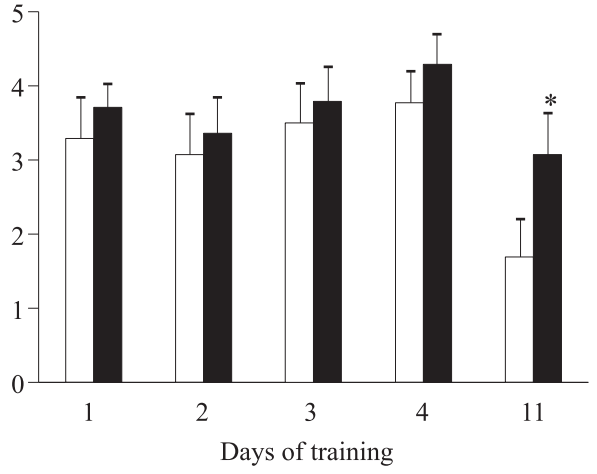

Fig 1. Number of executed reactions in the CFM test for rats aging 35-46 days. Light bars, control group (n=14); dark bars, treatment group (n=14). Here and in Figs. 2 and 3: *$p<0.05$ compared to the control.

CFM training of adult rats revealed no differences between the control and treatment groups. Therefore, early neonatal administration of the peptide has no effect on training with positive reinforcement in adult animals.

During AA conditioning, the number of short-latency avoidance reactions in treated rats was lower than in control animals (days 1 and 3 of training; Fig. 3, *a*). Skill testing showed that AVP-treated rats exhibit a lower number of intersignal reactions compared to controls (Fig. 3, *b*). This attests to more effective inhibition of conditioned response relationships not directly involved in the reaction of "ring – jump on the shelf" in animals of the treatment group (compared to control specimens). This process continued even after the last training session. The number of intersignal reactions in control rats did not differ on day 4 of training and during skill testing. However, this parameter tended to decrease in treated animals (Fig. 3, *b*). These data indicate that the nootropic effect of AVP during training with negative reinforcement is also observed in pubertal animals.

It can be concluded that early postnatal administration of AVP on days 8-14 of life improved training with positive reinforcement in "adolescent" rats and pubertal animals. During training with negative reinforcement, the nootropic effect of the peptide was observed in animals of all age groups.

Learning is one of the most complex tasks for the highly-organized nervous system. CNS structures involved in the process of learning are presented by subcortical nuclei responsible for certain requirements (hypothalamus and midbrain) and centers of positive and negative reinforcement (positive and negative emotions, "award" and "punishment", *etc*.). Each of these centers includes a variety of nervous structures.

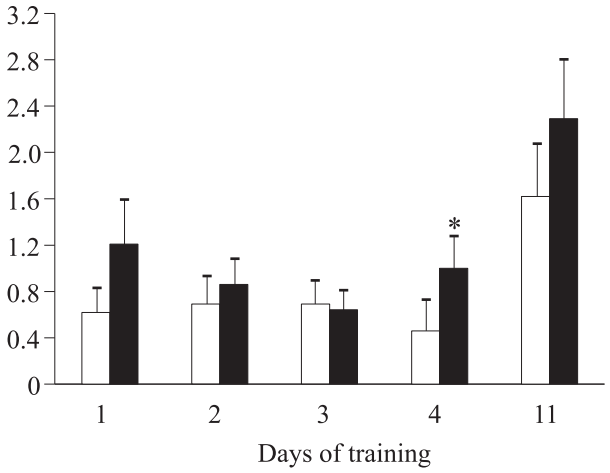

Fig. 2. Number of short-latency AA responses for rats aging 49-59 days. Light bars, control group (*n*=13); dark bars, treatment group (*n*=14).

For example, the reward system consists of the central gray matter, ventrotegmental area, lateral hypothalamic zones, ventral striatum and pallidum, dorsomedial amygdala, hippocampus, prefrontal cortex, and other structures [1]. These structures analyze the incoming sensory information, organize the autonomic or motor response, provide coupling between afferent and efferent components of the reflex arch (*i.e.*, closing of temporal relations), and form essential requirements and motivations.

The dopaminergic system is the major neurotransmitter component, which provides realization of positive reinforcement. Much attention is paid to GABA, glutamate, serotonin, norepinephrine, opioid peptides, AVP, purines, and corticosterone. Strong evidence exists that AVP affects a variety of neurotransmitter systems, including the noradrenergic and dopaminergic systems.

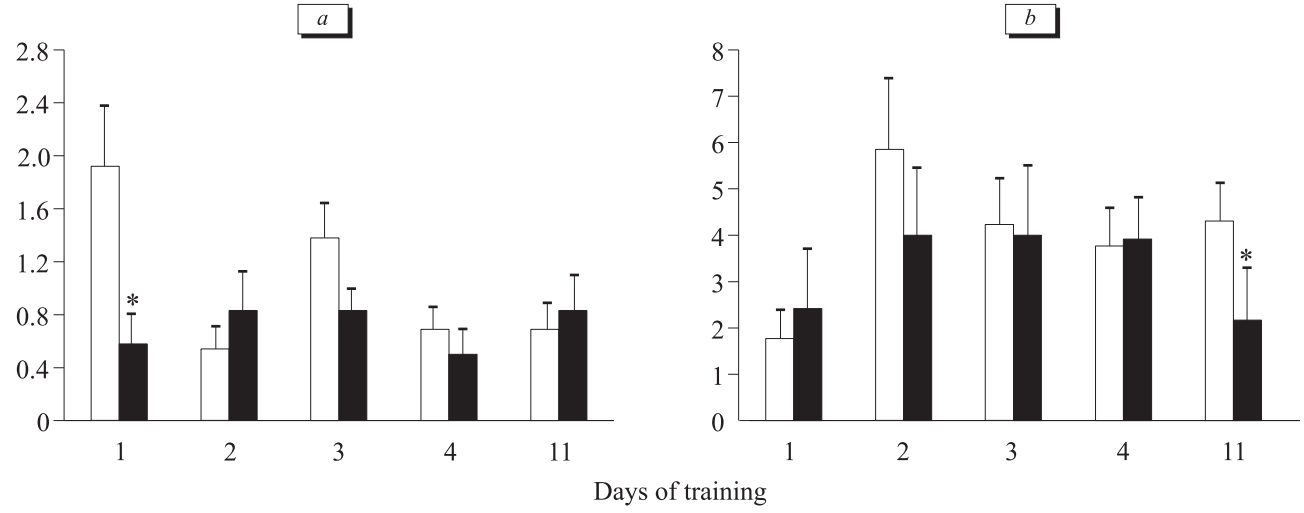

Fig. 3. Number of short-latency AA responses (*a*) and intersignal reactions (*b*) for rats aging 63-73 days. Light bars, control group (*n*=13); dark bars, treatment group (*n*=12).

Previous studies showed that the effect of AVP on learning and memory is realized via projections of dopaminergic fibers into the lateral septal nuclei [5]. It was hypothesized that noradrenergic projections from the locus coeruleus to the telencephalon play a role in the action of AVP on memory consolidation [3]. The influence of AVP on memory consolidation is associated with binding to noradrenergic and dopaminergic neurons of the lateral septum. AVP binds to V1 receptors on catecholaminergic cells of this brain region [4].

Anatomically, the punishment system is composed by the septal area, lateral amygdala, and ventromedial hypothalamus. The effect of these structures on CNS function is realized under conditions of a biologically unfavorable result and provides various types of conditioned inhibition. However, the system of negative reinforcement is less studied than the award system.

The question arises: how can we explain our results? The main constituents of learning are signal perception, memory consolidation, and formation of requirements and motivations. AVP has a similar effect on training with positive and negative reinforcement. Therefore, this peptide affects the first two constituents. During training with negative reinforcement, temporal relations with the involvement of a specific conditioned signal (bell sound) are formed on the basis of requirements for safety. The decrease in these requirements upon realization of an adequate behavior (jump on the shelf) contributes to activation of award centers, which triggers the process of memory consolidation. When the basal level of requirements for safety is reduced, the strength of final reinforcement will be low.

Our results indicate that early postnatal chronic administration of AVP has a strong nootropic effect under conditions of training with positive and negative reinforcement.

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