The parasympathetic responsiveness in young and aged rats
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Chapter 8

BEHAVIORAL AND NEUROENDOCRINE EFFECTS OF VASOPRESSIN IN RESTING AND STRESS CONDITIONS

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ABSTRACT

In order to understand the mechanisms by which systemically administered arginine-vasopressin (AVP) modulates behavior and autonomic functioning, (neuro)endocrine and behavioral measurements were taken in young adult male Wistar rats. The effects of subcutaneously administered AVP (6 μg/kg b.wt.) were determined in resting and mild emotional stress conditions before and 5 to 92 min after treatment.

Systemic administration of AVP caused a biphasic increase in blood glucose level, a long-lasting increase in CORT secretion, and a decrease in circulating NE under resting condition. It did not affect adrenal medullary E secretion. The stress induced sympathetic activation as reflected in plasma NE level was inhibited 60 min after AVP administration. In resting condition AVP caused a 60 min lasting increase in grooming behavior with a concomitant decrease in time spent resting. Sixty min after administration, AVP-treated rats were less active after the mild emotional stress of transportation and placement in a novel environment than vehicle-treated control rats.

The results suggest that AVP may modulate behavior not only by its hypothesized direct action in the brain or by its systemic pressor effect, but also by enhancing blood glucose and adrenal CORT secretion. The vasopressinergic sympato-inhibitory action may play a role in the previously reported vasopressinergic enhancement of parasympathetic cardiac stress responsiveness. Furthermore, this action of AVP and the suppression of stress induced active behavior suggest that AVP may also be a neuroendocrine factor with dearousing properties.

INTRODUCTION

Apart from its classically described antidiuretic and vasopressor action the neurohypophyseal peptide arginine-8-vasopressin (AVP) produces various behavioral changes. It prolongs extinction and improves consolidation and retrieval processes in avoidance learning tasks (5,16,24,39,50), and modifies behavioral responses to novelty stress (8,47,52). Previous research in this laboratory showed that AVP also serves as an important modulator of a vagally mediated bradycardiac response to an emotional stressor (2,4,8,25).

As to the mechanisms by which AVP exerts its behavioral (cognitive) actions there is ambiguity in the literature. De Wied and colleagues (16,17) hypothesized that the modulatory effect of AVP on memory processes is predominantly exerted through direct actions in the brain. Others have challenged this view suggesting that peripheral effects of AVP leading to increased arousal are responsible for the behavioral effects of the peptide (19,39). Based on observations that the cognitive (mnemonic) behavioral actions of systemically applied AVP could be reversed by antagonizing the pressor effect of AVP, the arousal changes secondary to increases in blood pressure were assigned as responsible mechanisms for the behavioral action of AVP (39). Since application of the vasopressin analogue desglycinamide-AVP, which lacks virtually all pressor activity (16), and AVP administered directly into the brain in doses that do not enhance systemic blood pressure also clearly elicited behavioral effects (16,33,36), it was hypothesized that systemically and
centrally administered AVP can influence behavior in a homologous manner but by different mechanisms of action (33,35).

Since it is not necessary to produce peripheral vasoconstriction to obtain the behavioral effects of AVP, vasopressin may modulate behavior not only by a direct vasopressinergic action in the brain but alternatively also by eliciting peripheral (neuro)endocrine changes via central or peripheral mechanisms. AVP affects sympathoadrenal (37) and adrenocortical activity (9,20,41). Therefore changes in these adrenal hormones may also play a role in the behavioral effects of AVP. The adrenal hormones corticosterone (CORT) (15), and epinephrine (E) (7,22,27,43) are known to play an important role in regulating cognitive processes. Changes in blood glucose represent another memory enhancing humoral factor (21,54).

In order to understand the dynamics and the mechanisms by which AVP modulates behavior, the effects of systemically applied AVP on the level of blood glucose and plasma levels of the catecholamines E and NE, and CORT in resting and mild emotional stress conditions were investigated. Observed effects of AVP on sympathetic activity may also yield information on the mechanisms contributing to the enhanced vagal stress-responses after administration of AVP. Behavior was analyzed to study whether systemically administered AVP alters the emotional "state" in animals during these conditions.

METHODS

Animals and housing

Male Wistar rats (4 months old), originating from the Winkelman substrain, were housed individually in clear Plexiglas cages (25x25x30 cm), with food and water ad libitum, on a 12h light-dark regime (light on between 07.30h - 19.30h) at a room temperature of 21 ±2°C.

Surgery

To study the effects of AVP administration on blood glucose levels, plasma catecholamines and CORT, half of the animals (n=12) were provided with a permanent silicon catheter (0.95 mm OD., 0.50 mm ID.) in the right atrium. The catheter was inserted via the right jugular vein and externalized on the top of the skull according to the techniques described earlier (49). These catheters allow frequent blood sampling in unrestrained and undisturbed freely moving rats (55). After surgery, the rats were allowed to recover for one week before the start of the experiments. During this week animals were handled and connected to the blood sampling tubing twice to habituate to the sampling procedure.
Blood sampling procedure

Blood samples of 0.45 ml were taken at each sampling point. After each sample the same quantity of heparinized (25 units per ml) donor blood was given to minimize the changes in blood volume with related changes in hemodynamics (49).

Experimental procedure

Neuroendocrine effects of AVP.

All experiments were performed between 09.00 and 13.30 hr, -i.e. in the period of stable and low plasma levels of E, NE and CORT (13). Six rats were injected subcutaneously (SC) with AVP. Saline (SAL) was administered to 6 other animals as a control treatment. At least one hour before the start of the experiment the animals were connected to the sampling tubing in order to obtain reliable, stress-free basal values. Blood samples were taken in the homecage before and after AVP or SAL administration (at t=0 min). Basal samples were taken at t=-11 and t=-1 min. Neuroendocrine and glucose response measures were taken at t=5, 10, 40, 60, 70, 77, and 92 min.

The effects of AVP on endocrine responses to the mild stress of environmental change were studied one week later in the same animals. Basal blood samples were taken at -11 and -1 min. At t=0 min SAL or AVP was administered. Stress was presented one hour after administration of the peptide. At t=60 min the homecage of each rat was transferred to another, similar room where a constant background "white" noise (65 dB, 2-8 kHz) was produced by a noise generator. During the transfer, which lasted for about 2 min, rats were not handled. Stress values were sampled at t=65; 70; 77; and 92 min. A delay of one hour before stress presentation was chosen to interpret the results in comparison with studies that have considered the effects of SC applied AVP on retention or autonomic functioning. In these studies the peptide was often administered ±1 hr prior to the test (2,8). It has further been shown that the pressor effect of AVP in the dosage as used returned to within the normal range after 1 hour (38,40).

Behavioral effects of AVP.

In the other group of rats (n=12) behavioral effects of systemically applied AVP were studied in resting and stress conditions. The experiment was started when all animals were at rest, -i.e. with high scores for resting behavior and low scores for activity and grooming behavior. After administration of the vehicle or AVP, behavior was sampled in every 10 sec. The observer was not familiar with the treatment schedule. After a preliminary analysis 8 separately recorded behavioral elements were pooled in three categories. Sniffing with head movements, walking and rearing were considered to represent active behavior. Face and fur washing, scratching and genital grooming were considered to be grooming behavior. Sitting with eyes open or eyes closed, and sleeping were considered as resting behavior, because the majority of behavioral samples in this category was classified as sleeping behavior. Sixty behavioral scores were added in blocks of 10 min.
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Treatment

Arginine-8-vasopressin (AVP) was dissolved in saline and injected subcutaneously (SC) in a dose of 6 µg/kg b.wt. at t=0 min. Saline (SAL) injections (1 ml/kg b.wt.) served as vehicle. The selection of the single dose of the peptide was based on both the results of previous studies of young and aged rats' cardiac response to emotional stress (4,8) and on several other studies indicating well defined behavioral effects of AVP (19,34,38,39). EEG recordings (18) further indicated a similarity between this SC dose and a "behaviorally relevant" intracerebroventricular dose of 1 ng. The rats were injected in a cross-over design with SAL or AVP. A 7-day wash-out period was allowed between injections in resting and stress conditions to minimize interactions of treatments.

Chemical determinations.

Blood samples of 0.45 ml were withdrawn for determination of plasma epinephrine (E), norepinephrine (NE) and corticosterone (CORT) concentration. The samples were immediately transferred to chilled (0°C) centrifuge tubes containing 0.01 % EDTA as antioxidant and 10 µl heparin solution (500 IU/ml) as anticoagulant. Blood was centrifuged at 4°C for 10 min at 5000 rpm, and 100 µl of the supernatant were stored at -20°C for corticosterone and at -80°C for the catecholamine measurements. Plasma CORT was measured by means of reversed phase high performance liquid chromatography, as described earlier (12). Determination of plasma catecholamine concentrations was performed by HPLC in combination with electrochemical detection (ECD) as described earlier (48), with minor modifications. The absolute detection limit for catecholamines is 0.5 pg per injection with a signal to noise ratio of 2.

Statistics

Results are presented as means ± SEM. Data were analyzed using a multivariate analysis of variance with repeated measures (MANOVA) followed by a two-tailed Student's t-test, or a Mann-Whitney U-test. Since stress responses were measured 60-90 min after administration of the substances, MANOVA testing was divided in analyzing the first 60 min and the last 30 min (60-90 min). A probability level of p<0.05 was taken as statistical significance for all tests.
In the left panel the effects of SAL (○-○) or AVP (●-●) administration on mean increases/decreases in blood glucose level (IA), plasma CORT (IB), NE (IC), and E (ID) levels in resting condition are shown. The right panel shows the effects of transfer of the home cage to a novel environment on alterations in glucose (IE), CORT (IF), NE (IG), and E (IH) 60 min after administration of SAL or AVP. The time of injection is indicated with an arrow. Means ± SEM of 6 rats are shown. *p<0.05; **p<0.01; ***p<0.001, determined by MANOVA and a two-tailed t-test.
In the left panel the effects of SAL (o-o) or AVP (••) administration on mean active (2A), grooming (2B), and resting behavior (2C) in resting condition are shown. The right panel shows one hour after administration the effects of AVP or SAL on stress induced behavioral changes in activity (2D), grooming (2E), and resting behavior (2F). Behavior was recorded every 10 sec. Since behavior was pooled in blocks of 10 min, each time point includes 60 behavioral recordings per rat. Means ± SEM of 6 rats are shown. *p<0.05; **p<0.01, determined by MANOVA and Mann-Whitney U-test.
RESULTS

Neuroendocrine effects of AVP

Resting condition.

**Blood glucose.** Basal blood glucose levels were equal in SAL- (92±4 mg/dl) and AVP-treated (86±2 mg/dl) animals. During the first 40 min AVP administration caused a strong elevation of blood glucose, with a peak level (23±4 mg/dl) at t=10 min (Fig. 1A). MANOVA indicated a significant treatment effect over the first 60 min after AVP administration, F(1,10)=9.09; p=0.01, and an interaction of treatment with time, F(4,40)=7.78; p=0.0002. However, in SAL- and AVP-treated rats blood glucose increased in a similar way after t=40 min in comparison to pretreatment values (p=0.01). A treatment effect was therefore absent during this period.

**Plasma corticosterone.** Basal CORT level was 1.8±1.2 μg/dl in SAL-treated rats, and 1.0±0.7 μg/dl in AVP-treated animals. Figure 1B shows that AVP administration caused a long lasting increase in CORT secretion. During the first 60 min a treatment effect was present, F(1,8)=17.79; p=0.003, as well as a significant interaction of treatment with time, F(3,24)=13.37; p=0.0001. A peak level (21.8±5.6 μg/dl) was reached at t=20 min. The last 30 min a treatment effect was still present, F(1,9)=39.16; p=0.0003, although CORT level in SAL treated rats increased during this period in comparison to pretreatment values, F(4,20)=4.52; p=0.009.

**Plasma norepinephrine.** Basal levels of plasma NE were similar in SAL- (182±25 pg/ml) and AVP-treated (156±22 pg/ml) rats. Figure 1C shows that AVP caused a strong decrease in plasma NE level. This effect was significant during the first hour resulting in a treatment effect, F(1,8)=10.38; p=0.01, and a significant interaction of treatment with time, F(4,32)=5.53; p=0.002. The lowest NE level was reached 20 min after AVP was administered (-150±17 pg/ml). The last 30 min NE levels in AVP treated rats were back to baseline.

**Plasma epinephrine.** Basal plasma E level was 4.5±2.8 pg/ml in SAL-, and 4.5±4.5 pg/ml in AVP-administered rats. Neither AVP nor SAL treatment did cause a change in plasma level of E (Fig. 1D).

Stress condition

**Blood glucose.** Transportation of the home cage to another environment 60 min after administration of SAL or AVP caused in both SAL-and AVP-treated animals a significant
(\(p<0.0001\)) increase in blood glucose, with peak levels reached at the 15\textsuperscript{th} min (\(t=77\) min) in the novel environment (Fig. 1E). AVP treatment per se failed to affect the glucose response to stress.

**Plasma corticosterone.** Stress of transfer caused an increase in plasma CORT in both SAL-, \(F(4.8)=5.59; p=0.02\), and AVP-treated rats, \(F(4.8)=46.7; p=0.0001\), as indicated in figure 1F. Although a treatment effect was present, \(F(1,4)=8.43; p=0.04\), it was probably caused by the higher initial CORT level on \(t=60\) min after AVP administration. The absence of a significant interaction between treatment and time, after transportation, indicates that AVP failed to alter the stress induced CORT response. Peak levels in both SAL- (20.7±9.6 \(\mu g/dl\)) and AVP-administered (31.4±1 \(\mu g/dl\)) rats were reached at \(t=92\) min.

**Plasma norepinephrine.** One hour after the application of the vehicle or AVP, transportation stress caused a sharp and brief increase in plasma NE in SAL- (263±92 pg/ml) but not in AVP-treated animals (Fig. 1G), resulting in a significant interaction between treatment and time, \(F(3,30)=4.52; p=0.01\). Five min later plasma NE in vehicle-treated animals was back to baseline level.

**Plasma epinephrine.** Figure 1H shows that the transportation stress caused a very small increase in E in both groups of animals (\(p=0.04\)). Peak levels were 69.3±25.3 pg/ml in SAL- and 56.2±27.8 pg/ml in AVP-treated rats. Vasopressin did not elicit a treatment effect on E secretion.

**Behavioral effects of AVP**

**Resting condition**

*Active behavior.* Figure 2A shows that injection per se resulted in an increase in active behavior that was similar in SAL- and AVP-treated animals. The reduction of activity in time was also similar after both treatments.

*Grooming behavior.* The injection procedure caused some initial grooming behavior in both vehicle and AVP treated animals (Fig. 2B). This behavior diminished during the first 30 min in SAL-treated rats. Grooming behavior remained elevated in AVP-administered animals until \(t=60\) min, resulting in a significant treatment effect over the first hour, \(F(1,10)=11.47; p=0.009\). The last 30 min the vasopressin treatment effect was absent. No interaction between treatment and time was observed.

*Resting behavior.* During the first 10 min the amount of time spent resting was relatively low (Fig. 2C). Subsequently, resting behavior increased up to near peak level during the first 30
min in SAL-treated rats. This coincided a decrease in grooming behavior. The increase in resting behavior exhibited after AVP administration was much less and remained below that of the vehicle-treated rats up to sixty min. After 60 min the major behavioral components in both groups were equal. MANOVA over the first hour shows a treatment effect, $F(1,10) = 11.47; p = 0.009$. The treatment effect of AVP was absent during $t=60-92$ min.

**Stress condition**

*Active behavior.* Figure 2D shows that transportation to a novel environment 60 min after administration of SAL or AVP caused an initial increase in active behavior that was similar in both groups. Active behavior after AVP administration, however, progressively decreased, while activity in vehicle-treated animals remained elevated during the 30 min observation period. This is reflected in a vasopressinergic treatment effect, $F(1,10) = 7.14; p = 0.02$.

*Grooming behavior.* Transfer stress did not elicit substantial grooming behavior (Fig. 2E) when compared to the same period in resting conditions. MANOVA failed to show a treatment effect either.

*Resting behavior.* Figure 2F shows that both groups spent the same amount of time resting during the first 10 min after home cage transfer. In AVP-treated rats, however, the time spent resting progressively increased, while this increase was less in vehicle-treated animals. Statistical analysis yielded a treatment effect that was close to significance, $F(1,10) = 4.2; p = 0.06$.

**DISCUSSION**

The main findings were that peripherally administered AVP has differential neuroendocrine and behavioral effects, both during resting and mild stress conditions. Administration of AVP caused an increase in blood glucose level and CORT secretion, and markedly depressed NE level during resting conditions. It did not affect adrenal medullary E secretion. AVP caused a 60 min lasting increase in grooming behavior with a concomitant decrease in time spent resting during this period. Sixty min after administration the increase in active behavior caused by the mild emotional stress of transportation and placement in a novel environment extinguished more rapidly in AVP- than in vehicle-treated control rats. The stress induced sympathetic activation was inhibited one hour after AVP administration as indicated by the absence of a plasma NE response.

The observed physiological and behavioral effects of AVP during resting condition
are particularly apparent within the first hour after systemic injection, similar to previously reported pressor effects caused by this dose of AVP (38). Some of the effects observed after AVP administration may be indirectly caused by changes in one of the other presented variables.

During resting conditions AVP caused a sharp initial increase in blood glucose followed by a second and slower increase in blood glucose in both SAL- and AVP-treated rats. Since plasma NE decreased and E remained stable after AVP administration, the first rise in glucose is probably caused by the direct action of AVP on hepatic glycogenolysis (51). The second phase in the glucose enhancement after AVP administration cannot be easily explained because it is also present in the controls.

The stimulating effect of AVP on the secretion of adrenocorticotropic (ACTH), inducing adrenal CORT secretion is well known (9,20,41). The stress induced CORT response, however, was not potentiated by AVP pre-treatment, although CORT level at the onset of the stress was much higher.

AVP diminished plasma NE levels during resting conditions. This probably is caused by the inhibitory action of circulating AVP on sympathetic preganglionic neurons (26,28,32,37), although an increased utilization or degradation rate of NE after AVP cannot be excluded. Since sympathetic ganglia are protected by a blood-nerve barrier (46), a direct inhibitory action of systemically administered AVP on sympathetic preganglionic neurons implies transportation of AVP over this barrier. The ganglionic sympatho-inhibitory vasopressinergic effect does not exclude additional central actions of systemically applied AVP. It has been reported that circulating AVP acts at the level of the area postrema and locus coeruleus by altering noradrenergic function, and enhances in this way the inhibitory influence on the sympathetic system (45,53). One hour after AVP administration, plasma NE returned to the baseline level. Stress, however, at that moment failed to enhance sympathetic activity in AVP-treated animals, indicating that the inhibition of sympathetic activation is still present.

The finding that AVP fails to affect adrenal medullary E secretion in both resting and stress conditions supports the results obtained by Kvetnansky et al. (37).

Some of the endocrine and metabolic alterations caused by AVP may play a role in the behavioral properties of systemically administered AVP. Increases in blood glucose level similar in magnitude to the observed AVP-induced rise in glucose, have been associated with enhanced memory performance (21,54). The glucose enhancing effect shortly after administration of AVP may therefore play a role in earlier reported posttrial learning modulating properties of systemically administered AVP (17,24). Whether the enhancement of CORT secretion by AVP plays a role in the vasopressinergic modulation of cognitive functioning is not clear yet. The effects of CORT and AVP on extinction of inhibitory avoidance are even opposite (3,5). Postlearning administration of CORT facilitates, however, the consolidation of acquired immobility in the Porsolt swimming test (29). CORT further was shown to reduce the efficacy of E to consolidate a passive avoidance response (14). Since AVP and CORT are reported to have a common central site of action in the
hippocampal norepinephrine-stimulation of cyclic AMP formation (42), an interaction between AVP and CORT actions, however, cannot be excluded.

Previous studies showed that memory enhancing actions of AVP are absent in adrenomedullectomized rats (6). These and other data of Borrell et al. (7) indicated that AVP requires the presence of peripheral E in order to enhance memory function. The present study shows that spontaneous behavior during resting and stress conditions are affected by AVP in the absence of a detectable E response, suggesting that the AVP action on behavior is not mediated via an increased E release. Although less potent then E, systemic injections of NE can enhance retention in rats (23). The vasopressinergic inhibition of sympathetic activity as observed in this study suggest that cognitive effects of the peptide cannot be explained by changes in the levels of plasma NE.

Several observations in this laboratory demonstrated the vasopressinergic enhancement of parasympathetic cardiac stress responses (2,8,25). The inhibitory action of AVP on stress induced sympathetic activation may be of major importance in the potentiating effect of AVP on the vagally mediated bradycardiac response to stressors evoking behavioral immobility (2,8,25). During stressors of this nature sympathetic and parasympathetic outflow are activated in parallel. Together with an increased vagal activity following systemical application of AVP (11), the vasopressinergic blockade of stress induced sympathetic activity may cause the autonomic balance to shift towards the parasympathetic side, leading to a dominant vagal action on the heart.

In resting condition, the elevation of grooming behavior and the associated decrease in time spent resting appeared as major behavioral effects of AVP. These changes diminished one hour after peptide treatment. Induction of grooming behavior immediately after ICV administration of AVP has been reported earlier (44). The early post-treatment period of excessive grooming as caused by AVP coincided multiple physiological and endocrine changes. Excessive grooming may represent a displacement activity during an increased arousal state and serve restoration of the homeostatic status (10,30).

A remarkable effect of AVP is the rapid termination of the stress-induced behavioral activation. This effect of the peptide was visible after the disappearance of the intrinsic behavioral actions of AVP. Since glucocorticoids are implicated as modulators of extinction of stress-related behavior (1), the long-lasting elevation of CORT level in AVP-treated rats may play a role in this accelerated "dearousal".

Stress is often associated with increased grooming behavior (31). After the mild stress of transfer of the home cage, grooming behavior was not elevated when compared to the same period under resting conditions. The avoidance of handling the animals is probably responsible for this very low grooming activity.

Together the state- and time dependent modulation of spontaneous behavior suggest that AVP has arousing properties shortly after administration when marked pressor (38) and endocrine vasopressinergic effects are apparent. In a later phase AVP may facilitate dearousal mechanisms following stress-induced behavioral activation.

The neuroendocrine changes indicate that AVP may modulate cognitive functioning
not only by its hypothesized direct action on the brain or by its systemic pressor effect, but also by enhancing the level of blood glucose and adrenal CORT secretion.

REFERENCES


