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Vasopressin Levels in Peripheral Blood and in Cerebrospinal Fluid During Passive and Active Avoidance Behavior in Rats[1]

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Vasopressin (AVP) levels were measured in plasma and cerebrospinal fluid (CSF) of rats during acquisition and retention of a passive avoidance response. Only 5 min after the onset of the retention session a significantly higher level of AVP was found in plasma of animals which displayed a long latency, as compared with the levels of animals which showed a weak passive avoidance response (short latencies), or no passive avoidance behavior at all (controls). Moreover no changes in plasma AVP levels were found in plasma of rats submitted to acquisition or extinction of an active avoidance response. It is suggested that, although an elevated plasma AVP level is associated with strong retention of a passive avoidance response the peripheral circulation as well as the CSF are of minor importance for the transport of this neuropeptide to its site of behavioral action.

INTRODUCTION

During the last decade conclusive data have been obtained about the effect of the neurohypophysial hormone arginine-8-vasopressin (AVP) on the maintenance of avoidance behavior of rats. Administration of AVP induces a dose-dependent long lasting inhibition of extinction of active avoidance behavior and an improvement of passive avoidance behavior (Van Wimersma Greidanus and De Wied, 1977). Concerning endogenous AVP it has been shown that Brattleboro rats, homozygous for diabetes insipidus, which lack AVP completely, show disturbances in avoidance behavior (De Wied, Bohus, and Van Wimersma Greidanus, 1975; Bohus, Van Wimersma Greidanus, and De Wied, 1976; Bailey and Weiss, 1979) and similar phenomena are observed in Wistar rats treated intracere-

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broventricularly (i.c.v.) with AVP-antibodies in order to neutralize bioavailable AVP in the brain (Van Wimersma Greidanus, Dogterom, and De Wied, 1975; Van Wimersma Greidanus, Bohus, and De Wied, 1975a, Bohus, Urban, Van Wimersma Greidanus, and De Wied, 1978). In search for a physiological role of endogenous vasopressin in avoidance behavior it was felt necessary to determine AVP levels in cerebrospinal fluid (CSF) and in peripheral blood of rats submitted to behavioral manipulation.

MATERIALS AND METHODS

Animals. Male rats of an inbred Wistar strain (CPB-TNO, Zeist, The Netherlands) weighing 110–130 g were used in active avoidance behavior and of 150–170 g in passive avoidance behavior. The rats were maintained five per cage with food and water *ad libitum* and the lights were on between 5 AM and 7 PM. They were transported on each day of the experimental procedure from the animal house to the experimental room.

For collecting CSF a polyethylene cannula was inserted into the right lateral ventricle of the brain, fixed with dental cement and two stainless screws (Brakkee, Wiegant, and Gispen, 1979). These rats were allowed to recover from this operation for a period of 3–4 days, while they were singly housed.

Behavioral experiments. Active avoidance behavior was studied in a pole jumping avoidance situation (Van Wimersma Greidanus and De Wied, 1971). Animals were trained to jump onto a pole placed vertically in the box, in order to escape or avoid a scrambled electric foot shock (EFS, 0.25 mA) which could be presented through the grid floor of the box. As conditioned stimulus (CS) light emitted by a 60 W bulb placed on top of the box was used. The CS was presented 5 sec prior to the unconditioned stimulus (US) of EFS. As soon as the animals escaped or avoided the EFS the light was switched off.

Ten acquisition trials were presented during 3 consecutive days with a mean intertrial interval of 60 sec. On Day 4 extinction trials were run during which the EFS was not applied anymore.

Trunk blood was collected immediately after the three acquisition sessions and after the extinction session as well as after a 3-min exposure to the pole jumping box on the Days 1, 2, 3, and 4. In addition, basal AVP levels were determined.

The passive avoidance procedure was adopted from Ader *et al.* (Ader, Weijnen, and Moleman, 1972) with minor modifications. The animals were adapted to enter a dark compartment from an illuminated platform. Adaptation consisted of one trial on the 1st day and three trials on the 2nd day. After the last adaptation trial on the 2nd day the animals received an inescapable EFS of 3 seconds in the dark compartment. Three different shock intensities were used in the various groups of animals: 0.00 mA, 0.25 mA, and 1.00 mA during 3 sec. Latencies to enter were recorded

during a retention session 24 hr after the single learning trial of EFS with a maximal observation time of 300 sec. Trunk blood was collected at either 5 sec, 1 min, or 5 min after the onset of the retention trial. From one group of rats blood was collected at 5 min after the learning trial (1.00 mA, 3 sec).

CSF was collected from cannulated rats at 5 min after the onset of the retention session. Since only very small amounts (0.1–2 μl) of CSF could be obtained from each animal, several CSF samples obtained from different animals from the same group were pooled together for AVP determination.

Radioimmunoassay (RIA) of vasopressin. AVP was measured after extraction from the plasma with activated Vycor glass powder. The details of the extraction procedure and the RIA are described elsewhere (Dogterom, Van Wimersma Greidanus, and De Wied, 1978). The antiserum used is highly specific for AVP, the cross-reactivity with vasotocin being approximately 0.25% and with oxytocin less than 0.1%. The sensitivity of the standard curve is 0.25 pg/tube allowing measurements of 0.5 pg AVP/ml plasma, using a maximal sample volume of 2 ml. Plasma of Brattleboro rats, homozygous for diabetes insipidus, which lack AVP completely, served as control for aspecific effects in the RIA and for the determination of the recovery of added amounts of standard AVP. The recovery over a wide range of concentrations was 69.4 ± 6.5% (n = 167). The measurements of AVP were performed on the same day as the samples were collected. The results are corrected for recovery. The differences between the AVP levels of various groups of animals during active avoidance behavior and between those of groups with various shock intensities during passive avoidance training were tested for significance using Mann–Whitney's U test or Student's t test. A P value of < 0.05 was considered as significant.

RESULTS

Plasma Levels

Basal AVP levels in peripheral blood prior to active avoidance conditioning amounted to 1.9 ± 0.2 pg and no significant changes were observed during acquisition or extinction of the pole jump avoidance response (Fig. 1). Generally plasma AVP levels tended to be higher prior to the behavioral sessions on Days 1, 2, and 3, than immediately after the acquisition sessions on Days 1, 2, and 3 but these tendencies were not statistically significant differences. Neither AVP level during extinction was different from the other values.

The basal level of AVP in peripheral plasma during passive avoidance retention was 1.7 ± 0.5 pg/ml (n = 9), and generally no differences between the AVP levels were found in the various groups of controls (Table 1). Only the group of animals with the highest shock intensity,

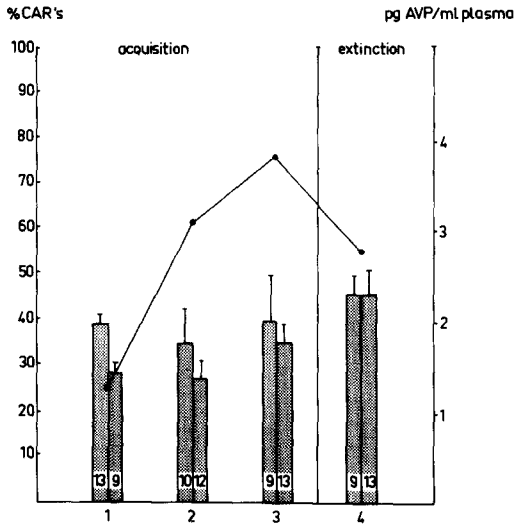

FIG. 1. Plasma AVP levels (vertical bars, right abscissa) during acquisition and extinction of pole jumping avoidance behavior. Behavioral performance (black line) is indicated as percentage conditioned avoidance responses (CARs) (left abscissa). The numbers on the horizontal axis indicate the days of successive behavioral sessions. Each left bar represents the presession level of AVP, whereas each right bar represents the postsession level of AVP. The number in the gray bars indicate the number of animals per group.

which were decapitated after the maximal observation time, showed a significantly higher AVP value as compared to the non- and low-shocked groups (see Table 1). The same tendency was seen in the group that was decapitated 5 sec after the beginning of the retention trial, but this difference was not significant. AVP levels in peripheral plasma of a group of rats decapitated at 5 min after a shock of 1.00 mA were the same as the basal level: 1.5 ± 0.2 pg/ml (n = 9).

CSF Levels

AVP levels in the CSF as collected from the lateral ventricle of the brain appeared to be much higher than the levels in the peripheral blood and also higher than the levels determined in the CSF collected from the cisterna magna (11.5 pg AVP/ml, Dogterom, Van Wimersma Greidanus, and Swaab, 1977). However, no significant differences between AVP levels in the CSF collected from the lateral ventricle could be found in the various groups of animals during retention of the passive avoidance response. Whereas the median avoidance latencies varied from 7 to >300 sec, the AVP levels ranged from 34.8 ± 11.8 pg/100 μl (no shock) to 44.1 ± 10.5 pg/100 μl (high shock) (see Table 2).

TABLE 1
Median Latency Scores during Retention of a Passive Avoidance Response after Different Shock Levels and the AVP Levels in Peripheral Plasma Collected 5 sec, 1 min, or 5 min after the Onset of the Retention Session

Shock intensity:	0.00 mA		0.25 mA		1.00 mA	
Maximal length of the retention session	Median latency (sec)	pg AVP/ml[a]	Median latency (sec)	pg AVP/ml[a]	Latency scores (sec)	pg AVP/ml[a]
5 sec	4	1.8 ± 0.4 (n = 10)	—	2.2 ± 0.5 (n = 10)	—	2.6 ± 0.5 (n = 9)
1 min	3	1.5 ± 0.4 (n = 9)	21	2.1 ± 0.7 (n = 8)	—	1.5 ± 0.2 (n = 10)
5 min	3	1.7 ± 0.1 (n = 11)	21	1.2 ± 0.1 (n = 13)	300	3.0 ± 0.5[b] (n = 14)

[a] Mean ± SEM.
[b] $P < 0.025$, 1.00 mA versus 0.00 mA.
$P < 0.005$, 100 mA versus 0.25 mA.

TABLE 2
Median Latency Scores during Retention of a Passive Avoidance Response after Different Shock Levels, and the AVP Levels in CSF as Measured 5 min after the Onset of the Retention Session

Shock intensity (mA)	Median latency (sec)	pg AVP/100 μl[a]
0.00	7 (22)[b]	34.8 ± 11.8 [7][c]
0.25	40 (25)	38.9 ± 9.5 [4]
1.00	>300 (15)	44.1 ± 10.5 [6]

[a] Mean ± SEM.
[b] Number of animals per group in parentheses.
[c] Number of pooled CSF samples in brackets.

DISCUSSION

It is generally accepted that emotional stimuli induce changes of the release of hormones from the anterior as well as from the posterior lobe of the pituitary. This includes an increase of the release of α-MSH, ACTH, and prolactin (Mason, 1968; Seggie and Brown, 1975) and of AVP and oxytocin (O'Connor and Verney, 1942; Pickford, 1969) and a decrease of the release of growth hormone in the rat (Seggie and Brown, 1975). These processes are interpreted as an adaptation mechanism to sustain the organism's homeostasis according to the concept of Selye (1950). Since it has well been established that ACTH, MSH, and AVP have direct effects on brain mechanisms related to learning, motivation, and memory (De Wied, 1974; De Wied, Bohus, Gispen, Urban, and Van Wimersma Greidanus, 1976; Miller, Kastin, and Sandman, 1977; Van Wimersma Greidanus and De Wied, 1977) it was proposed that an increased release of α-MSH (Sandman, Kastin, Schally, Kendall, and Miller, 1973) and of AVP (Thompson and De Wied, 1973) is related to these processes. Indeed a relationship was found between antidiuretic activity of eye plexus blood of rats and their passive avoidance behavior (Thompson and De Wied, 1973). The data presented in this study do not confirm such a relationship between the plasma AVP levels and behavioral parameters, though a significantly augmented level was found in peripheral plasma of highly shocked animals at 5 min after the beginning of the retention trial, associated with long avoidance latencies. Animals decapitated at 5 min after a 1.00 mA shock showed AVP levels which were not different from control values. Husain, Manger, Weiss, Hart, and Franz (1976) reported that several other noxious stimuli like loud noise and forced exercise did not cause a rise of plasma AVP levels. Thus, it may be that the concept that emotional stimuli generally cause an increase of plasma AVP levels

has to be modified. In addition the peripheral circulation as a route of transport for behaviorally active peptides seems not the most likely way.

CSF levels of AVP were not changed at 5 min after the onset of the retention session. Although direct release of AVP into the CSF of rats is likely to occur (Dogterom, Van Wimersma Greidanus, and Swaab, 1977) the absence of a relationship between AVP levels in CSF and passive avoidance latencies scores makes this transport route probably of less importance. However correlation between individual attributes could not be computed.

The recent demonstration of vasopressin-containing fibers in extrahypothalamic structures such as septum, hippocampus, and amygdala of rats (Buys, 1978; Buys, Swaab, Dogterom, and Van Leeuwen, 1978; Kozlowski, Brownfield, and Hostetter, 1978) strongly suggests that direct connections exist between hormone producing cells in the hypothalamic nuclei and these brain areas which all seem to be important for the behavioral effect of AVP (Van Wimersma Greidanus, Bohus and De Wied, 1975b; Van Wimersma Greidanus and De Wied, 1977; Van Wimersma Greidanus, Croiset, Bakker, and Bouman, 1979). These observations favor the view that peptides (i.e., vasopressin) may be released in the CNS in a similar way as neurotransmitters. The measurement of vasopressin in discrete brain areas during behavioral processes is needed in order to give more information on the physiological function of AVP in this respect.

REFERENCES

Ader, R., Weijnen, J. A. W. M., and Moleman, P. (1972). Retention of a passive avoidance response as a function of the intensity and duration of electric shock. *Psychon. Sci.* **26,** 125–129.

Bailey, W. H., and Weiss, J. M. (1979). Evaluation of a "memory deficit" in vasopressin deficient rats. *Brain Res.* **162,** 174–178.

Bohus, B., Van Wimersma Greidanus, Tj. B., and De Wied, D. (1976). Behavioral and endocrine responses of rats with hereditary hypothalamic diabetes insipidus (Brattleboro strain). *Physiol. Behav.* **14,** 609–615.

Bohus, B., Urban, I., Van Wimersma Greidanus, Tj. B., and De Wied, D. (1978). Opposite effects of oxytocin and vasopressin on avoidance behaviour and hippocampal theta rhythm in the rat. *Neuropharmacology* **17,** 239–247.

Brakkee, J. H., Wiegant, V. M., and Gispen, W. H. (1979). A simple technique for rapid implantation of a permanent cannula into the rat brain ventricular system. *Lab. Animal Sci.* **29,** 78–81.

Buys, R. M. (1978). Intra- and extrahypothalamic vasopressin and oxytocin pathways in the rat. *J. Cell. Tissue Res.* **192,** 423–435.

Buys, R. M., Swaab, D. F., Dogterom, J., and Van Leeuwen, F. W. (1978). Intra- and extrahypothalamic vasopressin and oxytocin pathways in the rat. *J. Cell. Tissue Res.* **186,** 423–433.

Dogterom, J., Van Wimersma Greidanus, Tj. B., and Swaab, D. F. (1977). Evidence for the release of vasopressin and oxytocin into cerebrospinal fluid: Measurements in plasma and CSF of intact and hypophysectomized rats. *Neuroendocrinology* **24,** 108–118.

Dogterom, J., Van Wimersma Greidanus, Tj. B., and De Wied, D. (1978). Vasopressin in cerebrospinal fluid and plasma of man, dog and rat. *Amer. J. Physiol.* **234,** E463–467.

Husain, M. K., Manger, W. M., Weiss, R., Hart, C., and Franz, A. G. (1976). Vasopressin release in the rat after manual restraint: An effect of abdominal compression and not of stress, Abstract, *Vth International* Congress of Endocrinology, Hamburg, July 18–24, No. 481.

Kozlowski, G. P., Brownfield, M. S., and Hostetter, G. (1978). Neurosecretory supply to extrahypothalamic structures: Choroid plexus, circumventricular organs and limbic system. *In* W. Bargmann, A. Oksche, A. Polenov, and B. Scharrer (Eds.), *Neurosecretion and Neuroendocrine Activity*, pp. 217–227. Springer-Verlag, Berlin.

Mason, J. W. (1968). A review of psychoendocrine research on the pituitary adrenal cortical system. *Psychosomatic Med.* **38,** 576–607.

Miller, L. H., Kastin, A. J., and Sandman, L. A. (1977). The psychobiological actions of MSH in man. *In* F. J. H. Tilders, D. F. Swaab, and Tj. B. Van Wimersma Greidanus (Eds.), *Control, Chemistry and Effects of α-MSH: Front. Horm. Res.*, Vol. 4, pp. 153–161. Karger, Basel.

O'Connor, W. J., and Verney, E. B. (1942). The effect of removal of the posterior lobe of the pituitary on the inhibition of water diuresis by emotional stress. *Quart. J. Exp. Physiol.* **31,** 393–408.

Pickford, M. (1969). Neurohypophysis—antidiuretic (vasopressor) and oxytocic hormones. *In* W. Haymaker, E. Anderson, and W. J. H. Nauta (Eds.), *The hypothalamus*, pp. 463–505. Thomas, Springfield, Ill.

Sandman, C. A., Kastin, A. J., Schally, A. V., Kendall, J. W., and Miller, L. M. (1973). Neuroendocrine responses to physical and psychological stress. *J. Comp. Physiol. Psychol.* **84,** 386–390.

Seggie, J. A., and Brown, G. M. (1975). Stress response patterns of plasma corticosterone, prolactin, and growth hormone, following handling as exposure to novel environment. *Canad. J. Physiol. Pharmacol.* **53,** 629–637.

Selye, H. (1950). *The Physiology and Pathology of Exposure to Stress*, p. 6. Acta Inc., Montreal, Canada.

Thompson, E. A., and De Wied, D. (1973). The relationship between the antidiuretic activity of rat eye plexus blood and passive avoidance behavior. *Physiol. Behav.* **11,** 377–380.

De Wied, D. (1974). Pituitary-adrenal system hormones and behavior. *In* F. O. Schmitt and F. G. Worden (Eds.), *The Neurosciences; 3rd Study Program*, pp. 653–666. MIT Press, Cambridge, Mass.

De Wied, D., Bohus, B., and Van Wimersma Greidanus, Tj. B. (1975). Memory deficit in rats with hereditary diabetes insipidus. *Brain Res.* **85,** 152–156.

De Wied, D., Bohus, B., Gispen, W. H., Urban, I., and Van Wimersma Greidanus, Tj. B. (1976). Hormonal influences on motivational, learning, and memory processes. *In* E. J. Sachar (Ed.), *Hormones, Behavior and Psychopathology*, pp. 1–14. Raven Press, New York.

Van Wimersma Greidanus, Tj. B., and De Wied, D. (1971). Effects of systemic and intracerebral administration of two opposite acting ACTH related peptides on extinction of conditioned avoidance behavior. *Neuroendocrinology* **7,** 291–301.

Van Wimersma Greidanus, Tj. B., and De Wied, D. (1977). The physiology of the neurohypophyseal system and its relation to memory processes. *In* A. N. Davison (Ed.), *Biochemical Correlates of Brain Structure and Function*, pp. 215–248. Academic Press, New York.

Van Wimersma Greidanus, Tj. B., Bohus, B., and De Wied, D. (1975a). The role of vasopressin in memory processes. *In* W. H. Gispen, Tj. B. Van Wimersma Greidanus, B. Bohus, and De Wied (Eds.), *Hormones, Homeostasis and the Brain, Progress in Brain Research 42*, pp. 135–141. Elsevier, Amsterdam.

Van Wimersma Greidanus, Tj. B., Bohus, B., and De Wied, D. (1975b). CNS sites of action of ACTH, MSH and vasopressin in relation to avoidance behavior. *In* W. E. Stumpf and L. D. Grant (Eds.), *Anatomical Neuroendocrinology*, pp. 284–289. Karger, Basel.

Van Wimersma Greidanus, Tj. B., Dogterom, J., and De Wied, D. (1975). Intraventricular administration of anti-vasopressin serum inhibits memory consolidation in rats. *Life Sci.* **16,** 637–644.

Van Wimersma Greidanus, Tj. B., Croiset, G., Bakker, E. A. D., and Bouman, H. J. (1979). Amygdaloid lesions block the effect of neuropeptides (vasopressin, $ACTH_{4-10}$) on avoidance behavior. *Physiol. Behav.* **22,** 291–295.