Vasopressin and Memory Consolidation

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INTRODUCTION

Vasopressin is one of the hormones which is stored and released by the posterior lobe of the pituitary. The name vasopressin is based on the pressor action of the hormone which is caused by contraction of peripheral blood vessels. However, in much lower concentrations it acts to conserve body water by concentrating the urine, thus reducing the amount of water required to excrete waste solutes. The term antidiuretic hormone (ADH) may therefore be more adequate for this substance.

ADH is a nonapeptide which is synthesized in cell bodies of neurosecretory neurons, situated in the anterior hypothalamus, in particular in the supraoptic and paraventricular nuclei (Sachs et al., 1969; Scharrer, pp. 125–137 in this volume). It is bound to a specific protein, neurohypophysin II, which is found throughout the entire neurosecretory neuron. The hormone protein complex is packed in granules from which it is released by exocytosis, which involves fusion of the granule with the plasma membrane and subsequent diffusion of its contents into the bloodstream (Douglas, 1973; Pickering, pp. 161–179 in this volume). High quantities of vasopressin-neurophysin have also been found in the hypophyseal portal vessel system (Zimmermann et al., 1973). In addition, morphological evidence points to connections between neurosecretory cells and the infundibular recess of the third ventricle (Wittkowski, 1968). These findings indicate that vasopressin may be secreted into portal blood and cerebrospinal fluid (CSF) from neuron terminals (Goldsmith and Zimmermann, 1975). Thus, the hypothalamic-neurohypophyseal system not only uses the general circulation for peripheral effects of posterior pituitary principles, but also the portal vessel system for regulation of anterior pituitary function, and the CSF for action on the central nervous system.

The latter function has been disclosed only recently, and is involved in memory processes. Memory is defined here as the retention of acquired behavior. According to current points of view memory storage in general comprises at least two stages. The first stage represents a very short period immediately after the learning experience, and involves electrical events with metabolic processes which accompany these events. This stage can be erased

easily. It is transformed into a stage of a more permanent character, the so-called "long-term" memory. This consolidation process is accompanied by growth of axon collaterals, inducing changes in connectivity within the neural network, and also involves metabolic changes (Entingh et al., 1975; Matthies, 1974; Perumal et al., 1975).

EFFECT OF VASOPRESSIN ANALOGUES ON ACQUISITION AND MAINTENANCE OF ACTIVE AND PASSIVE AVOIDANCE BEHAVIOR

It is probably the consolidating phase of the memory process which is affected by vasopressin and its analogues. This notion arose from our observations in the early sixties when it was found that removal of the posterior lobe of the pituitary interfered with the maintenance of a conditioned avoidance response (de Wied, 1965). Shuttle-box avoidance behavior of posterior lobectomized rats markedly differs from that of sham-operated control animals. Although acquisition of the avoidance response is normal in the posterior lobectomized rat, the rate of extinction is much faster. Pitressin, a relatively crude extract of posterior pituitary origin, in amounts which normalize the water intake of the mildly diabetic posterior lobectomized rat, at the same time restores the ability to maintain non-reinforced avoidance behavior. Pitressin was subsequently found to increase resistance to extinction of shuttle-box avoidance behavior in intact rats (de Wied and Bohus, 1966). The same was found for purified lysine-vasopressin (LVP) on extinction of a pole-jumping one-way avoidance procedure (de Wied, 1971; van Wimersma Greidanus et al., 1973). A single subcutaneous injection of a moderate amount of this peptide increased resistance to extinction of the avoidance response which lasted several days, depending on the dose administered. Since the effect extended beyond the demonstrable presence of the peptide in the body, these studies indicated that vasopressin triggers a long-term effect on the maintenance of avoidance behavior probably by facilitating consolidation.

The inhibitory effect of vasopressin on extinction of active avoidance behavior might be explained by an increase in the general level of activity. However, administration of this peptide does not affect rearing, grooming or ambulation in a so-called "open-field" situation. In addition, vasopressin also affects passive avoidance behavior, as studied in a simple step-through one-trial passive avoidance situation (Ader and de Wied, 1972). In this situation, it facilitates retention of passive avoidance behavior, and the effect once again is of a long-term nature. Subsequent experiments indicated that the behavioral effect of vasopressin is independent of its vasopressor and antidiuretic activities. Desglycinamide-lysine-vasopressin (DG-LVP), which was isolated from hog pituitary material (Lande et al., 1971), is practically devoid of classical endocrine activities (de Wied et al., 1972). It effectively normalizes the lower rate of acquisition of shuttle-box avoidance behavior of hypophysectomized rats and, like vasopressin, it induces a long-term resistance to extinction of active and passive avoidance behavior.

VASOPRESSIN ANALOGUES ON APPROACH BEHAVIOR AND RETROGRADE AMNESIA

Vasopressin analogues do not seem to affect extinction of a food running response in rats. Garrud et al. (1974) failed to observe an effect of LVP or DG-LVP on extinction of a straight runway approach response for food in food-deprived rats. Although DG-LVP is practically devoid of antidiuretic activity, an interaction between the peptide, hunger drive and water metabolism cannot be excluded. However, the behavior of food-deprived rats in a continual punishment situation, which were trained to hold a lever down for 8 sec in order to obtain a food reward, can be affected by vasopressin. When electric shock was introduced after training to a stable level contingent upon the completion of an 8 sec lever hold at the same time as the food, administration of lysine-vasopressin prolonged the time needed to make the next response after each shock, and increased the efficiency with which the animals performed. The latter effect may have been due to the vasopressin-induced increased tendency to freezing (Garrud, 1975). However, vasopressin is also active in approach behavior. It was found to increase resistance to extinction of sexually rewarded learning behavior. Male rats trained in a T-maze to run for a receptive female chose the correct arm of the maze at a significantly higher percentage following DG-LVP treatment after each acquisition session. The effect again is of a long-term nature. Copulation reward appeared to be essential for this effect since non-rewarded rats do not make more correct choices than do placebo-treated animals after cessation of the treatment (Bohus, in preparation). DG-LVP also delays the disappearance of intromission and ejaculatory behavior of male rats following castration. Thus, vasopressin analogues not only affect the maintenance of aversively motivated behavior but also extinction of a hormone determined behavioral pattern. Evidence for an effect on memory processes was further obtained when it appeared that vasopressin analogues could reverse amnesia. Lande et al. (1972) found that DG-LVP protects against puromycin-induced memory loss in mice. Rigter et al. (1974) demonstrated that amnesia for a one trial passive avoidance response in rats, as induced by CO_2 or by electroconvulsive shock, is reversed by treatment with DG-LVP immediately after the learning trial. These authors suggested that the peptide promotes memory consolidation either by facilitating the consolidating process or by protecting memory consolidation from the adverse effects of the amnesic treatment. The possibility that vasopressin influences retrieval was considered as well since DG-LVP exhibited antiamnesic effects also if injected 1 hr prior to the retention test.

BEHAVIORAL AND ASSOCIATED ENDOCRINE DEFECTS IN HEREDITARY DIABETES INSIPIDUS RATS

The availability of rats with hereditary diabetes insipidus (DI) provided a model to study memory function in the absence of vasopressin. Homozygous hereditary DI rats of the Brattleboro strain (HO-DI) lack the ability to

synthetize vasopressin while their heterozygous litter mates (HE-DI) have a relatively normal water metabolism (Valtin, 1967; Valtin et al., 1965; Valtin and Schroeder, 1964). HO-DI rats are inferior in acquiring and maintaining active and passive avoidance behavior (Bohus et al., 1975). The behavioral deficits are most obvious in a step-through one-trial passive avoidance paradigm. The rate of acquisition of a shuttle-box avoidance response is slower in HO-DI rats as compared to that of heterozygous litter mates or homozygous normal rats of the Brattleboro strain. These are identical to Wistar strain rats in shuttle-box avoidance and passive avoidance behavior.

Extinction of the behavior is very rapid in HO-DI and somewhat less rapid in HE-DI as compared to that of Wistar rats. The rate of acquisition of the pole-jumping response appeared to be the same in HO-DI and HE-DI rats but Wistar rats acquire the response much faster. Extinction is very rapid in HO-DI rats, somewhat slower in HE-DI animals and least rapid in the Wistar rats. Memory function of HO-DI rats is completely impaired in a one-trial passive avoidance situation when retention is tested 24 hr or later following shock exposure. Arginine-vasopressin (AVP) and DG-LVP given immediately after the learning trial readily restore passive avoidance behavior in HO-DI rats (de Wied et al., 1975). This favors the hypothesis that memory rather than learning processes are disturbed in the absence of vasopressin. Indeed, HO-DI rats are able to acquire "fear" motivated responses in the shuttle-box or in the pole-jumping test. Furthermore, full retention of passive avoidance behavior is obtained in HO-DI rats when retention is tested shortly after the learning trial. The main disturbance is in the ability to maintain the behavior. The passive avoidance response is only partially absent 3 hr after, and completely gone 24 hr after the learning trial (de Wied et al., 1975; van Wimersma Greidanus et al., 1975). These observations suggest that consolidation of memory is selectively impaired in the absence of vasopressin.

Vasopressin levels in eye plexus blood are augmented during passive avoidance response and the rate of increase is related to avoidance latency, which in turn is related to shock intensity (Thompson and de Wied, 1973). It might be, therefore, that an association between endogenous release of vasopressin and specific environmental cues is of physiological significance in the maintenance of new behavior patterns (Bohus et al., 1972). The pituitary-adrenal response also shows a marked relationship with passive avoidance behavior in DI rats (Fig. 1). At the immediate retention test, when full passive avoidance behavior is displayed by HO-DI rats, an elevation of plasma corticosterone is found which is of the same magnitude as that of HE-DI rats. At the 3 hr retention test a partial avoidance behavior is associated with a reduced corticosterone response, while retention impairment is coupled with the absence of a significant increase in plasma corticosterone (Bohus et al., 1975) These observations, therefore, indicate that the absence of vasopressin in HO-DI rats, which results in an impairment of a psychological mechanism, also results in an impairment of the accompanying endocrine response in an otherwise "fear" provoking environment. This would suggest that the behavioral effect of vasopressin is mediated by ACTH or other pituitary hormones but vasopressin effectively restores avoidance behavior in hypophysectomized rats (Bohus et al., 1973; Lande et al., 1971).

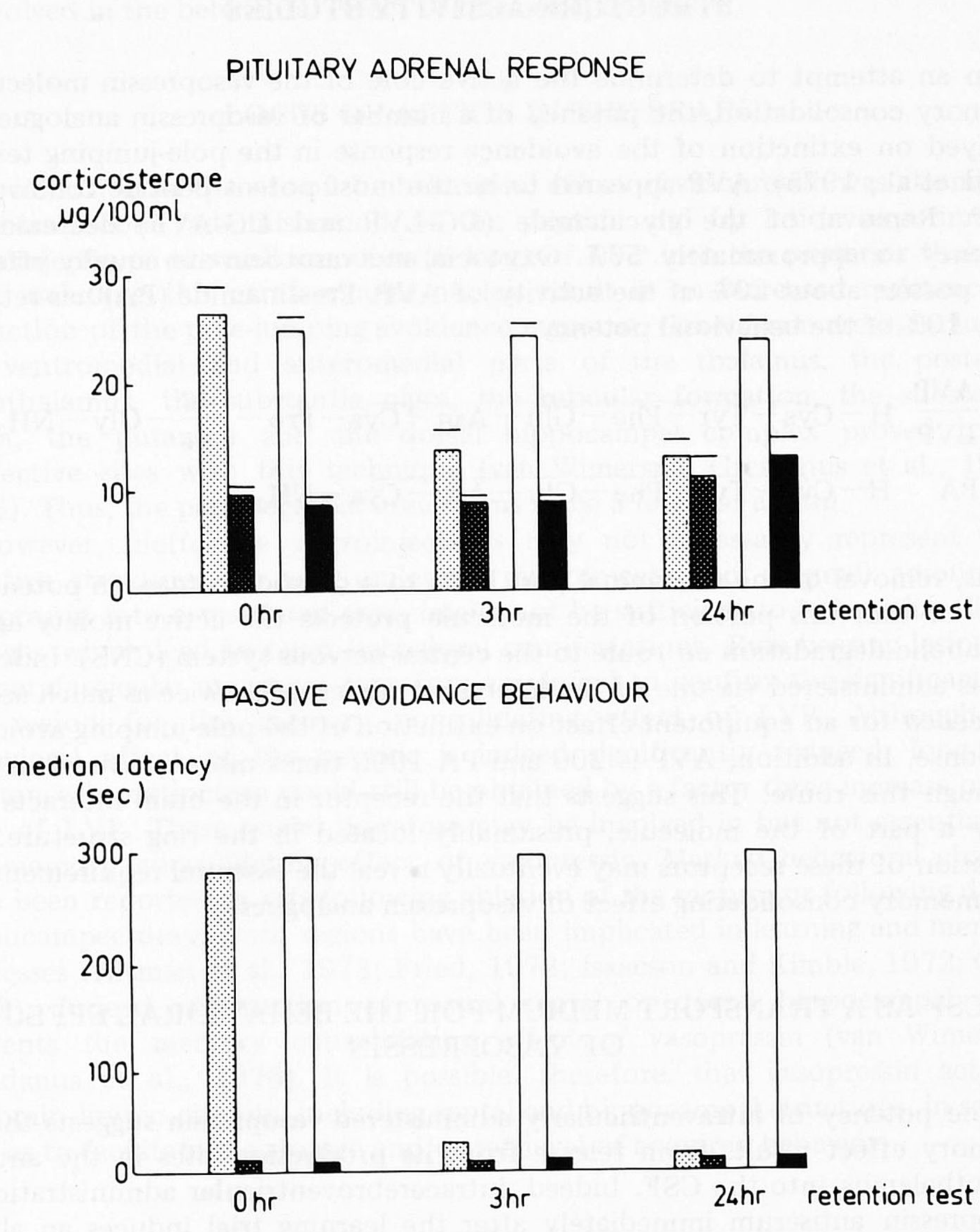

Fig. 1. Plasma corticosterone response in homozygous (HO) and heterozygous (HE) diabetes insipidus (DI) rats during passive avoidance retention. Rats (6–10 per group) were exposed to electric shocks (1 mA) or sham shock for 3 sec in the dark box and tested for retention at various intervals after the learning trial. Plasma corticosterone was determined in trunk blood from rats decapitated 15 min after the onset of the retention test.

STRUCTURE-ACTIVITY STUDIES

In an attempt to determine the active core of the vasopressin molecule in memory consolidation, the potency of a number of vasopressin analogues was assayed on extinction of the avoidance response in the pole-jumping test (de Wied et al., 1975). AVP appeared to be the most potent peptide followed by LVP. Removal of the glycinamide (DG-LVP and DG-AVP) decreases the potency to approximately 50%. Oxytocin and vasotocin are equally effective and possess about 20% of the activity of AVP. Pressinamide (PA) has retained only 10% of the behavioral potency.

$$\frac{\text{AVP}}{\text{LVP}}\quad \text{H}-\overline{\text{Cys}-\text{Tyr}-\text{Phe}-\text{Glu}-\text{Asn}-\text{Cys}}-\text{Pro}-\frac{\text{Arg}}{\text{Lys}}-\text{Gly}-\text{NH}_2$$

$$\text{PA}\quad \text{H}-\overline{\text{Cys}-\text{Tyr}-\text{Phe}-\text{Glu}-\text{Asn}-\text{Cys}}-\text{NH}_2$$

Thus, removal of the C-terminal part leads to a drastic decrease in potency. It may be that this portion of the molecule protects the active moiety against metabolic degradation en route to the central nervous system (CNS). Indeed, if PA is administered via one of the lateral ventricles only twice as much as AVP is needed for an equipotent effect on extinction of the pole-jumping avoidance response. In addition, AVP is 200 and PA 1000 times more active when given through this route. This suggests that the receptor in the brain interacts with only a part of the molecule, presumably located in the ring structure. The isolation of these receptors may eventually reveal the essential requirements for the memory consolidating effect of vasopressin analogues.

CSF AS A TRANSPORT MEDIUM FOR THE BEHAVIORAL EFFECT OF VASOPRESSIN

The potency of intraventricularly administered vasopressin suggests that its memory effect results from release from its production sites in the anterior hypothalamus into the CSF. Indeed, intracerebroventricular administration of vasopressin antiserum immediately after the learning trial induces an almost complete deficit in retention of passive avoidance behavior when tested at various intervals after the learning trial (van Wimersma Greidanus et al., 1975a). In contrast, avoidance latencies of control animals treated with rabbit serum or animals treated with oxytocin- or growth hormone-antiserum continue to avoid maximally. Only if animals treated with vasopressin antiserum are tested at 2 min, 1 hr or at 2 hr after the learning trial is maximum avoidance obtained. Thus, as in HO-DI rats, long-term memory rather than learning itself is disrupted in rats in which vasopressin in the CSF has been neutralized by antibodies. Peripheral (i.v.) injection of 100 times as much vasopressin antiserum as required to neutralize the peptide in the general circulation, as indicated by a virtual absence of vasopressin in the urine and by a marked increase in urine production, still fails to affect passive avoidance behavior.

These results indicate that centrally released rather than circulating vasopressin is involved in the behavioral effect of this nonapeptide.

LOCUS OF ACTION IN THE BRAIN

Localization of the site of the behavioral effect of vasopressin was attempted by intracerebral administration of the peptide and by lesion experiments. Microinjections of small amounts (0·1 μg) of LVP into the posterior thalamic area, including the parafascicular nuclei, result in an increased resistance to extinction of the pole-jumping avoidance response. Other brain areas, including the ventromedial and anteromedial parts of the thalamus, the posterior hypothalamus, the substantia nigra, the reticular formation, the substantia grisea, the putamen and the dorsal hippocampal complex proved to be ineffective sites with this technique (van Wimersma Greidanus et al., 1973, 1976). Thus, the parafascicular area seems to be a locus of action.

However, ineffective microinjections may not necessarily represent non-sensitive structures, since a unilateral microinjection of a small amount of vasopressin into a restricted area, might not be sufficient to induce functional changes which lead to overt behavioral manifestations. Rats bearing lesions in the parafascicular area were therefore employed to confirm the significance of this region for the memory consolidating effect of LVP. Although the behavioral effect of the peptide is indeed significantly reduced, long-term resistance to extinction could still be obtained by a factor three increase of the dose of LVP. These nuclei therefore may be involved in but not essential for the memory consolidating effect of vasopressin. Marked behavioral changes have been reported in rats following ablation of the septum or following dorsal hippocampectomy. Both regions have been implicated in learning and memory processes (Altman et al., 1973; Fried, 1972; Isaacson and Kimble, 1972; Olds, 1972). Indeed, destruction of rostral septal or dorsal hippocampal areas prevents the memory consolidating effect of vasopressin (van Wimersma Greidanus et al., 1976). It is possible, therefore, that vasopressin acts on midbrain limbic circuits, including septal and hippocampal structures, in such a way as to facilitate the storage and/or retrieval of acquired behavior.

DIABETES INSIPIDUS AND PARADOXICAL SLEEP

Electrophysiological data support the hypothesis that the hippocampus is involved in the memory consolidating effect of vasopressin analogues. Rhythmic slow activity (RSA) during paradoxical sleep (PS) episodes contain substantially slower hippocampal theta frequencies in HO-DI rats as compared to HE-DI or homozygous normal rats (Urban and de Wied, 1975).

Differences are found in various spectral parameters and the peak frequency of RSA of HO-DI rats is approximately 1 Hz lower than that of controls. Thus, PS causes a difference in RSA in the absence of vasopressin. Administration of DG-AVP enhances the generation of higher frequencies and almost completely restores the spectrum of hippocampal frequencies to control values. PS

deprivation leads to consolidation deficits (Fishbein, 1971; Leconte and Bloch, 1970; Stern, 1971). It might be, therefore, that the impaired memory of HO-DI rats is due to the different quality of PS found in these animals. Drugs which facilitate memory functions enhance the generation of theta frequencies in the post-learning period (Longo and Loizzo, 1973), suggesting that changes in hippocampal theta activity may be related to memory consolidation. Landfield et al. (1972) maintain that theta activity in the post-learning period may reflect a brain state which is optimal for memory storage. Hypophysectomized rats which also show learning deficits (de Wied, 1964) have shorter PS episodes and lack the normally present PS circadian rhythmicity (Valatx et al., 1975). PS is also markedly disturbed in the chronic pontine cat without hypothalamus or pituitary gland (Jouvet, 1965). These deficits can be restored by treatment with various pituitary principles (Jouvet, 1965).

VASOPRESSIN ANALOGUES AND THE DEVELOPMENT OF RESISTANCE TO THE ANALGESIC ACTION OF MORPHINE

The biochemical substrate of vasopressin analogues is as yet unknown. The protective effect of DG-LVP against puromycin-induced memory blockade in mice (Lande et al., 1972) suggests that vasopressin affects memory processes through protein synthesis. Experimental evidence for this at present is not available. Recently, Krivoy et al. (1974) reported that DG-LVP facilitates the development of resistance to the analgesic action of morphine in mice. This might suggest a physiological role of vasopressin in the development of tolerance to narcotic analgesics. Conversely, in the absence of vasopressin the development of resistance to the analgesic action of morphine as measured in rats on the hot plate, is greatly retarded (de Wied and Gispen, 1976). This disturbance can be restored by treatment with vasopressin analogues. Development of resistance to morphine analgesia may be regarded as a form of learning or memory (Cohen et al., 1965). This view is corroborated by observations showing that protein synthesis inhibitors which impair learning and memory, prevent the development of tolerance to narcotic analgesics as well (Cox and Osman, 1970). Thus, similar mechanisms as in learning and memory processes may be involved in the development of tolerance.

CONCLUSION AND SUMMARY

The hypothalamic-neurohypophyseal system possibly makes use of (a) the general circulation for peripheral effects of posterior pituitary hormones; (b) the portal vessel system for regulation of anterior pituitary function and (c) the cerebrospinal fluid for CNS activities. Evidence is presented that vasopressin and its analogues facilitate the consolidation of learned behavior patterns. Under certain conditions these peptides facilitate acquisition of active avoidance behavior and increase the resistance to extinction of active and passive avoidance behavior, and of sexually motivated approach behavior as well. Conversely, severe memory disturbances are found in the absence of

vasopressin, such as occurs in hereditary diabetes insipidus rats. Intraventricular administration of minute amounts of vasopressin analogues facilitate memory consolidation. This supports the idea that the behavioral effect of these polypeptides is centrally mediated. Vasopressin antibodies, which are assumed to neutralize in situ vasopressin released into the CSF, prevent memory consolidation. Studies on paradoxical sleep in diabetes insipidus rats reveal disturbances in hippocampal theta frequencies, and strengthen the hypothesis that memory consolidation is under the influence of vasopressin analogues. The development of resistance to the analgesic action of narcotic analgesics is facilitated by administration of vasopressin analogues and markedly retarded in diabetes insipidus rats. These and other results suggest that the memory consolidating effects of vasopressin analogues are of a more general nature.

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DISCUSSION

R. BALÁZS: May I ask you whether you see any change in behavior in diabetes insipidus patients?

D. DE WIED: We ourselves do not see patients, but of course we have asked this question to various clinicians. First of all there are many patients with a hereditary form of hypothalamic diabetes, and these have not been very cooperative so far. But Dr. R. Heath from New Orleans sent me a paper on a number of patients with diabetes insipidus: several of these complained about memory disturbances. In these cases however, diabetes insipidus

was caused by trauma or tumors, which makes an interpretation of such findings quite impossible.

J. OLDS: First, I wish to express a view of my own and of many of my colleagues: this is potentially the most important addition to behavioral psychology that has come along in a long time. I would like to ask to what degree you think that the action of vasopressin is due to the same vasopressin that is involved in diabetes insipidus and what degree, you think, is due to some peptide that might be in the brain already?

D. DE WIED: We cannot say anything for sure, but my personal feeling is that when consolidation takes place, vasopressin affects protein synthesis in a direct way. We think that at the place where vasopressin is acting we may find growth of axon collaterals.

J. OLDS: Doesn't it seem unreasonable to you that something like this diabetes state of the animal, which would not seen to be a necessary counterpart of consolidation, should appear from your research *to be* a necessity?

D. DE WIED: This is difficult to answer. Vasopressin has many effects: it acts on the kidney, on the blood vessels, on the anterior pituitary, it has metabolic actions, etc. etc. Apparently, another of its actions is in the CNS. For this effect, the peptide is probably released from the anterior hypothalamic nuclei into the CSF. It is of course possible that it in itself causes the production of a peptide in the brain which affects memory.

H. VAN DER LOOS: How do you think vasopressin is being released into the third ventricle?

D. DE WIED: There is morphological evidence for the presence of neurosecretory material in the third ventricle.

B. SCHARRER: There are indeed branches of neurosecretory neurons that enter the third ventricle, although actual release of vasopressin has not yet been proved.

D. DE WIED: It is necessary to measure the release of vasopressin into the CSF under learning conditions.

H. VAN DER LOOS: If rats are brought to secrete vasopressin under other circumstances, will that affect memory consolidation?

D. DE WIED: I don't know, but we have found that vasopressin is released in more than normal amounts into the eye plexus blood during the retention of passive avoidance behavior.

B.T. PICKERING: I really wanted to ask a question along the same line as Dr. Old's. If we are looking at the doses of vasopressin that you have used, it seems to me likely that what you are doing is to mimic the effects of some peptide other than vasopressin. Because, even when you put your dose directly into the ventricle it is quite a large dose, in terms of vasopressin.

D. DE WIED: The lowest dose of vasopressin that we have used to affect memory consolidation (in the lateral ventricle) is 25 pg. However, the physiological role of vasopressin in this respect follows from the disturbance in memory consolidation of hereditary hypothalamic diabetes insipidus rats.

S.P.R. ROSE: The important thing in these experiments is that you have shown that endogenously produced substances can affect attention. We must, however, not forget that substances like amphetamine or strychnine have related effects on attention.

D. DE WIED: The difference with amphetamine is that it has all kind of effects on the brain.

This can be shown, for instance, in open field tests. But the peptides seem to have a more specific effect on memory consolidation.

J. OLDS: I would like to ask Dr de Wied for a comment. I imagine that you think that peptide messages are sent from some areas to other areas in the brain. Because you mentioned the cerebrospinal fluid, moreover, I suppose that you imagine that the access is better for those parts of the brain that are located closer to the cisterns and the ventricles. When a command goes out, i.e., vasopressin is secreted, it would say that it is time to pay attention. There has to be a set of neurons that sends the message, i.e., one that controls the vasopressin secretion step, but there has also to be a set of neurons that receive the message, i.e., some sort of a detection system which is waiting for the message. Am I perhaps over-exaggerating the network that you are thinking of?

D. DE WIED: No, not at all: this is in fact the line along which we are now thinking. We need, however, to have proof of the release of these peptides in the CSF. Heller et al. (1968), who were the first to find vasopressin in the CSF, tried to determine roughly where this vasopressin came from. Peripheral injections of vasopressin did not augment the vasopressin content in the CSF. This is thus one piece of evidence that the vasopressin that has bèen found by bioassay or radioimmunoassay in the CSF probably comes from hypothalamic sites, and not from the peripheral circulation. We felt that we could obtain more evidence by the binding of vasopressin in the CSF to specific antibodies. We assume that these antibodies will not easily leave the CSF once they are in the lateral ventricle. In this way, behavioral disturbances were obtained which were similar to those found in the diabetes insipidus animal (Bohus et al., 1975). There is quite a lot of evidence that all kinds of stressful situations release vasopressin, ACTH, prolactin, and/or growth hormone from the pituitary.

J. OLDS: Do you think that this is a different axon which is carrying the vasopressin towards the cerebrospinal fluid, from that axon that would carry vasopressin just to the vascular system of the posterior lobe?

B. SCHARRER: Well, I recall the discussion and diagrams presented by Dr. Knowles at the IVth International Symposium at Strasbourg, in 1966 (Knowles, 1967). His evidence for a bidirectional secretory capacity of certain neurosecretory neurons of lower vertebrates argues against the view that different axons carry neurosecretory material to the cerebrospinal fluid and the vascular system, respectively. The same cell whose axon terminal makes contact with a blood vessel may reach into the third ventricle with short dendritic processes. This arrangement provides two "gates" in opposite directions, but the principal destination for the secretory product seems to be the vascular system.

D. DE WIED: Is it not easier to assume that most of the stimuli cause a release in all possible directions?

B. SCHARRER: Even if it is true that release of neurosecretory material into the vascular system involves membrane depolarization comparable to that in conventional axons, discharge from the apical pole of neurosecretory neurons need not necessarily be accomplished in the same way. Moreover, if we assume that an active peptide released into the circulation has a specific function, different from that of the same substance present in the cerebrospinal fluid, obligatory simultaneous discharge in all directions would seem to be less than optimal.

J. DOGTEROM: We have just recently tried to measure vasopressin in the cerebrospinal fluid in human, dog and rat. For all three species, there appears to be a concentration of vasopressin in the cerebrospinal fluid which is about 5 or 10 times higher than in the general circulation. Simultaneous plasma determinations in dog and rat also revealed high levels, however. So we have to study whether the high concentrations in the cerebrospinal fluid are not caused by the anesthesia in these latter species, or by stress in humans. From immunofluorescence studies at the Netherlands Central Institute for Brain Research, it appears that vasopressin-containing fibers of the hypothalamo-neurohypophyseal tract are

running very closely under the lining of the third ventricle. This looks as if the same fiber tract would be able to release vasopressin both into the general circulation and into the cerebrospinal fluid.

J. OLDS: I would like to ask one question about specificity. I wondered whether the Brattleboro rat might be deficient in a *variety* of vasopressin-like substances. Is there, for instance, an oxytocin deficit in the Brattleboros?

D. DE WIED: Brattleboros have about 50% of the normal oxytocin content in the pituitary, so there may be a defect also in the synthesis of this hormone. Oxytocin, moreover, has approximately 20% of the behavioral effects of vasopressin. In addition, these animals seem to have an increased vasotocin release, which could also contribute to the behavioral defects that we have seen.

J. OLDS: So you may have a whole field of peptides that can influence learning to some degree?

D. DE WIED: Yes, indeed ACTH and its analogues also have such effects. And there are now reports in the literature about such effects of releasing factors and other peptides in the brain. These peptides might give specific (but also non-specific) information to the brain.

P.B. BRADLEY: I think, in this connection, about the fact that substance P is coming back into the picture. And, of course, the morphine-like factor which has been found in the brain (Hughes et al., 1975) is also a peptide. So I think we have to consider many more of these substances.

D. DE WIED: Well, some of these peptides I have been talking about do in fact interfere with the binding of morphine (Terenius et al., 1975).

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