Chapter 6

EFFECTS OF NEONATAL ADMINISTRATION OF VASOPRESSIN ON CARDIAC AND BEHAVIORAL RESPONSES TO EMOTIONAL STRESS IN ADULT MALE RATS

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ARGININE-8-VASOPRESSIN (AVP) was administered subcutaneously on postnatal days 3-7 in a high (10 μg/100 g b.w.) or a low dose (1 μg/100 g b.w.) to male Wistar rats. Control pups were untreated or saline injected. Behavioral observations in a complex maze after maturation indicated that neonatal administration of AVP increases exploratory behavior in this novel environment in a dose-dependent way. Cardiac monitoring during the conditioned emotional stress of fear of inescapable electric footshock showed that only the high dose of AVP attenuates the bradycardiac stress response. The analysis of cardiac responses also suggested an adult hyposensitivity to AVP in rats treated neonatally with AVP. In addition, the low dose of neonatal AVP was impairing the retention of a passive avoidance behavior.

The data indicate that the neonatal administration of AVP exerts long-term effects upon the behavioral adaptation to novelty and memory processes related to emotional stress. That neonatal AVP is less effective in influencing adult vagally mediated cardiac stress responses suggests differences in the developmental sensitivity ("critical periods") of the central vasopressinergic systems involved in the regulation of behavior and autonomic functioning.

INTRODUCTION

Manipulation of the endocrine environment of the newborn rat may have remarkable impact on the "programming" and "organizational" processes in the developing central nervous system (3,11,20). As a result, long-term changes in a number of physiological and behavioral patterns, including responsiveness to the "activational" effects of hormones, can be observed later in life (9,15).

The neurohypophyseal peptide arginine-8-vasopressin (AVP) is an important modulator of physiological and behavioral stress responses via brain and peripheral mechanisms in adult animals. The facilitating effect of AVP on memory consolidation and retrieval processes has been repeatedly described (6,10,17,19). Reports on the way in which AVP can modulate behavioral activity are not equivocal, probably due to behavioral state- and dose-dependent effects of AVP and differences in route of administration. Centrally applied AVP is arousing at a low level, while it reduces arousal at high level of activation (7). Peripherally administered AVP facilitates behavioral activity to a novel environment in a low dose (26), while higher doses suppress activity (25). This behavioral suppression may be due to the marked peripheral hemodynamic effects caused by higher doses of AVP. Experiments in our laboratory in freely moving rats suggested that AVP also serves as a modulator of the vagally mediated cardio-inhibitory response to certain emotional stressors (5,8,12,22). The profound effect of neonatally administered AVP on learning and memory task performance in later life (21,27) suggests that the peptide may have important programming and/or organizational effects on brain and physiological processes. In this paper we present evidence that postnatal administration of AVP affects behavioral reactivity.
to novelty, the cardiac response to an emotional stressor and adult passive avoidance behavior. In addition, attention has been paid to the effect of neonatal exposure to AVP on the modulating properties of vasopressin on the bradycardiac stress response in adulthood.

METHODS

Animals

Thirty-two male Wistar rats (CpB TNO, Zeist, The Netherlands and bred in this laboratory) were used. Males in litters were culled to 8 pups each. Eight pups remained untreated. The other 24 pups received daily subcutaneous (s.c.) injections, beginning on postnatal day 3 and ending on day 7, of one of the following preparations: (1) saline; (2) AVP1, 1 µg/100 g bodyweight; (3) AVP10, 10 µg/100 g. AVP was dissolved in saline. The assignment of pups within a litter to treatment groups was done at random. Each mother raised pups of a variety of treatments. The pups were weaned on day 23 and housed 5 to a cage at a standard temperature of 21 ± 2°C in a light-controlled room (light on between 07.00-19.00h). All animals had free access to standard food and water. The behavioral and cardiac tests were performed at an age of 3 months old between 9.00 and 13.00 hr.

Surgery

In order to record the electrocardiogram (ECG) two transcutaneous stainless steel electrodes made of standard paperclips were implanted under light ether anesthesia by a technique described earlier (4). Two days of recovery were allowed after this minor operation.

Behavioral Reactivity to Novelty

Apparatus

Novelty-induced behavioral reactivity was tested in a complex maze. The apparatus was a modified Hebb-Williams (60x60x60 cm) maze made of plywood and covered by wire mesh. The maze was divided into alleys and subfields by means of wooden falls. The floor of the maze was divided into 10x10 cm squares. The maze was placed in a sound-attenuated semi-dark room.

Procedure.

In the complex maze the behavior of each rat was observed for 5 min. The number of floor units entered and the number and duration (sec) of rearings were recorded with the aid of a microprocessor. This behavioral test always preceded the study in the emotional stress situation.
Emotional Stress, Behavior and Cardiac Response

Apparatus

A step-through type passive avoidance apparatus as designed by Ader et al. (1) was used to investigate emotional stress-related behavior and cardiac response. Briefly, the apparatus consisted of a dark compartment (40x40x40 cm) and a well lit platform attached to the front center. A small sliding door separated the two compartments. An unavoidable painful electric footshock could be delivered in the dark box through the stainless steel bars which served as a floor.

Recording and analysis of the ECG

The ECG of freely behaving rats was monitored telemetrically by means of a miniature FM transmitter (model SNR 102F, Dynamic Electronics Ltd., London, England), attached to a velcro strap secured around the chest of the rat (4). The transmitted signals were received on a commercial FM receiver, amplified (Grass P5CR preamplifier) and stored on tape with the aid of an instrumentation recorder (Minilog, Philips) for off-line computer analysis. Prerecorded ECG samples were played back through a cardiotachometer pulsegenerator which generated a square wave electric pulse at each R wave. The time between the onset of two consecutive pulses, the interbeat interval (IBI), was measured by a personal computer (Olivetti M24). IBI's shorter than 100 and longer than 220 msec were discarded because these were likely to be due to artefacts. The heart rate was expressed as mean IBI: the longer the IBI, the lower the heart rate was.

Experimental procedure

The animals were first habituated to the experimental circumstances. Immediately before each of the daily sessions the strap holding the transmitter was fixed around the chest of the rat in the animals' room. After transportation to the experimental room, on day 1 the rat was allowed a 3-min adaptation to the dark compartment. Immediately afterwards a single training trial was given during which the animal was placed on the illuminated platform and allowed to enter the dark compartment. The sliding door was closed and the rat stayed another 3 min in this compartment. On days 2 and 3 the training procedure was repeated. The rat was directly placed on the platform. Following entering the dark compartment the rat was removed after 5 min. To accustom the animals to the injection method, s.c. saline injections were given 1 hour prior to the training on these days. At the 4th daily session ECG recordings started in order to obtain prestress heart rate values. Sixty min prior to the test, the animals were saline injected (s.c.) to achieve correct control values for poststress recordings. The ECG recording lasted for a period of 1 min in the dark compartment, starting immediately upon entrance. In a second session on day 4, an inescapable electric footshock (0.6 mA, 2 sec) was delivered immediately after entering the dark. The rat was removed after 1 min and returned to the home cage. To obtain postshock ECG values under emotional stress of fear of inescapable footshock, the rats were subjected to the experimental situation on days 5 and 6. They were placed directly into the dark compartment with closed sliding door. ECG's were recorded in the first min of the exposure. On these two days animals were injected subcutaneously with saline or AVP, in a dosage of 3 µg/kg, with a cross-over design. Accordingly, each animal served as its own control. The treatments were given 60 min prior to testing.
Avoidance latency

The latency of entering the dark compartment from the lit platform was used as the behavioral measure of the conditioned emotional behavior. This, usually designated as one-trial learning inhibitory or passive avoidance test was performed 1 week after the two short forced exposures to the dark compartment. The rat was placed on the platform facing away from the open sliding door to the dark compartment. The latency to reenter the dark was measured up to a maximum of 300 sec. No treatment was given at this time.

Statistical analysis

Postnatal saline injections did not significantly influence cardiac and behavioral responses as compared to untreated animals. Results obtained in untreated and neonatal saline treated rats were therefore pooled in a so-called control group. Avoidance latencies were expressed individually and as median latency in sec. The other results were calculated as means ± SEM. Cardiac data were analyzed using a one-way ANOVA and a paired t-test. Behavioral data were evaluated for significance using the non-parametric Kruskal-Wallis ANOVA and the Mann-Whitney U-test. A probability level of p < 0.05 was taken as statistical significance for all tests.

![Score](image)

**Fig.1**
Behavioral reactivity to novelty in a complex maze of control (8 neonally untreated and 8 saline treated) rats and of rats treated neonatally repeatedly with a low (AVP1; n=8; 1µg/100g b.w.) or a high (AVP10; n=8; 10 µg/100g b.w.) dose of AVP. Ambulation index is a combined measure of rearing and crossing activities ((1/2 x number of crossings) + (number of rearings + duration of rearings)). **p<0.01 vs. control (Mann-Whitney U-Test).
Heart rate expressed as interbeat interval (IBI) before (preshock) and one day after inescapable shock (postshock) in adult control animals; animals treated neonatally repeatedly with a low (AVP1) or a high (AVP10) dose of AVP during the first min of an emotional stress of forced exposure to the dark compartment of a passive avoidance apparatus where the footshock was delivered earlier. Preshock measurements were performed after saline injection one day before footshock was delivered. Prior to the two postshock tests the animals were injected in a cross-over design with saline and AVP (3 µg/kg). The substances were administered 60 min prior to testing. \*p<0.05; \**p<0.01; \***p<0.001 vs. preshock (paired t-test). For further details see Fig.1.

Avoidance latencies one week after inescapable footshock of controls and rats treated neonatally with AVP. Results are presented as individual scores and as medians in sec. \**p<0.01 vs. control (Mann-Whitney U-test). For further details see Fig.1 and 2.
RESULTS

Behavioral Reactivity to a Novel Environment

Fig. 1 shows that neonatal AVP administration dose-dependently increased adult activity in the complex maze compared to the saline treated group ($H(2,32) = 7.99, p = 0.01$).

Cardiac and behavioral responses to emotional stress

Fig. 2 shows the mean cardiac rate in preshock conditions after adult saline administration and in postshock conditions after the rats were treated with saline or AVP. Neonatal AVP administration had no effect on heart rate under preshock conditions.

As compared to the preshock condition the emotional stress of the exposure to the former shock compartment was accompanied by longer mean IBIs in the control group, suggesting a relative bradycardiac response ($p < 0.05$). AVP administration prior to the test caused a further increment of the bradycardiac stress response ($p < 0.001$). Rats receiving neonatal low doses of AVP (AVP1) showed a bradycardiac stress response similar to the one performed by the control animals. However, adult pre-test AVP administration failed to cause a further increase in the magnitude of the stress bradycardia in this group. The group receiving neonatal high doses of AVP (AVP10) did not show a significant bradycardiac response to stress, neither under saline nor AVP treatment conditions. One-way ANOVA failed to reinforce a significant neonatal treatment effect on cardiac stress response (postshock IBI- preshock IBI) following saline ($F(2,29) = 0.23, p = 0.8$) or AVP administration ($F(2,29) = 1.12, p = 0.3$). ANOVA also indicated that there was no neonatal treatment effect on absolute IBI in both preshock and postshock conditions.

Fig. 3 depicts the avoidance latencies measured one week after the last cardiac recording session. Rats receiving neonatal repeated injections of the lower dose of AVP showed significantly shorter avoidance latencies than the controls ($p < 0.01$). Avoidance latency in rats treated with the high doses of the peptide was not impaired.

DISCUSSION

The present findings suggest that increased availability of vasopressin during the first week after birth markedly affects adult memory to aversive events and behavioral reactivity in a novel environment. The adult cardiac response to an emotional stressor appears to be less sensitive to neonatal AVP treatment.

The analysis of cardiac stress responses suggests that an adult hyposensitivity to AVP may exist in rats treated neonatally with AVP. This suggestion, however, needs further extended investigation. While neonatal treatment with the lower doses of AVP did not alter
the magnitude of the stress bradycardia, this dosage clearly affected memory retrieval as indicated by the impaired avoidance behavior performed by these animals. The results presented here suggest that vasopressinergic systems involved in autonomic and behavioral stress responses are differentially affected by neonatal administration of AVP. In our view, at least two possible explanations may be given. The first may be the late development of tonic parasympathetic control of the rat heart. The vagal regulation of cardiac rate appears to be complete only after the second week of postnatal life (16). To manipulate the vagally mediated bradycardia to stress by neonatally administered AVP, it is perhaps necessary to extend or change the period of administration. The second explanation may be a different critical period to endocrine manipulation for the diverse central and peripheral vasopressinergic systems. This might originate from a differential development in time of vasopressinergic systems involved in autonomic and behavioral processes. As to central vasopressinergic systems it is known that the first appearance of AVP binding sites during embryonic life is coincident with the first detection of AVP mRNA and of immunoreactive AVP (2,18). Petracca et al. (23) showed, however, that in the amygdala, binding did not change after postnatal day 3, while binding sites in the septum proliferated slowly to attain adult (90 days) distribution. Tribollet et al. (28) also showed that the appearance of AVP receptors is not the similar in many areas of the rat brain and depends on the developmental stage.

The impaired passive avoidance behavior of animals treated neonatally with the low doses of AVP may be interpreted as a decreased sensitivity in adulthood of vasopressinergic systems involved in memory processes to endogenous vasopressin. It is generally accepted that AVP affects passive avoidance behavior (13,19,24). Differences in findings about facilitation or inhibition of avoidance retention by vasopressin are often dose or arousal related (7,13). This might be reflected in the failure of the high doses of AVP to impair later avoidance retention. Handelmann and Sayson (14) showed that postnatal injections of vasopressin decrease the number of binding sites for AVP in the adult kidney. This receptor downregulation probably also occurs in the certain brain areas. Chronic prenatal administration of AVP also impairs adult memory retrieval (27), suggesting a decreased receptor sensitivity to endogenous vasopressin. If Bohus' (7) hypothesis that certain vasopressinergic systems serve behavioral passivity is correct, the observed behavioral changes in the novel environment may also be attributed to a hyposensitivity of central vasopressinergic systems. However, the exact mechanism underlying the differential physiological and behavioral effects of neonatal administration of AVP needs further studies.
REFERENCES


