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Neurohypophysial hormones and their distribution in the brain

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ABSTRACT

Arginine vasopressin is produced in the supraoptic nucleus (SON), paraventricular nucleus (PVN), and suprachiasmatic nucleus, and oxytocin in the SON and PVN. Neurosecretory fibres are found to terminate in neurohaemal organs and in extrahypothalamic area, while there is evidence of direct endings on the cerebral ventricles only in early brain development. In adulthood the neurohypophysial hormones in cerebrospinal fluid are thus considered to result rather from the removal of the hormones released in extrahypothalamic brain area than from direct terminations on the ventricular surface. The release of neurohypophysial hormones in extrahypothalamic area seems to take place via peptidergic synapses. Neurohypophysial hormones and extrahypothalamic pathways are present early in fetal life, and are thought to be involved in brain development. Peptide transmitters may thus play an essential role both in brain maturation and in central processes in adult life.

INTRODUCTION

The two main subjects of this symposium, hormone production by the brain and the effects of these hormones on the central nervous system, can be traced

back more than 40 years in the literature. Around the time that the Scharrers (1940) were advocating the possibility that nerve cells were capable of hormone production, Popa (1938) suggested that the pituitary may produce 'neurotropic principles' that were transported via the portal vessels and the cerebrospinal fluid to the brain. He supposed the existence of a 'neurotropine' which would be the mother-substance of all posterior-pituitary hormones, and talked of a 'cerebrostimuline' and a 'cerebrorelaxine'. Although we shall never know exactly which substances were responsible for the central effects described by Popa, neurohypophysial hormones were early candidates as centrally active substances. As early as 1932, Harvey Cushing demonstrated strong central, mainly parasympathetic, effects of pituitrin after injection of this extract into human brain ventricles. It was, however, the behavioural experiments of de Wied (1965) which really opened the experimental field on the effects of neurohypophysial hormones on the brain. De Wied showed that posterior lobectomy resulted in a more rapid extinction of a conditioned shuttle-box avoidance response, and that the administration of pitressin-tannate restored the extinction rate.

The present paper reviews the accumulating evidence that endogenous arginine vasopressin (AVP) and oxytocin (OXT) reveal their central actions in the adult rat brain via exohypothalamic fibres and peptide synapses, and that these hormones seem, moreover, to be involved in brain development. The limited related data on such systems in the human brain are also presented.

Observations in mammalian fetuses have suggested the existence of a third neurohypophysial hormone, arginine vasotocin (AVT). It has been said that this is present in adulthood in the pineal and the subcommissural organ (Pavel, 1978; Rosenbloom and Fisher, 1975), and, during fetal development, also in the mammalian neurohypophysis (Pavel, 1975). However, as the use of a specific and sensitive radioimmunoassay has not succeeded in confirming the presence of this hormone in mammalian brain (Dogterom *et al.*, 1979 and 1980), significant amounts of this hormone are not present in the mammalian brain, and it will not, therefore, be included in the present dissertation.

SITES OF PRODUCTION OF AVP AND OXT

The 'classical' and sometimes still propagated (Knigge *et al.*, 1978) view on the distribution of AVP and OXT in the brain was that the paraventricular nucleus (PVN) was predominantly or entirely responsible for OXT production, while the supraoptic nucleus (SON) synthesized mainly vasopressin. The development of immunocytochemical techniques and antibody purification procedures has enabled the specific localization of the sites of production, transport and release of the neurohypophysial hormones in the brain, and the data obtained by means of these techniques do not support the supposed functional differentiation of the SON and PVN.

In the rat the two hormones have been found in similar percentages in the SON and in the PVN (Figure 1). Within the SON and PVN, oxytocin cells have been found mainly in the rostral part of the nuclei, and vasopressin cells have been found mainly in the caudal part (Swaab *et al.*, 1975b).

Figure 1 Percentage of vasopressin- and oxytocin-containing cells in the supraoptic nucleus (SON) and paraventricular nucleus (PVN) from the rostral (left) to caudal (right) part, and the percentage and total number (*n*) of these cells per nucleus. The values given are means $\pm$ SEM (calculated from five mean values). The blank areas between the oxytocin and vasopressin cell areas in this figure represent the percentages of cells without hormones. Note that the SON contains about two-and-a-half times more oxytocin-containing cells than the PVN (Swaab *et al.*, 1975b; reproduced with permission)

In the SON, oxytocin cells have been found principally in the dorsal portion, while vasopressin cells have been found mainly in the ventral areas (van Leeuwen *et al.*, 1976; Vandesande and Dierickx, 1975). In the PVN the vasopressin neurons have been found to have a tendency to group in the centre of the nucleus, while the majority of the oxytocin cells have been found in the periphery (Vandesande and Dierickx, 1975). AVP and OXT are produced in different cells (Vandesande and Dierickx, 1979 and 1975; van Leeuwen and Swaab, 1977; Aspeslagh *et al.*, 1976; Swaab *et al.*, 1975b). An additional source of AVP and its related neurophysin appears to be the parvocellular suprachiasmatic nucleus (Swaab and Pool, 1975; Swaab *et al.*, 1975a; Vandesande *et al.*, 1975). These compounds are present in the suprachiasmatic nucleus in much smaller granules (94 nm) than in the SON (143 nm) (van Leeuwen *et al.*, 1978). Since Brattleboro rats homozygous for diabetes insipidus do not reveal such an AVP reaction in the suprachiasmatic nucleus (Swaab and Pool, 1975; Swaab *et al.*, 1975a), and extracts from the isolated nucleus of Wistar rats run parallel in our AVP radioimmunoassay, this nucleus most probably contains genuine AVP rather than a related compound. Apart from the accessory neurosecretory cell groups that are situated

outside the SON and PVN (Palkovits *et al.*, 1974) and along the proximal parts of the exohypothalamic PVN tracts (Buijs *et al.*, 1978), these three nuclei—the SON, the PVN and the suprachiasmatic nucleus—are the only production sites of AVP and OXT in the brain.

In the human hypothalamus, separate AVP and OXT producing neurons have been found in the SON and PVN, while the suprachiasmatic nucleus only contains AVP. The total amount of AVP cells in the human hypothalamo-neurohypophyseal system is, however, two-and-a-half times larger than the amount of OXT cells (Defendini and Zimmerman, 1978), while the AVP concentration is in some areas as much as ten times higher than that of OXT (George, 1978). In the SON, 80–90% of the cells contain AVP and some 10–20% OXT, while in the PVN the figures are 60–80% and 20–40% respectively (Defendini and Zimmerman, 1978; Dierickx and Vandesande, 1977).

TRANSPORT AND RELEASE OF NEUROHYPOPHYSIAL HORMONES

The SON, PVN and suprachiasmatic nucleus cells project fibres into a number of brain regions. Three types of neurohypophyseal hormone-containing fibre terminations can be distinguished:

I. The classical sites of termination of the neurosecretory fibres are the neurohaemal regions. The fibres from the SON and PVN ending in the neurohypophysis (Sherlock *et al.*, 1975) are thought to be the main source of neurohypophyseal hormones in the peripheral blood. In addition, vasopressin-containing fibres from the PVN terminate in the external zone of the median eminence (Vandesande *et al.*, 1977); it may be these fibres which are responsible for the high levels of neurohypophyseal hormones found in portal blood (Zimmerman *et al.*, 1973). Electrophysiological observations suggest that these latter fibres are axon collaterals of those which run into the neurohypophysis (Blume *et al.*, 1978). The granular diameter in the external zone of the median eminence is, however, much smaller (90 nm) (Dube *et al.*, 1976) than that in the neurohypophysis, which would imply a selection mechanism for the small granula in the collaterals. Whether the suprachiasmatic nucleus contributes towards peripheral or portal AVP blood levels is not known. Although suprachiasmatic fibres run in the caudal direction (Buijs *et al.*, 1978; Swanson and Cowan, 1975; Makara *et al.*, 1972), they seem not to end in the external zone of the median eminence (Antunes *et al.*, 1977; Vandesande *et al.*, 1977). AVP-containing fibres from the suprachiasmatic nucleus seem to end in the organum vasculosum of the lamina terminalis (Buijs, 1978) and so may contribute to the cerebrospinal fluid (CSF) composition (Joseph and Knigge, 1978). However, since no correlation has been found between peripheral AVP levels and avoidance behaviour, the importance of this first class of neuro-

hypophysial hormone-containing endings for central processes is seriously doubted (Dogterom, 1977), the more so since AVP and OXT do not readily penetrate the blood-brain barrier (Landgraf *et al.*, 1979).

II. In parallel with findings in lower animals, neurohypophysial hormone-containing fibres ending directly on the cerebral ventricles, and thus capable of hormone release directly into the CSF, have been thought to be present and functional in mammals (Rodriguez, 1976). However, no evidence of a frequent existence of such terminations in adulthood has been found. Despite much effort, Buijs (unpublished observations), using immunoelectron-microscopy, has been unable to demonstrate any neurohypophysial hormone-containing terminations on the ventricular surface, while Rodriguez (personal communication), using conventional electron-microscopy techniques to make serial electron-microscopy sections throughout the median eminence, suspected the presence of only a few such endings. Rodriguez, however, has not confirmed the endocrine content of these terminations by means of immunocytochemistry. In addition, hormones released into the CSF near to the

Figure 2 Intra- and extrahypothalamic pathways originating in the suprachiasmatic nucleus (small triangles) and paraventricular nucleus (large triangles). The open triangles and broken lines indicate vasopressin-containing cell bodies and fibres respectively, and the closed triangles and dotted lines represent oxytocin-containing cell bodies and fibres. Fibres run from the suprachiasmatic nucleus to the organum vasculosum lamina terminalis (OVLT), the lateral septum (SL) and lateral habenula (LH). Paraventricular nucleus fibres run to the hippocampus (Hi), the amygdala (A), nuclei in the medulla oblongata, and into the spinal cord (Diagram by R. M. Buijs)

infundibulum would have to be transported upstream in order to reach rostral target areas such as the lateral septum. Sterba suggested recently (personal communication) that the neuroendocrine 'endings' that were visualized on the ventricular surface might even be due to fixation artifacts. On the other hand, observations in the fetal brain of the rat have shown the presence of a number

of such contacts during early brain development (Velis *et al.*, 1979). At present it thus seems most likely that in the adult rat the neurohypophysial hormones in the CSF come from the numerous fibres terminating in the brain (see below) and that they represent the elimination of centrally released peptides, rather than that they are using the CSF as the 'specific route' to their sites of action in the brain. In the fetal brain the CSF might be a transport medium for global central effects of neurohypophysial hormones, e.g. in brain development (see below).

III. The extensive networks of extrahypothalamic neurohypophysial hormone-containing fibres which are found in a number of brain areas (Figure 2) are currently thought to be the anatomical basis for the central effects described for these peptides. From the suprachiasmatic nucleus AVP-containing fibres run to the lateral septum and the lateral habenular nucleus. The PVN sends AVP- and OXT-containing fibres to the hippocampus, the medial septum, the amygdala, nuclei in the medulla oblongata, and to the spinal cord. The more caudally one looks, the relatively more OXT fibres one finds (Buijs, 1978). These observations have been confirmed by radioimmunoassay in dissected brain areas (Dogterom and Buijs, 1980; Dogterom *et al.*, 1978). Only a few fibres have been found to run into the pineal stalk, the anterior part of the pineal, the subcommissural organ, and the attachment place of the choroid plexus (Buijs, 1978).

PEPTIDE SYNAPSES

These neurosecretory fibres seem to terminate on other neurons either as 'boutons' on the level of the dendritic tree or as punctate perineural structures on cell bodies (Buijs, 1978). The latter structures (Figure 3A) were first described in 1956 by Barry, who terminated his important work on the existence of extrahypothalamic fibres and possible 'neuroendocrine synapses' because he was convinced, already at that time, that this hypothesis could only be confirmed by combined studies, including electron-microscopy (personal communication). The recently developed technique of immunoelectron-microscopy has indeed revealed that both the structures mentioned above represent synapses which are morphologically indistinguishable from the conventional amine-containing synapses, except for the fact that the 'synaptic vesicles' contain neurohypophysial hormones (Figure 3B) (Buijs and Swaab, 1979; Dogterom and Buijs, 1980). The existence of 'true' synapses containing neurohypophysial hormones confirms Sterba's earlier expectations (Sterba, 1974). Release of neurohypophysial hormones from such peptidergic synapses is strongly suggested by the observations of Cooper *et al.* (1979). No information is yet available on the localization or characteristics of AVP or OXT receptors in the brain. The AVP 'receptors' reported to be present in the

Figure 3 Peptidergic synapses in the central nervous system:

3A Camera lucida drawings of 'neurosecretory synapses' revealed by means of Gomori staining, by Barry (1956): 'Quelques types de synapses neurosécrétatoires. a et b: région du noyau périventriculaire postérieur et du noyau parafasciculaire chez *Rhinolophus ferrum equinum*. c: région du noyau de la strie semi-circulaire chez la souris blanche. d et f: région des noyaux amygdaliens basal et médian chez *Rhinolophus ferrum equinum*. e: région du noyau précommissural chez *Rhinolophus ferrum equinum*.' (Reproduced with permission)

3B Vasopressin positive reaction around a neuron (N) in the lateral septum, obtained by the unlabelled antibody enzyme technique. The bar represents 10 μ m. Note the similarity with Figure 3A

3C Immunoelectron-microscopically demonstrated vasopressin positive terminal, which forms a synapse (arrow) with an unlabelled dendrite in the lateral septum. The bar represents 0.20 μ m

(The photographs for Figure 3B and 3C were taken by R. M. Buijs)

intermediate lobe of the pituitary (Castel, 1978) are most probably the result of an artifactual staining of α -MSH (van Leeuwen *et al.*, 1979).

Since hypothalamic hormone-producing neurons seem to effect hormone release not only in hypothalamic neurohaemal organs but also via synaptoid contacts in the extrahypothalamic area, the question arises as to whether the same cell is able to execute both functions. Support for this possibility comes from the bi- or multi-polar shape of hypothalamic hormone-producing cells (Buijs, 1978; Buijs *et al.*, 1978) and from the finding that almost all cells of the supraoptic and paraventricular nuclei can be retrogradely stained by HRP from the neurohypophysis (Sherlock *et al.*, 1975). However, electrophysiological studies have so far failed to confirm this possibility (Blume *et al.*, 1978; Boer, unpublished observation). Recently, arguments have been made which support the alternative theory that whilst some cells project towards the neurohypophysis, others project towards the extrahypothalamic area. HRP injections into the neurohypophysis or the medulla are thought to label largely different populations of PVN cells (Armstrong *et al.*, 1979; Hosoya and Matsushita, 1979; Ono *et al.*, 1978; Schober, 1978). This opens the possibility of a differential release of neurohypophysial hormones into the peripheral bloodstream and into the brain.

THE PRESENCE OF NEUROHYPOPHYSIAL HORMONES DURING DEVELOPMENT

Yet unpublished radioimmunoassay data from our institute show that in rats AVP and OXT are present relatively early in development. At day 16 of pregnancy a content of approximately 100 pg of AVP and OXT per fetal brain has been found. While the AVP content in the fetal brain gradually increases during development, up to 3.6 ng on the first post-natal day, the course of the OXT content shows a U-shape, with its highest level (± 130 pg) at day 16, its lowest level (± 55 pg) at day 18 and a gradual increase (to ± 110 pg) up to the first post-natal day. These data suggest that considerable neuropeptide concentrations may be present in the fetal brain even before day 16.

Current immunocytochemical studies show that the fetal SON contains neurohypophysial hormones from at least day 17 and the PVN from day 18, while neurohypophysial hormone-containing cells or fibres of the supra-chiasmatic nucleus appear only between 7 and 10 days post-partum (Velis *et al.*, 1979). Exohypothalamic neurohypophysial hormone-containing fibres are present in the amygdala by day 18 and possibly earlier.

In the human fetal hypothalamus the earliest stainings of oxytocin and vasopressin neurons in the SON and PVN have been obtained in weeks 11–13 of gestation (Fellmann *et al.*, 1979), and oxytocin and vasopressin were already present in the neurohypophysis in the youngest fetus (15 weeks) of our series (Visser and Swaab, 1979). Preliminary observations in the human

nervous system reveal that exohypothalamic neurohypophysial hormone-containing fibres are present by mid-pregnancy (Buijs, unpublished observation).

ROLE OF NEUROHYPOPHYSIAL HORMONES IN THE ADULT BRAIN

The arguments in favour of AVP and OXT having a role in adaptive behaviour are dealt with by de Wied and by Bohus in the present volume, and other authors review the effects of these substances on the human central nervous system. It is suggested that in the rat the central effects of AVP are also of physiological importance, since the intraventricular injection of vasopressin antiserum has been found to inhibit memory consolidation (van Wimersma Greidanus *et al.*, 1975). Concerning the study of the physiological function of AVP in the brain, great expectations were raised by the finding of a complete absence of retention of a passive avoidance response in the homozygous Brattleboro rat, which is genetically lacking in AVP (de Wied *et al.*, 1975). However, homozygous animals showed only a partial performance deficit in a similar study by Bailey and Weiss (1979) and no deficit in a shuttlebox study by Celestian *et al.* (1975). In an extensive, as yet unpublished, study by van Oyen *et al.* on homozygous animals, no deficit was found at 60 days, while at 120 days the animals actually performed better than the heterozygous controls in the passive avoidance paradigm. Consequently, the Brattleboro rat does not seem to offer the simple and ideal model for endogenous AVP effects that we had all hoped. Interesting in this respect is the observation of van Oyen *et al.* that a slight but significant performance deficit was present in homozygous Brattleboro rats at 35 days of age, and the unpublished finding of Steinbusch that in the Brattleboro rat more serotoninergic endings are present in the suprachiasmatic nucleus, the lateral septum and the lateral habenula. If, in fact, during development the extra-hypothalamic AVP endings of the homozygous Brattleboro rat are replaced by other fibre systems the result is not just an AVP-less animal, but one which has a very different brain. Other central functions in which the neurosecretory fibres might be involved are drinking behaviour and the maintenance of water balance (Buijs, 1978), while the vasopressin-containing PVN fibres ending in the nucleus tractus solitarius might be involved in the regulation of blood pressure (Tanaka *et al.*, 1977), and those in the spinal cord might collect sensory information for the milk ejection reflex (Buijs, 1978).

It was felt that an alternative way of obtaining data on the physiological involvement of neurohypophysial hormones in central processes might be to measure the peptide content of isolated extrahypothalamic area during processes in which these hormones are involved. In order to show changes in the endogenous AVP and OXT content of brain structures during behaviour,

Dogterom and Buijs (1980) instituted a pilot study in which these hormones were measured in dissected areas during the passive avoidance paradigm 5 minutes after the learning trial. However, no changes were seen either in the AVP or in the OXT content in any of the structures studied. Of course, ideally such a study should be extended to longer intervals from the start of the learning trial, but here we are faced with the same type of problem that occurred before pituitary hormones could be determined in the peripheral blood and were measured in the pituitary itself. The hormone content in a brain area certainly reflects not only hormone release, but other variables as well, i.e. the rates of its production, transport and elimination. It is clear that more dynamic procedures of hormone release measurements have to be developed. So far we have not had success with push-pull procedures.

Our ignorance about the fundamental properties of the 'peptidergic synapse' is thus obvious. In view of this, neuronal systems with synapses that are more readily accessible for fundamental study than the mammalian nervous system have to be looked for. Neurons of the pond snail (*Lymnaea stagnalis*) which seem to contain peptides which are similar or identical to AVP and OXT (Schot *et al.*, 1979) might be a suitable model system. It is also possible that the co-culture of neurosecretory cells (Gähwiler *et al.*, 1978) and an extrahypothalamic area where neurosecretory fibres usually terminate might form a useful model.

ROLE OF AVP AND OXT DURING DEVELOPMENT

Several possible functions of neurohypophysial hormones in fetal life are being studied at present. One role which has been suggested is in the maintenance of the amniotic fluid, but this is not sufficiently supported by the available data at present (Swaab *et al.*, 1978). Fetal OXT seems to be essential for speeding up the course of labour (Boer *et al.*, 1979). The early presence of neurohypophysial hormones in an extrahypothalamic area of the fetal brain suggests a possible involvement in developmental processes. Since the hereditary absence of AVP in the Brattleboro rat appears to result in seriously stunted post-natal brain development, persisting throughout life, AVP might be involved not only in adult brain function but also in brain development. A lower brain weight and total DNA content appears most pronounced in the cerebellum and medulla oblongata, and more detailed studies of this are currently in progress (Boer *et al.*, 1980 and 1978). Whether this is indeed a direct effect of AVP on brain development has still to be studied, but *in vivo* AVP has shown to be a potent mitogen for fibroblast and possibly also for chondrocytes and bone marrow cells (Rozengurt *et al.*, 1979).

Vasopressin and oxytocin are thus not only acting as neurohormones, but also play an essential role as neurotransmitters in brain maturation in the neonatal period and in a number of other central processes in adult life.

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