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COMMENTARIES

NEUROHYPOPHYSEAL PRINCIPLES AND MEMORY PROCESSES

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In mammals, the neurohypophyseal hormones, vasopressin and oxytocin, are formed predominantly in cell bodies of two hypothalamic nuclei, the supraoptic nucleus (SON) and the paraventricular nucleus (PVN) [1]. Following their formation and storage in neurosecretory granules, these peptide hormones are transported, together with their carrier proteins (neurophysins), within the axons to neurosecretory terminals where they are stored. The producing cells have the essential electrical properties of ordinary neurons and a variety of stimuli can generate impulses which are conducted along the axons and which provoke the release of stored material. The posterior pituitary is regarded as the major depot of vasopressin and oxytocin. The arrangement in this organ is perfectly suited for the direct transfer of the hormones to the bloodstream, which subsequently acts as a transport route for these principles to their target organs. Vasopressin is physiologically involved in maintaining the osmolarity and volume of body fluids mainly by altering the renal excretion of solute-free water and, therefore, has also been designated as anti-diuretic hormone. Oxytocin acts on the mammary gland causing ejection of milk and may play an important role in the expulsion of the fetus at parturition. Specific stimuli arising from the external environment or from interoceptive mechanisms can elicit the release of vasopressin or oxytocin, e.g. changes in osmotic pressure or blood volume, suckling or manipulations of the genital tract. Many afferent pathways in the central nervous system (CNS) have been implicated in the transfer of information from the periphery to the neurons of the SON and PVN. In addition to this excitatory neuronal input, evidence has been presented for the existence of inhibitory pathways. These may be involved in feedback of the hormones on their own release.

Vasopressin and oxytocin both influence central neuronal activity and metabolic processes suggesting a direct action of these hormones on brain functions [1, 2]. Neurohypophyseal hormones may use the cerebrospinal fluid (CSF) in addition to the bloodstream as a transport system for actions at CNS sites [2]. Vasopressin and oxytocin are present in the CSF and produce sometimes different and even opposite effects following their injection into the general circulation or into the CSF e.g. antidiuresis versus diuresis [2–4]. Furthermore, neurohypophyseal hormones may be released directly into the CSF via neurosecretory fibers originating from the magnocellular hypothalamic nuclei [2, 4, 5]. A third route has been proposed for the transport of vasopressin and oxytocin to their central target sites. The peptidergic neurosecretory network ascending from the SON and PVN to limbic structures in particular and also to some more caudal brain sites may be an important channel for neurohypophyseal hormones to modulate brain mechanisms [5–8].

Avoidance behavior. Neurohypophyseal hormones affect brain processes involved in the maintenance of conditioned avoidance behavior. After removal of the posterior pituitary, including the intermediate lobe, extinction of two-way active avoidance behavior is markedly facilitated [9]. A crude extract of posterior pituitary origin (pitressin) as well as purified lysine^8-vasopressin (LVP), administered as long acting preparation during either acquisition or extinction, restores the ability to display normal extinction behavior [9, 10]. Furthermore, vasopressin improves disturbed shuttle-box avoidance acquisition in hypophysectomized rats [11]. In intact rats, a single subcutaneous (s.c.) injection of LVP or of its analog desglycinamide^9-lysine^8-vasopressin (DG-LVP) results in resistance to extinction of one-way active avoidance behavior [12–16]. DG-LVP is practically devoid of classical endocrine activities as displayed by the whole LVP molecule. It has been isolated from hog pituitary material [14]. This might indicate that peripheral and central receptor sites which are activated by vasopressin may be structurally different with respect to biological activity. A similar phenomenon has been observed for ACTH and various neuropeptides related to ACTH with respect to central and peripheral target sites [17]. Depending on the dose administered, the effects can persist for several

Abbreviations—SON—supraoptic nucleus, PVN—paraventricular nucleus, CNS—central nervous system, CSF—cerebrospinal fluid, LVP—lysine^8-vasopressin, AVP—arginine^8-vasopressin, DG-LVP—desglycinamide^9-lysine^8-vasopressin, DG-AVP—desglycinamide^9-arginine^8-vasopressin, PA—pressinamide, TA—tocinamide, PLG—pro-leu-gly, PAG—pro-arg-gly, ACTH—adrenocorticotrophic hormone, DI—diabetes insipidus, HTR—hippocampal theta rhythm, PS—paradoxical sleep, CA—catecholamines, DA—dopamine, NA—noradrenaline.

days indicating that vasopressin triggers a longterm effect on avoidance behavior, probably by facilitating consolidation [10, 13]. The finding that hereditary diabetes insipidus (DI) rats, which lack the ability to synthesize vasopressin, have difficulty in acquiring and maintaining responses in active and passive avoidance paradigms [18–20] supports this postulate. Furthermore, central blockade of vasopressin activity by the intracerebroventricular administration of vasopressin antiserum immediately after the learning trial, induces a deficit in retention of passive avoidance behavior [21]. This disturbance is not demonstrable shortly after the learning trial but only after several hours [21, 22]. Thus, in the absence of vasopressin, consolidation of the acquired response is selectively impaired rather than learning itself. In addition, vasopressin seems to play a role in retrieval processes or in the expression of stored information [23]. The action of vasopressin appears to be a specific one. Other peptides and hormones do not mimic its behavioral effects [14]. Treatment with vasopressin is critically time-related to the behavior [12]. Generalization to other aversively motivated responses does not occur [16]. Other kinds of behavior, e.g. locomotor activity, extinction of approach response for food, are not affected by peptide treatment [23, 24]. However, under certain circumstances, reward performance (e.g. sexually motivated behavior) is improved by vasopressin [25, 26]. Intracerebral microinjection and lesion experiments suggest a participation of specific brain areas, in particular of limbic midbrain structures, in the behavioral effects of vasopressin [27–29].

Arginine^8-vasopressin (AVP) is the most potent peptide yet found which delays extinction of active avoidance behavior following its subcutaneous administration [23, 30]. Removal of the C-terminal part of AVP yields a peptide with decreased potency. Desglycinamide^9-arginine^8-vasopressin (DG-AVP) has an activity of approximately 50 per cent and pressinamide (PA) has only 10 per cent of the behavioral activity of AVP (Fig. 1). Thus in spite of the finding that the C-terminal part of vasopressin (PAG) only slightly mimics the action of vasopressin in this respect, this part of the molecule seems to be important for the hormone to be completely effective, probably because the active moiety is protected against metabolic degradation. Accordingly, PA is relatively more potent when given directly into one of the lateral brain ventricles [31]. These data suggest that the ring structure of vasopressin contains the essential information for the effect of the molecule on the consolidation of active avoidance behavior, although a second active site in the hormone cannot yet be excluded. In particular, the amino acid residues phe [3] and asn [5] have been designated as the most critical sites of the molecule [32]. The covalent ring structure of oxytocin (tocinamide, TA) and the C-terminal tripeptide of this hormone (PLG) are practically devoid of activity whereas oxytocin itself, in relatively high doses, mimics the effect of vasopressin (Fig. 1). In contrast, small doses of peripherally administered oxytocin have been reported to facilitate active avoidance behavior, in particular in water deprived rats [33]. Opposite effects of vasopressin and oxytocin have also been observed on retention of passive avoidance behavior following the intracerebroventricular injection of the peptides [34] (Fig. 1). The significance of these actions is substantiated by the findings that intracerebroventricularly applied antiserum against oxytocin increases retention of passive avoidance behavior and inhibits extinction of active avoidance behavior and that antiserum against AVP has the opposite effect in both test paradigms [34]. As in the case of vasopressin, the ring structure of oxytocin seems to contain the essential information in this respect, since low doses of TA mimic this effect of oxytocin while PLG is inactive [35]. Higher doses of tocinamide affect passive avoidance behavior in a way similar to that observed with higher doses of oxytocin and with vasopressin. Intracerebroventricularly injected PA has a marked potency, while PAG is only slightly active, which is consistent with the effect of similar treatment in the active avoidance test.

It has been reported that deprivation of paradoxical sleep (PS) interferes with the consolidation of learned responses [36–38]. PS during the post training period seems to be specially important for memory storage [39, 40]. Hippocampal theta rhythm in this period has been considered as an optimal brain state for information storage [41, 42]. Hippocampal theta rhythm (HTR) during PS may thus be an optimal electrophysiological brain state for consolidating processes. This is in agreement with the finding that HTR during PS episodes of DI rats which have a disturbed consolidation, contains substantially lower frequencies than does that of heterozygous control animals [43]. Administration of DG-AVP, which normalizes the impaired memory function of DI rats, almost completely restores the spectrum of hippocampal frequencies to the one found in control animals [44]. In normal rats, intracerebroventricularly applied DG-LVP increases the proportion of high frequency components in HTR, while oxytocin has an opposite effect [34]. A physiological significance for this particular modulating role of the neurohypophyseal principles is substantiated by the finding that, on intracerebroventricular injection, AVP antiserum attenuates and oxytocin antiserum accelerates HTR. This fits rather well with the data on passive avoidance behavior. The frequency of HTR appears to be positively correlated with the intensity of neural activity reaching the septum via ascending midbrain limbic pathways [45–47]. Thus, the alterations in HTR induced by neurohypophyseal principles and which are presumably the result of functional changes in the reticulo-septo-hippocampal network, may be a means for these principles to exert their influence on consolidating processes.

Thus, brain functions involved in the consolidation of acquired avoidance responses are modulated in opposite ways by vasopressin and oxytocin. Structure-activity relationships reveal that in both cases, the ring structure is the most important part of the molecule. Yet, it is not clear whether the activity of the hormones is mediated by the same or by two different receptor sites. Assuming that one receptor is involved, oxytocin may behave like a

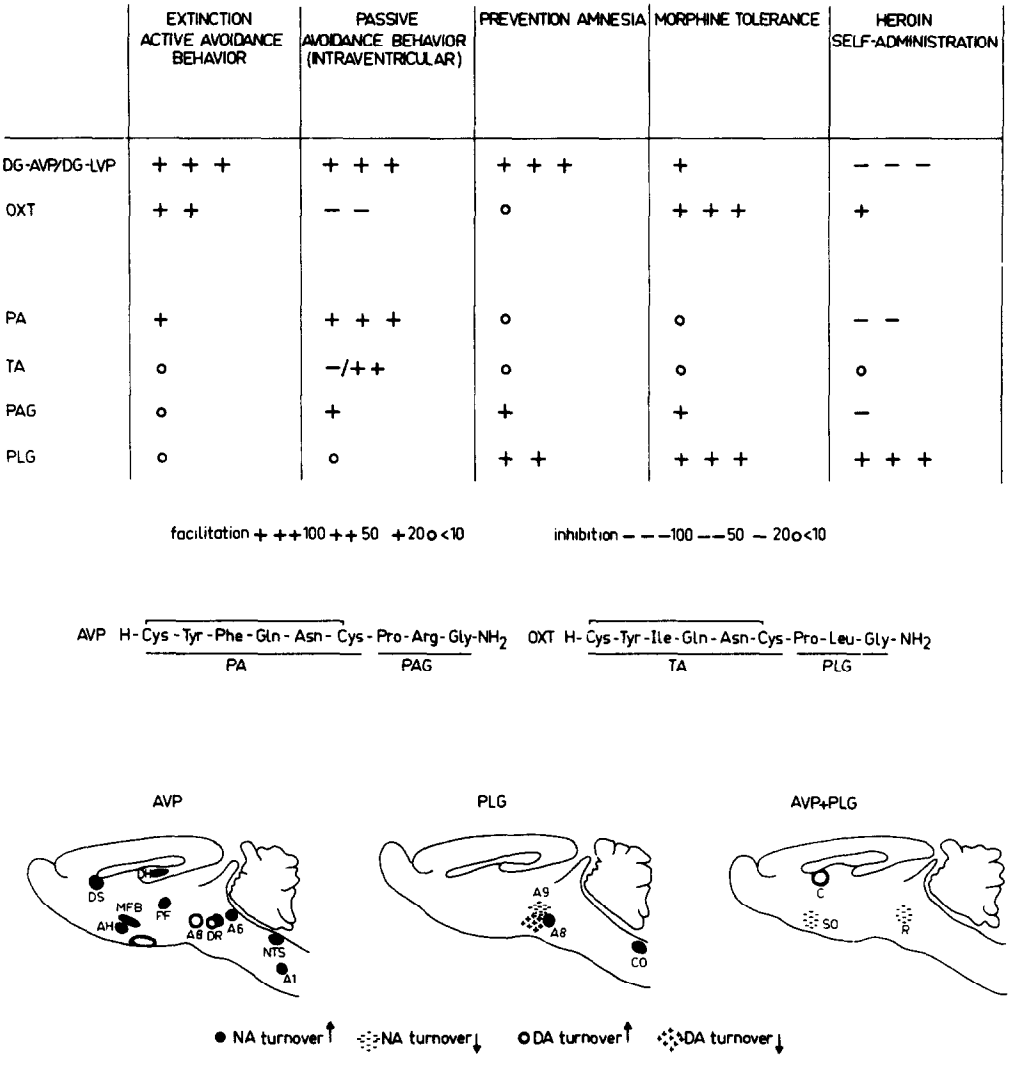

Fig. 1. Summary of the effects of neurohypophyseal principles on various memory tests and on catecholamine metabolism in restricted brain areas. The effectiveness of the peptides is expressed as approximate potency relative to the most effective peptide in that particular assay.

Abbreviations—A1, A6, A8, A9: refer to catecholaminergic cell body regions, AH: anterior hypothelamic nucleus, C: caudate nucleus, CO: nucleus commissuralis, DA: dopamine, DH: dorsal hippocampal nuclei, DS: dorsal septal nucleus, DR: dorsal raphe nucleus, EM: median eminence, NA: noradrenaline, NTS: nucleus tractus solitarii, MFB: medial forebrain bundle, PF: parafascicular nucleus, R: nucleus ruber, S: supraoptic nucleus.

partial agonist for vasopressin active sites. This might also be true for peripheral receptors involved in the antidiuretic activity of vasopressin [48–50]. Such an assumption might explain the finding that the facilitation of extinction by oxytocin treatment is more pronounced in water-deprived rats, which have higher blood levels of vasopressin than do non-deprived controls [3, 33].

Amnesia. Memory of maze learning in mice can be blocked for long periods by the intracerebral injection of puromycin after training [51]. Retrograde amnesia for a one-trial passive avoidance response can be induced by carbon-dioxide (CO_2) treatment of animals immediately following termination of the acquisition trial [52, 53]. Both test systems have been used to explore the action of neurohypophyseal principles on memory processes. The memory blocking action of puromycin is attenuated after treatment with DG-LVP either before or after the training session [54]. This peptide also counteracts CO_2-induced amnesia in rats [55]. Since treatment both before and immediately after the learning trial as well as treatment prior to the retention test is effective, vasopressin may influence both consolidation and retrieval processes [55, 56]. This is consistent with the effects of vasopressin on passive avoidance behavior outlined above. The covalent ring structure of vasopressin appears to be inactive in counteracting amnesia, but, interestingly, the C-terminal tripeptide is active [57, 58] (Fig.

1). The same pattern is seen for oxytocin and its analogues. Although oxytocin itself has only slight activity, gly-gly-gly-oxytocin is fully active [57]. PLG and the dipeptide, leu-gly, are very effective in the amnesia test systems, while the covalent structure of oxytocin apparently contains little information in this respect [57, 58].

In conclusion, memory processes which can be blocked by amnesic treatments are affected by neurohypophyseal hormones. The active sequence for this effect seems to be located in the C-terminal part of the molecule; some modifications in the ring structure of vasopressin however can reduce its potency drastically [57].

Morphine tolerance. Development of tolerance to narcotics can be considered as an analogous process of learning and memory [59–63]. Administration of morphine is a novel stimulus for the organism and repeated treatment with the drug results in an altered response to this stimulus. The validity of this postulate is supported by findings showing that both learning and memory and the development of tolerance are reliably attenuated by similar agents. Treatment with protein synthesis inhibitors or electroconvulsive shock shortly after either morphine administration or the learning trial, inhibits the development of tolerance to morphine as well as the expression of conditioned avoidance behavior [63–69]. Thus, it can be hypothesized that neurohypophyseal principles are involved in the development of tolerance to morphine. Indeed, Krivoy *et al.* [70] showed that DG-LVP facilitates the development of resistance to the analgesic action of morphine in mice. It was later found that the development of tolerance is delayed in DI rats [71]. This impaired development can be restored by treatment with AVP or DG-LVP. Neurohypophyseal principles may also affect the development of physical dependence on morphine, since the development of tolerance to, and the physical dependence on morphine seem to run parallel [62, 72]. Both DG-AVP and oxytocin appear to facilitate development of physical dependence on morphine [73, 74]. The potency of oxytocin is approximately 5-fold that of DG-AVP. PA and TA are inactive, whereas PAG and PLG have an activity similar to that of the parent hormones. Accordingly, PAG is approximately 5-fold less active than PLG.

Thus, brain processes activated by morphine injection and leading to the development of tolerance and dependence on subsequent presentation of the drug are affected by neurohypophyseal hormones. These activities appear to reside in the C-terminal part of the molecule. This compares rather well with the results obtained in the amnesia tests, but is at variance with the data obtained with avoidance behavior.

Heroin self-administration. Intravenous (i.v.) self-administration is widely used to determine the reinforcing efficacy of drugs, a property regarded as the most important factor in drug abuse [75–77]. Learning and memory processes have also been implicated in this behavior, in that drug injection gains and maintains control over behavior. Morphinomimetics in particular are self-administered due to their reinforcing action and the subsequent development of tolerance to and physical dependance on these drugs. Neurohypophyseal principles also affect self-administering behavior with narcotics. Daily treatment with DG-AVP reduces self-administration with heroin in rats in a dose-dependent manner [78]. Although less active, PA causes a similar inhibition of self-administering behavior; and relatively high doses of PAG mimic this effect. The inhibition appears more pronounced on subsequent test days. As in avoidance behavior, the effect of the neuropeptide appears to be of a long term nature. In contrast, oxytocin treatment slightly facilitates heroin self-administration. This stimulatory effect is more pronounced following treatment with PLG, whereas TA is inactive (Fig. 1). Inhibition of heroin self-administration can also be achieved after intracerebroventricular administration of DG-AVP, but with much lower doses than required with systemic injection [79]. Antiserum containing antibodies against AVP injected intracerebroventricularly facilitates heroin self-administration [79]. Control serum (normal rabbit serum) and sera containing antibodies against oxytocin and human growth hormone appear to be inactive. These data may be an indication that vasopressin is involved in self-administering behavior by attenuating the reinforcing efficacy of the drug. It is questionable whether the initial reinforcing effect of heroin is blocked by vasopressin, since the behavior is not affected during the first experience trial. However, such an action cannot be excluded, particularly since animals seem to display this behavior at a set point, related to the rewarding properties of the first narcotic injection and therefore dependent on the unit dose presented [80]. Nevertheless, in view of the involvement of vasopressin in memory processes, it is more likely that rewarding mechanisms triggered by heroin and involved in acquisition and maintenance of drug-seeking behavior are under the control of vasopressin. Consolidation of this behavior rather than acquisition of the response is affected by vasopressin analogues. Accordingly, the involvement of vasopressin in heroin self-administration resembles that in active avoidance behavior. With both behavior situations the vasopressin effect is of long term nature and the ring structure seems to contain the essential information. The increase in heroin intake after PLG treatment may be related to the enhancement of tolerance to and physical dependence on morphine. However, data concerning manipulations aimed at inducing tolerance and physical dependence do not support this assumption [80].

Catecholamines. Brain catecholamines (CA) are implicated in memory functions [81–87]. Alterations in the availability of central CA have a profound influence on consolidation and retrieval of active and passive avoidance behavior [81–84, 88]. The memory-blocking effect of protein synthesis inhibitors can be modulated with drugs which alter adrenergic functions [83, 89]. Inhibitors of CA synthesizing enzymes produce amnesia [89]. CA have been implicated in the process leading to development of tolerance to and dependence on morphine as well as in the self-administration of drugs [62, 90, 91]. Thus, evidence is available that

brain CA participate in the various processes which are modulated by neurohypophyseal principles.

Intracerebroventricular administration of AVP increases the nerve impulse flow in noradrenergic neurons located in the hypothalamus, the thalamus and the medulla oblongata [92]. It appears that AVP alters catecholamine metabolism in a restricted number of sites within these and other brain structures [92, 93] (Fig. 1). Noradrenaline (NA) disappearance is accelerated in the dorsal septal nucleus, in nuclei of the dorsal hippocampus, the parafascicular nucleus, the medial forebrain bundle, the anterior hypothalamic nucleus, the dorsal raphe nucleus, the locus coeruleus, the nucleus tractus solitarii and the A1-region. Conversely, a decreased NA disappearance is observed in the supraoptic nucleus and the nucleus ruber. Dopamine (DA) disappearance is accelerated in the caudate nucleus, the median eminence, the dorsal raphe nucleus and the A8-region. Interestingly, in most of these regions, PLG fails to affect CA metabolism [94]. This peptide mimics the effect of AVP in the caudate nucleus, the supraoptic nucleus and the nucleus ruber. In addition, PLG increases NA disappearance in the A8-region and the nucleus commissuralis and decreases that of NA and DA in the A-9 region.

AVP has been implicated in the regulation of a variety of autonomic and neuroendocrine processes and may be involved in the feedback action of its own release [1]. Structures in the hypothalamus and the brain stem are thought to be primarily involved in these regulatory effects. CA changes induced by AVP in these regions may therefore be a reflection of these interactions. Outside these regions, AVP increases NA nerve impulse flow in the dorsal septal nucleus, the dorsal hippocampus and the parafascicular nucleus. These structures play an essential role in behavioral performance and are implicated in learning and memory processes [95–99]. Destruction of septal or hippocampal structures prevents the consolidating effect of vasopressin and microinjection of LVP into the posterior thalamic area, including the parafascicular nucleus, increases the resistance to extinction of active avoidance behavior [13, 29, 100]. Thus, the increased NA nerve impulse flow in these areas and the involvement of these structures in the consolidating effect of vasopressin on active and passive avoidance behavior may be more than coincidental. PLG is seen not to affect NA metabolism in these regions and is also not active in these test situations.

The most prominent effect of PLG on CA metabolism is observed in the substantia nigra, especially the A8 and A9 regions, and in the nucleus caudatus. This points to a specific action of PLG on the nigrostriatal DA system. Moreover, PLG increases synthesis of DA in striatal tissue as has been shown in tyrosine incorporation studies [101]. Accordingly, PLG potentiates DOPA-induced behavioral changes in animals and preliminary data suggest that PLG reduces the symptoms of Parkinson's disease in humans [102, 103]. Evidence has been presented for a specific role of DA in morphine tolerance, drug self-administration and amnesia [62, 72, 90, 91, 104]. It may be that the action of PLG on morphine tolerance, heroin self-administration and amnesia is related to its effect on nigrostriatal DA. However, the involvement of other neurotransmitters, e.g. serotonin, in the interaction of PLG with brain function cannot be excluded at present [105, 106].

Concluding remarks. Pituitary hormones play an essential role in the homeostatic functions of organisms [1]. In particular, these hormones control a variety of endocrine activities by interfering with peripherally located target sites. During the past decade, it has become apparent that hypothalamic and pituitary hormones modulate various brain functions as well, suggesting that these peptides are also involved in behavioral homeostasis [107, 108]. Some of their fragments exert effects similar to those of the parent polypeptides, but these fragments have lost the inherent endocrine activities of their parent hormones (e.g. ACTH [4–10]) [17]. Other fragments have intrinsic activities different from those of the parent hormones (e.g. endorphins [107, 109]). These and other observations have led to the postulate that behaviorally active "neuropeptides" are generated enzymatically from precursor molecules and that this might be a general phenomenon [107].

Neurohypophyseal hormones may generate neuropeptides similarly. Hypothalamic extracts partially degrade oxytocin to yield PLG and the dipeptide leu-gly, and PLG has been isolated as such from hypothalamic tissue [110, 111]. DG-LVP, which is present in hog pituitary material, is probably derived enzymatically from LVP [14]. Thus, it is tempting to speculate that vasopressin and oxytocin, like other pituitary hormones, are putative prohormones for neuropeptides, which specifically modulate memory processes in the brain. It is unknown whether these neuropeptides are generated by enzymes present at the site of biosynthesis of their parent hormones and are subsequently transported to their site of action, or whether such enzymes are located in the vicinity of the central target sites of the neuropeptides. These enzymes might be activated by specific environmental or interoceptive stimuli. This activation might in turn lead to an increase in bio-active material at putative receptor sites.

Unfortunately, little is known concerning the mode of interaction of neuropeptides derived from neurohypophyseal hormones with brain tissue. As postulated for other biologically active peptides, the neuropeptides may serve as neurotransmitters or more likely as neuromodulators which alter the release of other transmitters [109, 112, 113]. Another possibility is that hypothalamic and pituitary neuropeptides interfere with postsynaptic receptor sites of other neurotransmitter systems (e.g. perhaps PLG with DA receptors, α- and β-endorphin with enkephalin receptors). Neurotransmitters activate the receptor site for a short period of time. The duration of activation of the receptor complex by neuropeptides might be longer-lasting and might therefore lead to temporary alterations in neuronal activity. Although such an action has not yet been demonstrated it would be consistent with the long term nature of the effects of neurohypophyseal principles and might be a putative mode of action of these substances in modulating memory processes.

The various CNS functions are controlled by diff-

erent neuropeptides (Fig. 1). Fragments of vasopressin and of oxytocin act in opposite directions to modulate avoidance behavior, heroin self-administration and HTA, while they affect the development of morphine tolerance and reversal of amnesia in the same direction. Furthermore, the most dominant feature for the action on avoidance behavior and for the inhibition of heroin self-administration resides in the ring structure of the hormones, while the active core for reversal of amnesia, development of morphine tolerance and facilitation of heroin self-administration is present in the C-terminal part of the molecules. It can be postulated that neurohypophyseal hormones selectively modulate brain mechanisms to consolidate, to retrieve and to repress recently acquired information. The ring structure of the hormones is important for consolidating processes while their C-terminal part seems to be more concerned with retrieval processes. Oxytocin and vasotocin both reduce passive avoidance behavior following intraventricular application. This is mainly a feature of the whole molecule. Thus, these molecules as a whole may act to repress the reproduction of recent information or to block consolidation processes. Such a postulate assumes a specific enzymatic cleavage of these hormones (see before) in order to enable the organism to adapt adequately to environmental changes.

The reviewed data are interpreted as indicating that neurohypophyseal principles interfere directly with brain tissue and that their most important action is to modulate memory processes. These effects may be mediated by different neurotransmitter systems: limbic midbrain NA in the case of consolidation of avoidance behavior; nigrostriatal DA in the case of amnesia and tolerance development; and both CA systems in the case of drug-seeking behavior. However, the complex character of the formation of new behavior patterns does not allow one neurotransmitter system only to be assigned to the full expression of the phenomena concerned. It is conceivable that the stimuli triggering learning and memory processes generate one or more selective neuropeptides of neurohypophyseal origin or, more likely, generate these principles randomly. Subsequently, these neuropeptides specifically affect the activity of brain neurotransmitter systems. The expression of these interactions may depend on the nature of the invading noxious stimulus or the environmental change.

REFERENCES

1. *Handbook of Physiology* (Eds E. Knobil and W. H. Sawyer), Section 7: *Endocrinology*, Vol. IV. (1974).
2. E. M. Rodriguez, *J. Endocr.* **71**, 407 (1976).
3. J. Dogterom, C. M. F. Rheenen-Verberg, Tj. B. van Wimersma Greidanus and D. F. Swaab, *J. Endocr.* **72**, 74P (1977).
4. B. S. Nashold, Jr., E. M. Mannarino and R. R. Robinson, *Nature, Lond.* **197**, 293 (1963).
5. A. Weindl, M. V. Sofroniew und I. Schinko, *Arzneimittel-Forsch.* **26**, 1191 (1976).
6. G. Sterba, in *The Final Neuroendocrine Pathway* (Eds F. Knowles and L. Vollrath), p. 38. Springer Verlag, Berlin (1974).
7. J. B. Martin, L. P. Renaud and P. Brazeau, *Lancet* 2 393 (1975).
8. G. P. Kozlowski, M. S. Brownfield and G. Hostetter, in *Evolutionary Aspects of Neuroendocrinology* (Eds A. L. Polenov, W. Bangman and B. Scharrer) Springer Verlag, Berlin (in press).
9. D. de Wied, *Int. J. Neuropharmac.* **4**, 157 (1965).
10. D. de Wied, in *Frontiers in Neuroendocrinology 1969* (Eds W. F. Ganong and L. Martini), p. 97. Oxford University Press, NY (1969).
11. B. Bohus, W. H. Gispen and D. de Wied, *Neuroendocrinology* **11**, 137 (1973).
12. D. de Wied, *Nature, Lond.* **232**, 58 (1971).
13. Tj. B. van Wimersma Greidanus, B. Bohus and D. de Wied, in *Endocrinology* (Ed. R. Scow), p. 197. Excerpta Medica Int. Congress Series No. 273 (1973).
14. D. de Wied, H. M. Greven, S. Lande and A. Witter, *Br. J. Pharmac.* **45**, 118 (1972).
15. R. Ader and D. de Wied, *Psychon. Sci.* **29**, 46 (1972).
16. B. Bohus, R. Ader and D. de Wied, *Horm. Behav.* **3**, 191 (1972).
17. D. de Wied, A. Witter and H. M. Greven, *Biochem. Pharmac.* **24**, 1463 (1975).
18. H. Valtin and H. A. Schroeder, *Am. J. Physiol.* **206**, 425 (1964).
19. B. Bohus, Tj. B. van Wimersma Greidanus and D. de Wied, *Physiol. Behav.* **14**, 609 (1975).
20. D. de Wied, B. Bohus and Tj. B. van Wimersma Greidanus, *Brain Res.* **85**, 152 (1975).
21. Tj. B. van Wimersma Greidanus, J. Dogterom and D. de Wied, *Life Sci.* **16**, 637 (1975).
22. Tj. B. van Wimersma Greidanus and D. de Wied, *Behav. Biol.* **18**, 325 (1976).
23. D. de Wied and W. H. Gispen, in *Peptides in Neurobiology* (Ed. H. Gainer), p. 397. Plenum Press, NY (1977).
24. P. Garrud, J. A. Gray and D. de Wied, *Physiol. Behav.* **12**, 109 (1974).
25. P. Garrud, in *Hormones, Homeostasis and the Brain* (Eds W. H. Gispen, Tj. B. van Wimersma Greidanus, B. Bohus and D. de Wied), p. 173. *Prog. Brain Res.* 42. Elsevier, Amsterdam (1975).
26. B. Bohus, *Horm. Behav.* **8**, 52 (1977).
27. Tj. B. van Wimersma Greidanus, B. Bohus and D. de Wied, *Neuroendocrinology* **14**, 280 (1974).
28. Tj. B. van Wimersma Greidanus, B. Bohus and D. de Wied, in *Hormones, Homeostasis and the Brain* (Eds W. H. Gispen, Tj. B. van Wimersma Greidanus, B. Bohus and D. de Wied), p. 135. *Prog. Brain Res.* 42. Elsevier, Amsterdam (1975).
29. Tj. B. van Wimersma Greidanus and D. de Wied, *Pharmac. Biochem. Behav.* **5**, 29 (1976).
30. D. de Wied, B. Bohus, I. Urban, Tj. B. van Wimersma Greidanus and W. H. Gispen, in *Peptides: Chemistry, Structure and Biology* (Eds R. Walter and J. Meienhofer), p. 635. Ann Arbor Science Publ. Inc., Ann Arbor, MI (1975).
31. D. de Wied, *Life Sci.* **19**, 685 (1976).
32. R. Walter, J. M. van Ree, D. de Wied (submitted for publication) (1977).
33. H. Schulz, G. L. Kovács and G. Telegdy, in *Cellular and Molecular Bases of Neuroendocrine Processes* (Ed. E. Endröczi), p. 555. Akadémiai Kiadó, Budapest (1976).
34. B. Bohus, I. Urban, Tj. B. van Wimersma Greidanus and D. de Wied, *Neuropharmacology* (in press).
35. B. Bohus, personal communication (1977).
36. P. Leconte and V. Bloch, *C.R. Acad. Sci (Paris)* **271**, 226 (1970).
37. W. C. Stern, *Physiol. Behav.* 7, 345 (1971).
38. W. Fishbein, *Physiol Behav.* **6**, 279 (1971).
39. P. Leconte, E. Hennevin and V. Bloch, *Brain Res.* **49**, 367 (1973).

40. P. Leconte, E. Hennevin and V. Bloch, *Physiol. Behav.* **13**, 675 (1974).
41. P. W. Landfield, J. L. McGaugh and R. J. Tusa, *Science* NY **175**, 87 (1972).
42. V. G. Longo and A. Loizzo, in *Pharmacology and Future of Man* (Eds F. E. Bloom and G. H. Acheson), p. 46. Karger, Basel (1973).
43. I. Urban and D. de Wied, *Brain Res.* **97**, 362 (1975).
44. D. de Wied, Tj. B. van Wimersma Greidanus, B. Bohus, I. Urbán and W. H. Gispen, in *Perspectives in Brain Research* (Eds M. A. Corner and D. F. Swaab), p. 181. *Prog. Brain Res.* Vol. 45. Elsevier, Amsterdam (1976).
45. T. Paiva, F. H. Lopes da Silva and W. Mollenvanger, *Electroenceph. clin. Neurophysiol.* **40**, 470 (1976).
46. W. R. Klemm, *Brain Res.* **41**, 331 (1972).
47. W. R. Klemm, *Brain Res.* **36**, 444 (1972).
48. M. Pickford, in *Oxytocin* (Eds R. Caldeyro-Barcia and H. Heller), p. 68. Pergamon Press, Oxford (1961).
49. W. H. Sawyer and H. Valtin, *Endocrinology* **76**, 999 (1965).
50. C. Roy, T. Barth and S. Jard, *J. biol. Chem.* **250**, 3157 (1975).
51. L. B. Flexner, J. B. Flexner and R. B. Roberts, *Science NY* **155**, 1377 (1967).
52. R. M. Paolino, D. Quartermain and N. E. Miller, *J. Comp. Physiol. Psychol.* **62**, 270 (1966).
53. R. I. Taber and A. Banuazizi, *Psychopharmacologia (Berl.)* **9**, 382 (1966).
54. S. Lande, J. B. Flexner and L. B. Flexner, *Proc. Natn. Acad. Sci. U.S.A.* **69**, 558 (1972).
55. H. Rigter, H. van Riezen and D. de Wied, *Physiol. Behav.* **13**, 381 (1974).
56. H. Rigter, R. Elbertse and H. van Riezen, in *Hormones, Homeostasis and the Brain* (Eds W. H. Gispen, Tj. B. van Wimersma Greidanus, B. Bohus and D. de Wied), p. 163. *Prog. Brain Res.* Vol. 42. Elsevier, Amsterdam (1975).
57. R. Walter, P. L. Hoffman, J. B. Flexner and L. B. Flexner, *Proc. Natn. Acad. Sci.* **72**, 4180 (1975).
58. H. Rigter, personal communication (1977).
59. S. Siegel, *J. Comp. Physiol. Psychol.* **89**, 498 (1975).
60. M. Cohen, A. S. Keats, W. A. Krivoy and G. Ungar, *Proc. Soc. Exp. Biol. Med.* **119**, 381 (1965).
61. J. Cochin, in *Drug Addiction: Experimental Pharmacology* (Eds J. M. Singh, L. Miller and H. Lal), p. 365. Futura Publ. Comp. Inc., Mount Kisco, NY (1972).
62. D. H. Clouet and K. Iwatsubo, *Ann. Rev. Pharmac.* **15**, 49, (1975).
63. R. P. Kesner, D. J. Priano and J. R. DeWitt, *Science, NY* **194**, 1079 (1976).
64. I. P. Stolerman, P. Bunker, C. A. Johnson, M. E. Jarvik, W. A. Krivoy and E. Zimmermann, *Neuropharmacology* **15**, 309 (1976).
65. B. M. Cox, M. Ginsburg and O. H. Osman, *Br. J. Pharmac.* **33**, 245 (1968).
66. A. Geller, F. Robustelli, S. H. Barondes, H. D. Cohen and M. E. Jarvik, *Psychopharmacologia* (Berl.) **14**, 371 (1969).
67. A. A. Smith, M. Karmin and J. Gavitt. *Biochem. Pharmac.* **15**, 1877 (1966).
68. B. M. Cox and O. H. Osman, *Br. J. Pharmac.* **38**, 157 (1970).
69. M. E. Jarvik, *Ann. Rev. Psychol.* **23**, 457 (1972).
70. W. A. Krivoy, E. Zimmermann and S. Lande, *Proc. Natn. Acad. Sci. U.S.A.* **71**, 1852 (1974).
71. D. de Wied and W. H. Gispen, *Psychopharmacologia (Berl.)* **46**, 27 (1976).
72. A. E. Takemori, *Biochem. Pharmac.* **24**, 2121 (1975).
73. J. M. van Ree and D. de Wied, *Life Sci.* **19**, 1331 (1976).
74. J. M. van Ree and D. de Wied, *Psychoneuroendocrinology* **2**, 35 (1977).
75. C. R. Schuster and T. Thompson, *Ann. Rev. Pharmac.* **9**, 483 (1969).
76. M. H. Seevers, in *Chemical and Biochemical Aspects of Drug Dependence* (Eds S. J. Mulé and H. Brill), p. 13. CRC Press, Cleveland (1972).
77. T. Thompson and R. Pickens, *Fedn Proc.* **34**, 1759 (1975).
78. J. M. van Ree and D. de Wied, *Eur. J. Pharmac.* **43**, 199 (1977).
79. J. M. van Ree and D. de Wied, *Life Sci.* **21**, 315 (1977).
80. J. M. van Ree, J. L. Slangen and D. de Wied, *J. Pharmac. exp. Ther.* (in press).
81. S. S. Kety, in *The Neurosciences: Second Study Program* (Ed. F. O. Schmitt), p. 324. Rockefeller Univ. Press, NY (1970).
82. C. T. Randt, D. Quartermain, M. Goldstein and B. Anagnoste, *Science, NY* **172**, 498 (1971).
83. R. B. Roberts, J. B. Flexner and L. B. Flexner, *Proc. Natn. Acad. Sci. U.S.A.* **66**, 310 (1970).
84. R. K. Dismukes and A. V. Rake, *Psychopharmacologia (Berl.)* **23**, 17 (1972).
85. L. Stein, J. D. Belluzzi and C. D. Wise, *Brain Res.* **84**, 329 (1975).
86. J. Ashford and B. J. Jones, *Brit. J. Pharmac.* **56**, 255 (1976).
87. B. R. Cooper, G. R. Breese, L. D. Grant and J. L. Howard, *J. Pharmac. exp. Ther.* **185**, 358 (1973).
88. M. Roffman and H. Lal, *Archs Int. Pharmacodyn.* **193**, 87 (1971).
89. D. Quartermain and C. Y. Botwinick, *J. Comp. Physiol. Psychol.* **88**, 386 (1975).
90. H. Lal, *Life Sci.* **17**, 483 (1973).
91. R. A. Yokel and R. A. Wise, *Science, NY* **187**, 547 (1975).
92. M. Tanaka, D. H. G. Versteeg and D. de Wied, *Neurosci. Lett.* **4**, 321 (1977).
93. M. Tanaka, E. R. de Kloet, D. de Wied and D. H. G. Versteeg, *Life Sci.* **20**, 1799 (1977).
94. D. H. G. Versteeg, M. Tanaka, E. R. de Kloet, J. M. van Ree and D. de Wied, *Brain Res.* (in press).
95. B. Bohus and D. de Wied, *J. Comp. Physiol. Psychol.* **64**, 26 (1967).
96. D. J. Albert and C. J. Mah, *Learning and Motivation* **3**, 369 (1972).
97. J. Altman, R. L. Brunner and S. A. Bayer, *Behav. Biol.* **8**, 557 (1973).
98. M. Segal and J. Olds, *J. Neurophysiol.* **35**, 680 (1972).
99. R. Hirsch, *Behav. Biol.* **12**, 421 (1974).
100. Tj. B. van Wimersma Greidanus, B. Bohus and D. de Wied, in *Anatomical Neuroendocrinology* (Eds W. F. Stumpf and L. D. Grant), p. 284, Karger, Basel (1975).
101. E. Friedman, J. Friedman and S. Gershon, *Science, NY* **182**, 831 (1973).
102. N. P. Plotnikoff and A. J. Kastin, *Biochem. Pharmac.* **25**, 363 (1976).
103. A. J. Kastin, N. P. Plotnikoff, C. A. Sandman, M. A. Spirtes, R. M. Kostrzewa, S. M. Paul, L. O. Stratton, L. H. Miller, F. Labrie, A. V. Schally and H. Goldman, in *Anatomical Neuroendocrinology* (Eds W. F. Stumpf and L. D. Grant), p. 290. Karger, Basel (1975).
104. H. C. Fibiger and A. G. Phillips, *Brain Res.* **116**, 23 (1976).
105. F. Ramaekers, H. Rigter and B. E. Leonard, *Brain Res.* **120**, 485 (1977).
106. M. A. Spirtes, R. M. Kostrzewa and A. J. Kastin, *Pharmac. Biochem. Behav.* **3**, 1011 (1975).
107. D. de Wied, *Life Sci.* **20**, 195 (1977).
108. A. J. Prange, Jr., C. B. Nemeroff, M. A. Lipton, G. R. Breese and I. C. Wilson, in *Handbook of Psychopharmacology* (Eds L. L. Iversen, S. D. Iversen and S. H. Snyder), Plenum Press, NY (in press).

109. S. H. Snyder and R. Simantov, *J. Neurochem.* **28**, 13 (1977).
110. R. Walter, E. C. Griffiths and K. C. Hooper, *Brain Res.* **60**, 449 (1973).
111. R. M. G. Nair, A. J. Kastin and A. V. Schally, *Biochem. biophys. Res. Commun.* **43**, 1376 (1971).
112. H. W. Kosterlitz and J. Hughes, *Life Sci.* **17**, 91 (1975).
113. R. Walter and P. L. Hoffman, Proc. Bicentennial Neuropeptide Conference, in *Neuropeptide Influences on the Brain and Behavior* (Eds L. H. Miller, C. A. Sandman and A. J. Kastin), p. 109, *Ad. Biochem. Psychopharmac.* **17**, Raven Press, NY (1977).