Development of peptidergic systems in the rat brain

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Abstract. The brain contains a large variety and number of peptides some of which were known earlier as hypothalamic hormones (vasopressin, oxytocin, luteinizing hormone-releasing hormone, thyrotropin-releasing hormone, somatostatin) or as pituitary hormones (the family of opiomelanocortins), while others, not primarily known as hypothalamic or pituitary hormones, may also have endocrine effects (substance P, angiotensin II, neurotensin, bombesin, vasoactive intestinal peptide (VIP), gastrin/cholecystokinin, glucagon, carnosine, bradykinin). These peptides, which form a new class of putative neurotransmitters, are present early in brain development and show important sex differences in both their pattern of innervation and their effects. Their peripheral effects may include intrauterine growth of the placenta and fetus, the timing of birth, acceleration of the course of labour and responses to haemorrhage (redistribution of cardiac output and stimulation of blood cell formation).

Endogenous peptides are probably involved in brain development, which may explain their general, permanent and sex-dependent effects when given in the period of rapid brain development. Although peptides might in the future be useful for stimulating recovery from retarded brain development, at present one should be aware of the potential dangers of their use in, for example, obstetrics.

Although in 1901 Lomonaco & Van Rynberk were still claiming that the pituitary was a rudimentary organ without any physiological function (Van Rynberk was later appointed Professor of Physiology at the University of Amsterdam!), in the thirties the possibility was investigated that the pituitary produced compounds which influence brain function. The hypothesis that pituitary hormones were transported to the brain via the cerebrospinal fluid, in order to elicit central functions, was tested in patients by Harvey Cushing in 1932. He injected 'puituitrin' (a posterior lobe extract) directly into the lateral ventricle and obtained in these human beings strong parasympathetic effects, namely vasodilatation and profuse sweating, and a fall in tempera-

ture, which—by sophisticated control experiments—were shown to be central effects. De Wied (1965) took up the animal experimental approach by showing that posterior lobectomy resulted in a more rapid extinction of the conditioned shuttle-box avoidance response, a behavioural deficit that could be corrected by for example giving vasopressin (for review see De Wied 1980).

Route of transportation of peptides

Since Scharrer's concept of neurosecretion had been only relatively recently accepted, the endogenous neurohypophysial hormones which influenced behaviour were primarily thought to reach their central sites of action by release from the neural lobe and transport in the *bloodstream* back to the brain. This route, however, was seriously questioned, since (1) Van Wimersma Greidanus et al (1979) found no correlation between peripheral vasopressin levels and passive avoidance behaviour, (2) the blood levels of neurohypophysial hormones were extremely low (e.g. Dogterom et al 1977), while (3) these peptides do not readily cross the 'blood-brain barrier' (Landgraf et al 1979). The second possible route, via the *cerebrospinal fluid* (CSF), was not supported experimentally, either: (1) the inhibition of passive avoidance behaviour by intracerebroventricular administration of anti-vasopressin, first explained by inactivation of vasopressin by binding to the antibodies in the CSF (Van Wimersma Greidanus et al 1975), could also be accounted for by the rapid ($300\ \mu\text{m h}^{-1}$) transfer of antibodies from the ventricles into the brain (Swaab & Boer 1980); (2) the high levels of vasopressin in the CSF of hypophysectomized rats (Dogterom et al 1977) apparently did not fit in with the disturbed avoidance behaviour of hypophysectomized or posterior-lobectomized animals (De Wied 1965); and (3) Buijs, in spite of much effort, failed to find immunocytochemically any neurohypophysial hormone-containing terminations on the ventricular surface, although such structures were considered to be the morphological substrate for the CSF route. (Later studies, however, demonstrated fibres on the cerebroventricular surface in the fetal rat, suggesting that this route might be important in early development: Boer et al 1980, Buijs et al 1980.)

In the meantime, immunocytochemical experiments revealed an alternative site of production for vasopressin—the suprachiasmatic nucleus (SCN) (Swaab et al 1975, Vandesande et al 1975)—and the existence of extensive *extrahypothalamic pathways* of neurohypophysial hormone-containing fibres, terminating from the olfactory bulb down to the spinal cord (Buijs et al 1978, Buijs 1978). These peptidergic pathways are now generally considered to be the most probable route for the central effects of endogenous neuropeptides.

From the SCN, vasopressin-containing fibres run to the organum vasculosum of the lamina terminalis, Broca's diagonal band, the lateral septum, the lateral habenular nucleus, the dorsomedial nucleus of the hypothalamus, and the amygdala. The paraventricular nucleus (PVN) and the supraoptic nucleus (SON) send arginine-vasopressin (AVP)- and oxytocin-containing fibres to the olfactory bulb, taenia tectae, ventral hippocampus, medial septum, amygdala, the pineal, the medulla oblongata and the spinal cord (Buijs et al 1978, 1980, Buijs 1978). Immunocytochemistry at both the light and electron microscopical levels revealed that these peptide-containing fibres terminate in brain areas on other neurons by means of synapses mainly on the dendritic tree, but also on cell bodies. These peptidergic synapses are morphologically indistinguishable from conventional amine- or amino acid-containing synapses, except that the synaptic vesicles contain neurohypophysial hormones (Buijs & Swaab 1979). Similar synaptic terminations are described for substance P (Chan-Palay & Palay 1977) and enkephalin (Pickel et al 1979). The existence of such peptidergic synapses had been predicted in 1956 by Barry. Although for no peptide in the central nervous system (CNS) have all criteria for a transmitter been fulfilled, there are sufficient arguments for considering the peptides as a new and possibly even the most extensive and varied group of putative neurotransmitters.

Peptides as putative neurotransmitters

For historical reasons, the centrally acting peptides can be subdivided into:

(1) Peptides that were first known as *hypothalamic hormones*: vasopressin, oxytocin, luteinizing hormone-releasing hormone (LRH), somatostatin and thyrotropin-releasing hormone (TRH). In contrast to various earlier reports, in which arginine-vasotocin was considered to be present as a mammalian fetal neurohypophysial hormone with rapid eye movement (REM) sleep-inducing potentialities (Pavel 1979), this peptide appears not to be present in the mammalian brain in any appreciable amount (Dogterom et al 1980). (Of course, this does not exclude the presence of vasotocin-like peptides.)

(2) Peptides that were first known as *pituitary hormones*, but are also produced by the nervous system. To this group belongs the ever-enlarging family of peptides derived from the opiomelanocortin prohormone(s), for example α -, β - and γ -melanocyte-stimulating hormone (MSH); ACTH; β - and γ -lipotropin (LPH); α -, β - and γ -endorphins, dynorphin and Met/Leu-enkephalin. The 'neuroproteins', growth hormone and prolactin, although not peptides, can also be considered to belong to this group.

(3) *Other peptides*, not primarily known as hormones: substance P, angiotensin II, neurotensin, bombesin, vasoactive intestinal peptide (VIP),

delta sleep-inducing peptide (DSIP), peptides of the gastrin/cholecystokinin (CCK) family, glucagon, carnosine and bradykinin. The way this group is defined does not exclude that these peptides have endocrine effects; thus substance P may alter prolactin and growth hormone release from the pituitary (Eckstein et al 1980). Angiotensin II stimulates vasopressin release (Sladek & Joynt 1979). VIP is found in hypophysial portal blood (Shimatsu et al 1981) and may be of physiological importance in prolactin release (Rotsztein et al 1980).

Results on the first group of neuropeptides suggest that a double function for the molecule, as neurohormone and as neurotransmitter, might be important for coupling central and peripheral adaptive mechanisms. In situations of stress, vasopressin released in the external zone of the median eminence might induce the release of ACTH (Gilles & Lowry 1979), which stimulates the adrenal and signals stress to the brain, while centrally released vasopressin might make possible the adaptation of autonomic functions (Bohus 1980) and facilitate the recollection of stressful situations (De Wied 1980). Oxytocin is involved in milk ejection (Lincoln & Wakerley 1974) and in maternal behaviour (Pedersen & Prange 1979). LRH induces ovulation and facilitates lordosis behaviour via the extrahypothalamic pathways (Sakuma & Pfaff 1980). Since TRH not only stimulates thyroid function but also raises body temperature (Brown et al 1977) and induces shivering (Cooper & Boyer 1978), probably via the extrahypothalamic fibres terminating on motoneurons (Hököfelt et al 1975), TRH might be the crucial factor in an integrated adaptive reaction to a cold environment.

Presence of peptides in development

Neuropeptides are present early in development. Radioimmunoassayable AVP and oxytocin have been detected in the *rat* brain from fetal Day 13 onwards, although the oxytocin content was considerably lower than the vasopressin content. Immunocytochemical techniques revealed AVP-containing cells in the SON and PVN from, respectively, fetal Days 16 and 18 onwards, and extrahypothalamic AVP-containing fibres from Day 17, while oxytocin-containing cells and fibres could be stained only postnatally (Boer et al 1980, Buijs et al 1980, E. L. Baruch-Virransale, unpublished observations). The first immunopositive AVP-containing neurons in the SCN were detected on the second postnatal day, while fibres reached the lateral septum and lateral habenular nucleus on postnatal Day 10 (Boer et al 1980, De Vries et al 1981). LRH immunofluorescence-positive material was found in the organum vasculosum of the lamina terminalis and the external layer of the median eminence of rat fetuses at Day 20.5 of gestation (Daikoku et al 1978) while

fetal hypothalamic LRH was undetectable by the radioimmunoassay of Araki et al (1975) until the sudden increase after birth. Although neonatal pituitary-thyroid function is not physiologically dependent on TRH secretion (Oliver et al 1981), TRH-like material was found in the rat brain from the 18th–19th day of gestation (Conklin et al 1973) and from fetal Day 12 in the mouse brain (Faivre-Bauman et al 1978). Radioimmunoassayable somatostatin was present at fetal Day 20 (Ghirlanda et al 1978).

From Day 15–16 of pregnancy the opiomelanocortin peptides α -MSH, endorphin and enkephalin and the 16K fragment were found in the fetal rat brain, their ratio changing during development (see Swaab & Martin 1981).

Measurable amounts of substance P were found in the CNS of the rat fetus on Day 16 of gestation. The highest levels were found in the spinal cord. Postnatally, the substance P values increased for up to four weeks (Gilbert & Emson 1979). Although the considerable amounts of VIP in the neonatal brain (Robberecht et al 1979) suggest that this peptide might be present much earlier in the fetus, in the cerebral cortex VIP neurons are localized immunocytochemically only postnatally, first in the deep cortical layers, but later particularly in layers II–IV (Emson et al 1979). Peptides of the CCK/gastrin family increase from concentrations of 5–10% of the adult level at birth up to adult concentrations three weeks postnatally. Around birth the larger molecular forms were more abundant (Noyer et al 1980).

In the *human*, the first vasopressin-neurophysin-containing cells were observed in the fetal hypothalamus from 11 weeks of pregnancy (Fellman et al 1979), while oxytocin-neurophysin neurons were stained in the 14th week of gestation (Paulin et al 1978). Extrahypothalamic neurohypophysial hormone-containing fibres were found in the spinal cord from 17 weeks of gestation (Figs. 1 and 2). Fetal hypothalamic and cortical tissue contained radioimmunoassayable LRH at 4.5–6 weeks of pregnancy (Aksel & Tyrey 1977, Siler-Khodr & Khodr 1973, Winters et al 1974) and LRH-producing cells were stained at nine weeks of gestation (Paulin et al 1977). TRH was also assayed in the fetal brain as early as 4–5 weeks of pregnancy (Winters et al 1974) and in the fetal hypothalamus and cortex from eight weeks of gestation (Aubert 1979). The presence of TRH in the cerebellum of an anencephalic child (Winters et al 1974) tends to suggest an extrahypothalamic production site for this material.

Somatostatin-containing neurons were stained in the 14th–16th weeks of gestation (Paulin et al 1976, Bugnon et al 1977) and assayed in the fetal hypothalamus and cerebral cortex from the 10th week of gestation (Aubert et al 1977), while α -endorphin staining appeared in the infundibular region from the 11th week of development (Bloch et al 1978).

Since most of the studies mentioned are based only on immunocytochemical procedures, in which specificity is the most difficult criterion to fulfil

FIG. 2. Immunocytochemical staining of extrahypothalamic neurohypophysial hormone-containing fibres; bar represents 50 μm . (a) Medulla oblongata (male, no. T 79.38.92, 20 weeks of gestation, 350 g, sectio parva). Note the arborization; arrow heads indicate the continuation of the stained fibres out of focus. (b/c) Septal area of the brain of the patient of Fig. 1. Note the arborization of the fibre in (c).

FIG. 1. Immunocytochemical staining of 100 μm vibratome sections of human formalin-fixed material using a combined incubation with non-purified anti-vasopressin (#125) and anti-oxytocin (O_2D). (Female, no. S4645, born at 34 weeks of gestation, body weight 1560 g, first of twins, congenital heart failure and pancreas annulare, death 6 weeks after birth (body weight 2260 g) from pneumonia after operation; cerebral cortex showed focal nodular dysplasia.) (a) Paraventricular nucleus with multipolar magnocellular neurons; bar represents 250 μm . III, third ventricle. The arrow head points to the neuron magnified in (b). (b) Paraventricular nucleus neuron (see arrow in a); bar represents 25 μm . (c) Suprachiasmatic nucleus neuron; bar represents 25 μm .

(Swaab et al 1977), additional techniques have to be applied to prove that the compounds stained in the developing brain are chemically *identical* to the peptides mentioned, and not only chemically related.

Peripheral effects and functions of peptides in development

Various reviews have discussed the possible role of fetal peptides in fetal and placental growth (Visser & Swaab 1979, Swaab & Martin 1981), while fetal peptides might also be important in the birth process. In order for the fetal brain to influence such a complicated process as the initiation of birth, one would expect it to have reached a certain stage of maturity. The degree to which the fetal brain is involved in the birth process in various species (the sheep, human and rat) corresponds in fact with some general measures of the degree of maturity of the fetus at birth, such as the moment of the brain growth spurt and the proportion of paradoxical (rapid eye movement) sleep at birth (Swaab et al 1979). Of these three species, the fetal brain is most mature at birth in the sheep and is dominant with regard to the origin of the stimulus for the initiation of parturition, whereas in the immature rat fetus the fetal brain seems not to be involved in this process. The primate fetal brain is intermediate in this respect; it is able only to adjust the time of birth around a fixed species-specific mean. Various observations point to the fetal opiomelanocortins as factors influencing this process (Swaab et al 1979, Visser & Swaab 1979, Swaab & Martin 1981). This agrees with the high incidence of prematurity in the deliveries of heroin and methadone addicts (cf. Swaab & Martin 1981), while blockade of opiate receptors by naloxone in the relatively immaturesly born rat does not influence pregnancy length (Hetta & Terenius 1980).

Although labour was induced in one sheep by large amounts of vasopressin (P. W. Nathanielsz, personal communication), vasopressin is probably not *the* corticotropin-releasing factor controlling the timing of birth via the pituitary opiomelanocortins. Four children of a Dutch family with a hereditary form of hypothalamic diabetes insipidus and their five healthy siblings, all delivered by mothers with diabetes insipidus, were born within the normal range of gestation length (P. Oosting, unpublished observation from our institute). Since, in addition, no further disturbance was observed in these deliveries, an essential role of vasopressin in human labour is questionable, although it cannot be excluded that a function of vasopressin in this process is taken over by a different compound in this disease.

Fetal oxytocin seems to accelerate the course of labour in the human and the rat, rather independently of the state of maturity of the fetal brain at birth (Swaab et al 1979), which agrees with the concept that the release of this

peptide is secondary to increases in intracranial pressure or a response to a hypoxic stimulus (Hadeed et al 1979), rather than the result of a primarily fetal brain stimulus. The massive release of vasopressin by the fetus during delivery is associated with bradycardia, increased blood pressure and reduced skin temperature, indicating peripheral vasoconstriction. This is thought to reflect a favourable haemodynamic fetal adaptation to the hypoxia and stress of delivery, intended to redistribute cardiac output to vital organs like the heart, brain and adrenals (Pohjavuori & Fyhrquist 1980, Iwamoto et al 1979). The stimulatory effect of AVP on blood cell formation (Hunt et al 1977) might be an important adaptive response to haemorrhage at birth.

Central effects and functions of peptides in development

There is increasing evidence that, in common with the classical neurotransmitters (the amines and amino acids), sex hormones and thyroid hormones, neuropeptides also obey the rule (cf. Swaab 1980) that factors that are important for adult brain function are also involved in brain development. It may therefore not be surprising that the supposed effects of neuropeptides on the formation of cells, neurites and synapses will often result in more general rather than in well-defined functional changes in the network.

The possibility that neuropeptides have central effects in the developing brain is strengthened by the limited evidence available for the early presence of receptors for neuropeptides in the brain. Opiate receptor binding has been found in the rat brain as early as fetal Day 14 (Clendeninn et al 1976) and VIP receptors in the brain are present at birth (Robberecht et al 1979). The finding of Csaba et al (1980) that one neonatal injection of vasopressin or oxytocin causes permanent changes in peripheral receptors for these peptides might explain a number of the permanent central effects of neuropeptides given during development (see below). Neonatal administration of lysine-vasopressin (Wright & Kutscher 1977), 1-desamino-8-D-arginine-vasopressin (Lichardus et al 1978) or AVP (G. J. Boer, unpublished observations) interferes with the development of osmoregulation in rats, sometimes permanently. It is not yet clear whether this is a central effect of vasopressin. The protracted brain development found in Brattleboro rats also points to an effect of vasopressin on brain development (Boer et al 1980). Our current experiments are directed to showing that brain growth can be restored by vasopressin replacement in the Brattleboro rat.

Another peptide possibly involved in the development of the regulation of water balance is angiotensin II. The organization of the drinking response to angiotensin II matures between postnatal Days 4 and 5, while this peptide stimulates milk intake early in postnatal life (Misantone et al 1980).

Neonatal administration of TRH enabled rats to run faster towards a palatable food reward one month later. They were less emotional than normally and more active in an open field, while tooth eruption occurred earlier than usual and the pineal and hypothalamus weighed more. However, no separation of the possible central or peripheral actions of TRH as an explanation of these effects has been made (Stratton et al 1976).

Neonatal treatment with α -MSH alters later social behaviour and improves the performance on learning, memory and attention tasks in male rats; neonatal injection of an ACTH(4-9) analogue enhances attention in adult rats; and ACTH improves 'following' behaviour during imprinting in ducklings. One subcutaneous injection of ACTH(1-39), (1-24) or (1-16) to neonatal female rats was sufficient to accelerate eye opening, while injections of ACTH(1-39), (1-24), (1-10) or α -MSH accelerated motor behaviour (for references see Swaab & Martin 1981). The results with α -MSH may indicate a function for endogenous α -MSH in brain development, since injection of α -MSH antibodies subcutaneously into 19-day-old rat fetuses not only retarded body growth on Day 21, but also impaired brain maturation (Swaab et al 1978).

The various trophic effects of neuropeptides on the developing brain (cf. Swaab & Martin 1981; and see earlier) and the accelerated regeneration when ACTH(1-39) was given after crush denervation of peripheral nerves (Strand & Kung 1980) may suggest neuropeptides as valuable tools for correcting developmental disturbances of the nervous system. On the other hand, detrimental effects of neuropeptides on brain development are also known. ACTH given to children with petit mal epilepsy caused reversible enlargement of the cerebral ventricles and subarachnoid space, apathy, drowsiness and pseudodementia (Lagenstein et al 1979), while the deleterious effects of opiates, methadone and naloxone on fetal brain development (see Chasnoff et al 1980, Swaab & Martin 1981) might also be explained by an action via this peptide family. Since, moreover, the neonatal administration of β -endorphin or naloxone to the rat causes a permanent insensitivity to temperature stimuli (Sandman et al 1979), the proposed clinical use of β -endorphin as an analgesic at the time of delivery (Oyama et al 1980) or of naloxone to improve fetal heart rate (Goodlin 1981) should be strongly discouraged. For the reasons mentioned above one should, in general, be very cautious about using peptides during pregnancy and in the developing child. It will take some time to get gynaecologists used to the idea that even oxytocin can be considered to be a 'psychopharmakon' (cf. Buijs & Swaab 1979), but this compound too might be detrimental to the child (Cerutti et al 1979, Friedman et al 1979), possibly via a direct action on the brain.

In our 'unigender' era sexual dimorphism is often simply denied. Germaine Greer (1972) states: '... although sex hormones do enter the brain, ... no

correlation between that physiological fact and mental capacity or behaviour has ever been established . . .'. Yet an extensive literature exists on sex differences in brain morphology, function and sensitivity to various compounds, differences that are present early in development and might be of utmost importance for the evaluation of experimental results. This holds not only for systems directly involved in reproduction, like the sex differences in the human fetal brain content of LRH (Siler-Khodr & Khodr 1978) and human hypothalamic feedback actions (Seyler et al 1978). Increased body contact in the open field 120 days after neonatal administration of α -MSH was found only in males, and the improvement by neonatal α -MSH treatment of learning, memory and attention was also found only in males. ACTH(1-39) had more effect on the advancement of eye opening in females than in males and caused more ambulation and less grooming than normal in females, while in males no effect was found (for references see Swaab & Martin 1981).

Recent observations suggest a morphological basis for sex-dependent effects. The morphologically observed sex differences in the suprachiasmatic nucleus (Gorski et al 1978) are also reflected in its peptidergic neurons. A permanent sex difference was found in the vasopressinergic innervation of the lateral septum and lateral habenula from postnatal Day 10 onwards (De Vries et al 1981). This difference—the male rats having a much denser innervation than the female rats—can be manipulated by sex steroids in the neonatal period (G. J. De Vries et al, unpublished). Apart from these peptidergic differences, sexual dimorphism has been described in the rat in aminergic transmitters (Demarest et al 1981), in dendritic trees (Greenough et al 1977) and synaptic distribution (Matsumoto & Arai 1980), in brain structures like the sexually dimorphic nucleus of the preoptic area and the suprachiasmatic nucleus (Gorski et al 1978), in septal driving of the hippocampal theta rhythm (Drewett et al 1977), and even in the presence of spinal cord motor nuclei (Breedlove & Arnold 1980). In the human brain no morphological sexual dimorphism has (yet) been reported, although it is probably present, since the existence of functional sex differences—for example, in lateral differentiation (Levy & Reid 1978) and in mathematical abilities (Benbow & Stanley 1980)—is known. Since, in the rat, even the influence exerted by the uterine environment on fetal body growth affects male and female fetuses unequally (Ward et al 1977), we should pay attention to sex differences in *all* our experimental studies.

Conclusions and summary

A rapidly increasing number of peptidergic systems which contact other neurons by means of synapses is being detected throughout the brain. These

systems are already present early in development and influence not only 'peripheral' processes such as endocrine organs, fetal and placental growth, labour and circulation dynamics, but also brain development itself, often in a sex-dependent way. Peptidergic systems are thus crucial to the preparation for and the adaptation of the fetus to the outside world and might even be a means of promoting recovery in the damaged brain. However, at present the use of peptides in obstetrics should be severely limited because of their possible permanent effects on the developing organism.

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REFERENCES

- Aksel S, Tyrey L 1977 Luteinizing hormone-releasing hormone in the human fetal brain. *Fertil Steril* 28:1067-1071
- Araki S, Toran-Allerand CD, Ferin M, Vande Wiele RL 1975 Immunoreactive gonadotropin-releasing hormone (Gn-RH) during maturation in the rat: ontogeny of regional hypothalamic differences. *Endocrinology* 97:693-697
- Aubert ML 1979 Ontogenèse des fonctions hypothalamiques chez le foetus humain. *J Physiol (Paris)* 75:45-53
- Aubert ML, Grumbach MM, Kaplan SL 1977 The ontogenesis of human fetal hormones. IV. Somatostatin, luteinizing hormone releasing factor, and thyrotropin releasing factor in hypothalamus and cerebral cortex of human fetuses 10-22 weeks of age. *J Clin Endocrinol Metab* 44:1130-1141
- Barry J 1956 De l'existence probable de synapses interneuronales de type 'neurosecrétoire'. In: Ariëns Kappers J (ed) *Progress in neurobiology*. Elsevier, Amsterdam, p 36-44
- Benbow CP, Stanley JC 1980 Sex differences in mathematical ability: fact or artifact? *Science (Wash DC)* 210:1262-1264
- Bloch B, Bugnon C, Fellman D, Lenys D 1978 Immunocytochemical evidence that the same neurons in the human infundibular nucleus are stained with anti-endorphins and other related peptides. *Neurosci Lett* 10:147-152
- Boer GJ, Buijs RM, Swaab DF, De Vries GJ 1980 Vasopressin and the developing rat brain. *Peptides* 1 suppl 1:203-209
- Bohus B 1980 Effects of neuropeptides on adaptive autonomic processes. In: De Wied D, Van Keep PA (eds) *Hormones and the brain*. MTP Press, Lancaster, p 129-139
- Breedlove SM, Arnold AP 1980 Hormone accumulation in a sexually dimorphic motor nucleus of the rat spinal cord. *Science (Wash DC)* 210:564-566
- Brown M, Rivier J, Vale W 1977 Actions of bombesin, thyrotropin releasing factor, prostaglandin E₂ and naloxone on thermoregulation in the rat. *Life Sci* 20:1681-1688
- Bugnon C, Bloch B, Fellman D 1977 Etude immuno-cytologique des neurones hypothalamiques à LH-RH chez le foetus humain. *Brain Res* 128:249-262
- Buijs RM 1978 Intra- and extrahypothalamic vasopressin and oxytocin pathways in the rat.

- Pathways to the limbic system, medulla oblongata and spinal cord. *Cell Tissue Res* 192:423-435
- Buijs RM, Swaab DF 1979 Immuno-electron microscopical demonstration of vasopressin and oxytocin synapses in the rat limbic system. *Cell Tissue Res* 204:355-365
- Buijs RM, Swaab DF, Dogterom J, Van Leeuwen FW 1978 Intra- and extrahypothalamic vasopressin and oxytocin pathways in the rat. *Cell Tissue Res* 186:423-433
- Buijs RM, Velis DN, Swaab DF 1980 Ontogeny of vasopressin and oxytocin in the fetal rat: early vasopressinergic innervation of the fetal brain. *Peptides* 1:315-324
- Cerutti R, Stoppa G, Drago D et al 1979 Oxytocin and foeto-neonatal stress. In: Zichella L, Pancheri P (eds) *Psychoneuroendocrinology in reproduction*. Elsevier, Amsterdam, p 529-532
- Chan-Palay V, Palay SL 1977 Ultrastructural identification of substance P cells and their processes in rat sensory ganglia and their terminals in the spinal cord by immunocytochemistry. *Proc Natl Acad Sci USA* 74:4050-4054
- Chasnoff IJ, Hatcher R, Burns WJ 1980 Early growth patterns of methadone-addicted infants. *Am J Dis Child* 134:1049-1051
- Clendeninn NJ, Petraitis M, Simon EJ 1976 Ontological development of opiate receptors in rodent brain. *Brain Res* 118:157-160
- Conklin PM, Schindler WJ, Hull SF 1973 Hypothalamic thyrotropin releasing factor. Activity and pituitary responsiveness during development of the rat. *Neuroendocrinology* 11:197-211
- Cooper BR, Boyer CE 1978 Stimulant action of thyrotropin releasing hormone on cat spinal cord. *Neuropharmacology* 17:153-156
- Csaba G, László V, Darvas Z, Berzétei I 1980 Amplification of hormone receptors by neonatal oxytocin and vasopressin treatment. *Horm Metab Res* 12:28-31
- Daikoku S, Kawano H, Matsumura H, Saito S 1978 In vivo and in vitro studies on the appearance of LHRH neurons in the hypothalamus of perinatal rats. *Cell Tissue Res* 194:433-445
- Demarest KT, McKay DW, Riegle GD, Moore KE 1981 Sexual differences in tuberoinfundibular dopamine nerve activity induced by neonatal androgen exposure. *Neuroendocrinology* 32:108-113
- De Vries GJ, Buijs RM, Swaab DF 1981 Ontogeny of the vasopressinergic neurons of the nucleus supra-chiasmaticus and their extrahypothalamic projections in the rat brain. *Brain Res* 218:67-78
- De Wied D 1965 The influence of posterior and intermediate lobe of the pituitary and pituitary peptides on the maintenance of a conditioned avoidance response in rats. *Int J Neuropharmacol* 4:157-167
- De Wied D 1980 Peptides and adaptive behaviour. In De Wied D, Van Keep PA (eds) *Hormones and the brain*. MTP Press, Lancaster, p 103-113
- Dogterom J, Van Wimersma Greidanus TJB, Swaab DF 1977 Evidence for the release of vasopressin and oxytocin into cerebrospinal fluid: measurements in plasma and CSF of intact and hypophysectomized rats. *Neuroendocrinology* 24:108-118
- Dogterom J, Snijdwint FGM, Pévet P, Swaab DF 1980 Studies on the presence of vasopressin, oxytocin and vasotocin in the pineal gland, subcommissural organ and foetal pituitary gland: failure to demonstrate vasotocin in mammals. *J Endocrinol* 84:115-123
- Drewett RF, Gray JA, James DTD, McNaughton N, Valero J, Dudderidge HJ 1977 Sex and strain differences in septal driving of the hippocampal theta rhythm as a function of frequency: effects of gonadectomy and gonadal hormones. *Neuroscience* 2:1033-1041
- Eckstein N, Wehrenberg WB, Louis K et al 1980 Effects of substance P on anterior pituitary secretion in the female monkey. *Neuroendocrinology* 31:338-342
- Emson PC, Gilbert FFT, Loren I, Fahrenkrug J, Sundler F, Schaffalitzky de Muckadell OB 1979 Development of vasoactive intestinal polypeptide (VIP) containing neurones in the rat brain. *Brain Res* 177:437-444
- Faivre-Bauman A, Grouselle D, Nemeskeri A, Tixier-Vidal A 1978 Ontogenesis of thyroliberin in the mouse hypothalamus. *Brain Res* 154:382-387

- Fellman D, Bloch B, Bugnon C, Lenys D 1979 Etude immunocytologique de la maturation des axes neuroglandulaires hypothalamo-neurohypophysaires chez le fœtus humain. *J Physiol (Paris)* 75:37-43
- Friedman EA, Sachtleben MR, Wallace K 1979 Infant outcome following labor induction. *Am J Obstet Gynecol* 133:718-722
- Ghirlanda G, Bataille D, Dubois MP, Rosselin G 1978 Variations of the somatostatin content of gut, pancreas, and brain in the developing rat. *Metab Clin Exp* 27 suppl 1:1167-1170
- Gilbert RFT, Emson PC 1979 Substance P in rat CNS and duodenum during development. *Brain Res* 171:166-170
- Gillies G, Lowry PJ 1979 The relationship between vasopressin and corticotropin-releasing factor. In: Jones MT et al (eds) *Interaction within the brain-pituitary-adrenocortical system*. Academic Press, London, p 51-60
- Goodlin RC 1981 Naloxone and its possible relationship to fetal endorphin levels and fetal distress. *Am J Obstet Gynecol* 139:16-19
- Gorski RA, Gordon JH, Shryne JE, Southam AM 1978 Evidence for a morphological sex difference within the medial preoptic area of the rat brain. *Brain Res* 148:333-346
- Greenough WT, Carter CS, Steerman C, DeVoogd TJ 1977 Sex differences in dendritic patterns in hamster preoptic area. *Brain Res* 126:63-72
- Greer G 1972 *The female eunuch*. Paladin, London, p 99
- Hadeed AJ, Leake RD, Weitzman RE, Fisher DA 1979 Possible mechanisms of high blood levels of vasopressin during the neonatal period. *J Pediatr* 94:805-808
- Hetta J, Terenius L 1980 Prenatal naloxone affects survival and morphine sensitivity of rat offspring. *Neurosci Lett* 16:323-327
- Hökfelt T, Fuxe K, Johansson O, Jeffcoate S, White N 1975 Distribution of thyrotropin-releasing hormone (TRH) in the central nervous system as revealed with immunohistochemistry. *Eur J Pharmacol* 34:389-392
- Hunt NH, Perris AD, Sandford PA 1977 Role of vasopressin in the mitotic response of rat bone marrow cells to haemorrhage. *J Endocrinol* 72:5-16
- Iwamoto HS, Rudolph AM, Keil LC, Heymann MA 1979 Hemodynamic responses of the sheep fetus to vasopressin infusion. *Circ Res* 44:430-436
- Lagenstein I, Willig RP, Kühne D 1979 Cranial computed tomography (CCT) findings in children treated with ACTH and dexamethasone: first results. *Neuropädiatrie* 10:370-384
- Landgraf R, Ermisch A, Hess J 1979 Indications for a brain uptake of labelled vasopressin and oxytocin and the problem of the blood-brain barrier. *Endokrinologie* 73:77-81
- Levy J, Reid M 1978 Variations in cerebral organization as a function of handedness, hand posture in writing, and sex. *J Exp Psychol* 107:119-144
- Lichardus B, Ponec J, Brlicová A 1978 The application of dDAVP in pregnancy interferes with ontogenesis of osmoregulation in rats. In: Dörner G, Kawakami M (eds) *Hormones and brain development*. Elsevier, Amsterdam, p 247-252
- Lincoln DW, Wakerley JB 1974 Electrophysiological evidence for the activation of supraoptic neurones during the release of oxytocin. *J Physiol (Lond)* 242:533-554
- Lomonaco D, Van Rynberk G 1901 Recherche sulla funzione della ipofisi cerebrale. *Riv Mens Neuropatol Psichiatri* 9:1-28
- Matsumoto A, Arai Y 1980 Sexual dimorphism in 'wiring pattern' in the hypothalamic arcuate nucleus and its modification by neonatal hormonal environment. *Brain Res* 190:238-242
- Misantone LJ, Ellis S, Epstein AN 1980 Development of angiotensin-induced drinking in the rat. *Brain Res* 186:195-202
- Noyer M, Diem Bui N, Deschodt-Lanckman M et al 1980 Postnatal development of the cholecystokinin-gastrin family of peptides in the brain and gut of rat. *Life Sci* 27:2197-2203
- Oliver C, Giraud P, Lissitzky JC, Conte-Devolx B, Gillioz P 1981 Influence of thyrotropin-releasing hormone on the secretion of thyrotropin in neonatal rats. *Endocrinology* 108:179-182

- Oyama T, Matsuki A, Taneichi T, Ling N, Guillemin R 1980 β -Endorphin in obstetric analgesia. *Am J Obstet Gynecol* 137:613-616
- Paulin C, Li J, Bégeot M, Dubois PM 1976 La somatostatine et la fonction somatotrope antéhypophysaire chez le fœtus humain. In: *Coll Neuroendocrinologie INSERM*, Paris, p 129-138
- Paulin C, Dubois MP, Barry J, Dubois PM 1977 Immunofluorescence study of LH-RH producing cells in the human fetal hypothalamus. *Cell Tissue Res* 182:341-345
- Paulin C, Dubois PM, Czernichow P, Dubois MP 1978 Immunocytological evidence for oxytocin neurons in the human fetal hypothalamus. *Cell Tissue Res* 188:259-264
- Pavel S 1979 The mechanism of action of vasotocin in the mammalian brain. *Prog Brain Res* 52:445-458
- Pedersen CA, Prange AJ 1979 Induction of maternal behavior in virgin rats after intracerebro-ventricular administration of oxytocin. *Proc Natl Acad Sci USA* 76:6661-6665
- Pickel VM, Joh TH, Reis DJ, Leeman SE, Miller RJ 1979 Electron microscopic localization of substance P and enkephalin in axon terminals related to dendrites of catecholaminergic neurons. *Brain Res* 160:387-400
- Pohjavuori M, Fyhrquist F 1980 Hemodynamic significance of vasopressin in the newborn infant. *J Pediatr* 97:462-465
- Robberecht P, Deschodt-Lanckman M, De Neef Ph, Christophe J 1979 Vasoactive intestinal peptide: levels and functional receptors in rat brain before and after weaning. *Life Sci* 25:1001-1008
- Rotsztejn WH, Benoist L, Besson J et al 1980 Effect of vasoactive intestinal peptide (VIP) on the release of adenohipophyseal hormones from purified cells obtained by unit gravity sedimentation. *Neuroendocrinology* 31:282-286
- Sakuma Y, Pfaff DW 1980 LH-RH in the mesencephalic central grey can potentiate lordosis reflex of female rats. *Nature (Lond)* 283:566-567
- Sandman CA, McGivern RF, Berka C, Walker M, Coy DH, Kastin AJ 1979 Neonatal administration of β -endorphin produces 'chronic' insensitivity to thermal stimuli. *Life Sci* 25:1755-1760
- Seyler LE, Canalis E, Spare S, Reichlin S 1978 Abnormal gonadotropin secretory responses to LRH in transsexual women after diethylstilbestrol priming. *J Clin Endocrinol Metab* 47:176-183
- Shimatsu A, Kato Y, Matsushita N, Katakami H, Yanaihara N, Imura H 1981 Immunoreactive vasoactive intestinal polypeptide in rat hypophysial portal blood. *Endocrinology* 108:395-398
- Siler-Khodr TM, Khodr GS 1978 Studies in human fetal endocrinology. I. Luteinizing hormone-releasing factor content of the hypothalamus. *Am J Obstet Gynecol* 130: 795-800
- Sladek CD, Joynt RJ 1979 Angiotensin stimulation of vasopressin release from the rat hypothalamo-neurohypophyseal system in organ culture. *Endocrinology* 104:148-153
- Strand FL, Kung TT 1980 ACTH accelerates recovery of neuromuscular function following crushing of peripheral nerve. *Peptides* 1:135-138
- Stratton LO, Gibson CA, Kolar KG, Kastin AJ 1976 Neonatal treatment with TRH affects development, learning, and emotionality in the rat. *Pharmacol Biochem Behav* 5 suppl 1:65-67
- Swaab DF 1980 Neuropeptides and brain development—a working hypothesis. In: Di Benedetta C et al (eds) *A multidisciplinary approach to brain development*. Elsevier/North-Holland Biomedical Press, Amsterdam, p 181-196
- Swaab DF, Boer K 1980 Technical developments in the study of neuroendocrine mechanisms in rat pregnancy and parturition. In: Nathanielsz PW (ed) *Animal models in fetal medicine*, I. (Monographs in fetal physiology vol 2) Elsevier/North-Holland Biomedical Press, Amsterdam, p 169-234
- Swaab DF, Martin JT 1981 Functions of α -melanotropin and other opiomelanocortin peptides in

- labour, intrauterine growth and brain development. In: Peptides of the pars intermedia. Pitman Medical, London (Ciba Found Symp 81) p 196-217
- Swaab DF, Pool CW, Nijveldt F 1975 Immunofluorescence of vasopressin and oxytocin in the rat hypothalamo-neurohypophyseal system. *J Neural Transm* 36:195-215
- Swaab DF, Pool CW, Van Leeuwen FW 1977 Can specificity ever be proved in immunocytochemical staining? *J Histochem Cytochem* 25:388-391
- Swaab DF, Boer GJ, Visser M 1978 The fetal brain and intrauterine growth. *Postgrad Med J* 54 suppl 1:63-73
- Swaab DF, Visser M, Dogterom J 1979 The maturity of the foetal brain and its involvement in labour. In: Zichella L, Pancheri P (eds) *Psychoneuroendocrinology in reproduction*. Elsevier, Amsterdam, p 483-495
- Vandesande F, Dierickx K, De Mey J 1975 Identification of the vasopressin-neurophysin producing neurons of the rat suprachiasmatic nuclei. *Cell Tissue Res* 156:377-380
- Van Wimersma Greidanus TjB, Dogterom J, De Wied D 1975 Intraventricular administration of anti-vasopressin serum inhibits memory consolidation in rats. *Life Sci* 16:637-644
- Van Wimersma Greidanus TjB, Croiset G, Goedemans H, Dogterom J 1979 Vasopressin levels in peripheral blood and in cerebrospinal fluid during passive and active avoidance behavior in rats. *Horm Behav* 12:103-111
- Visser M, Swaab DF 1979 Life span changes in the presence of α -melanocyte-stimulating-hormone-containing cells in the human pituitary. *J Dev Physiol* 1:161-178
- Ward WF, Karp CH, Aceto H 1977 Developmental effects of the uterine environment: dependence on fetal sex in rats. *J Reprod Fertil* 50:269-274
- Winters AJ, Eskay RL, Porter JC 1974 Concentration and distribution of TRH and LRH in the human fetal brain. *J Clin Endocrinol Metab* 39:960-963
- Wright WA, Kutscher CL 1977 Vasopressin administration in the first month of life: effects on growth and water metabolism in hypothalamic diabetes insipidus rats. *Pharmacol Biochem Behav* 6:505-509

DISCUSSION

Ellendorff: If the injection of vasopressin into the rat fetus shows positive effects, the control experiment would be to deprive the rat of vasopressin. What happens in the Brattleboro rat, which lacks vasopressin? Rats of this strain should show all the deficiency symptoms.

Swaab: We thought initially that the Brattleboro rat would be an excellent control for all the functions of vasopressin, in particular for the central functions which are so hard to study. However, the behavioural deficiencies are rather controversial. In this respect it is interesting to consider the lateral septum and lateral habenula, which are major sites of vasopressinergic termination. This innervation is lacking in the homozygous Brattleboro rat. In the adult Brattleboro these areas are more densely innervated by serotonergic terminations. This suggests that the missing peptidergic fibres are replaced by a different system and that the Brattleboro rat is not the simple and ideal model for endogenous central vasopressin effects that we had all hoped. This rat has in fact a different brain (Swaab 1980).

Nathanielsz: As I understand it, in the Brattleboro rat you try to make good the vasopressin deficit in the cerebellar region. One wonders about the presence of receptors. You touched on up-regulation of receptors by their hormone. Is there any deficiency of the receptor in the Brattleboro rat, which has lacked vasopressin throughout its development?

Swaab: The cerebellum is a problem, because we couldn't find any extrahypothalamic peptidergic innervation of importance in that area, while it was one of the most severely affected areas in Brattleboro rats. Tracer studies show considerable vasopressin binding in the cerebellum (Willumsen & Bie 1969), so endogenous vasopressin might reach the cerebellum in the cerebrospinal fluid (CSF).

There is no information on vasopressin receptors in the brain as yet. We are trying to localize such receptors cytochemically. Behavioural experiments by David De Wied and his group show that the Brattleboro rat reacts to vasopressin given directly into the brain, so receptors should be present.

Challis: On the question of sex differences, some of the effects of injected peptides seemed to happen around the time when the indifferent gonad would still be present in the rat fetus. Has hormonal sexual differentiation begun then, or are we looking at an effect on the genome?

Swaab: Hormonal sexual differentiation starts well before birth in the rat. The sex differences in the brain that I mentioned, however, have been described only after birth in the rat.

Ellendorff: Jacobson & Gorski (1981) have shown in the rat that around Day 15 of fetal life there is a clear sex difference in neuroblast proliferation within the sexually dimorphic nucleus of the preoptic area. So sexual differentiation of the brain must take place very early in the rat, possibly under the influence of testosterone.

Swaab: On the other hand, organizational effects of steroids can be demonstrated in the neonatal period, not only on ovulation but also on behaviour (Van Oyen 1981), and on sex differences in the peptidergic innervation of the brain (G. J. De Vries et al, unpublished results). However, the exact critical phase in which the various phenomena are influenced by sex hormones might depend on the system studied.

Gluckman: One problem is to determine whether the effects of these peptides are direct or not. Many of them could be mediated by more classical neurotransmitter systems that have been stimulated by or inhibited by the peptide given. For example, when α -MSH is administered intravenously to the ovine fetus, fetal plasma prolactin concentrations are lowered (Gluckman et al 1980). This effect is likely to be mediated by the increased hypothalamic release of dopamine. What do you think about direct as opposed to secondary effects mediated by the more 'classical' neurotransmitters that are likely to be involved in brain growth and in the regulation of steroid hormone effects?

Swaab: Peptides like vasopressin and oxytocin are found in synapses that don't contain any classical transmitters and cannot be distinguished morphologically from the classical aminergic or amino acid-containing synapses. This suggests a direct synaptic effect of peptides on other neurons. Little is known about the electrical properties of such peptidergic synapses in the mammalian brain. Morris et al (1980) showed that iontophoretically applied oxytocin inhibited spontaneous and glutamate-induced firing of brainstem neurons. All the observations suggest a direct effect on neurons.

Gluckman: The long-term effects you find with vasopressin are similar to the long-term effects of perinatal drug therapy, which are presumably mediated by changes in neurotransmitter systems (Lundborg & Engel 1978, Slotkin et al 1979).

Swaab: Yes, but it has never been checked whether such drugs have any effect on peptides. People looked at the classical transmitters only, because at the time that was the most logical thing to do. There is no reason yet to think that peptides are acting differently from the classical neurotransmitters. The classical transmitters can also be released into the blood; this is true for amines like dopamine and probably also for GABA (Tappaz et al 1981). So the distinction between a neurohormone and a neurotransmitter is based only on the site where the compound is released. The name is, however, not specific for a given type of compound. A neurohormone or a neurotransmitter can be an amine, an amino acid or a peptide.

Thorburn: You said that vasopressin neurons were present in the suprachiasmatic nucleus. Where do their axons end? Is it possible that vasopressin, which can act as a corticotropin-releasing factor, is released from these neurons into the portal circulation and hence reaches the anterior pituitary, and stimulates release of ACTH?

Swaab: Vasopressin is found in the external zone of the median eminence, but it comes from the paraventricular nucleus, not from the suprachiasmatic nucleus. We think that the suprachiasmatic vasopressinergic fibres terminate in quite a number of brain areas, but final proof of these connections is still lacking. The putative sites of termination point, however, to central functions rather than to release into the blood, with the possible exception of the organum vasculosum of the lamina terminalis.

Thorburn: Is there any way that the suprachiasmatic nucleus could be driving the paraventricular nucleus?

Swaab: Yes. We think there is a loop between the SCN and the PVN via the lateral septum which might be important for osmoregulation (De Vries et al 1981).

Davis: Do any of these peptides cross the placenta? I would have thought it unlikely that they would.

Swaab: We have found oxytocin to be present in normal amounts in the

amniotic fluid of anencephalic fetuses, which do not produce this hormone themselves, so it must come from the mother.

Gluckman: The ability of neuropeptides to cross the placenta is very species-dependent. In some mammals, such as the rhesus monkey, thyrotropin-releasing factor (TRF) appears to cross the placenta (Azukizawa et al 1976). In the sheep, it doesn't (Thomas et al 1975).

Swaab: Some of these are poor experiments, done with iodinated hormones. This changes the physicochemical characteristics of the peptides considerably and does not give relevant physiological information.

Gluckman: In the experiments I am referring to the synthetic peptide was given and changes in the dependent hormone on the other side of the placenta were looked for. A further problem with studies where peptides are given to the mother and fetal effects are investigated is the problem of excluding indirect effects. For example, administration of growth hormone to the pregnant rat was reported to affect the neural development of the rat fetus (Sara & Lazarus 1974). However, growth hormone does not cross the rat placenta. The explanation of this experiment is not clear. One possibility is that growth hormone slightly prolonged gestation (Croskerry & Smith 1975). Alternatively, maternal nutrition or metabolism might have been affected.

Swaab: But growth hormone is not a peptide!

Thorburn: Substances given to the mother don't have to cross the placenta to affect the fetus. Tim Chard showed high levels of vasopressin and oxytocin in cord plasma and showed that the levels varied markedly, depending on the nature of the labour (Chard et al 1971). One could imagine that in a difficult labour, or with the administration of oxytocin to the mother inappropriately, there might be large variations in the levels of these peptides in the fetal circulation.

Swaab: Endogenous fetal peptides will probably be tremendously important for brain development, but we shouldn't disregard the possibility that compounds injected into the mother, such as oxytocin, do pass to the child. Now that peptides have become the fashion we should make the clinicians aware of the theoretical dangers.

Ellendorff: Clarke et al (1979) have proposed a model of an enkephalin and β -endorphin mechanism regulating oxytocin release at oxytocin terminals in the posterior pituitary. Martin & Voigt (1981) have shown enkephalin to co-exist with oxytocin and vasopressin in nerve terminals of rats, Met-enkephalin in oxytocin cells and Leu-enkephalin frequently in vasopressin cells. You used essentially the same technique. Have you any evidence that something similar happens in prenatal life?

Swaab: F. W. Van Leeuwen in our institute is studying this by immunoelectron microscopy in the adult rat. Met-enkephalin and vasopressin are always present in different terminals and in different types of granules. Interestingly,

the terminations of Met-enkephalin fibres seem always to be found in the vicinity of glial cells in the neurohypophysis. This suggests that Met-enkephalin has a regulatory action on the release of vasopressin, mediated via the glial cells.

Wealthall: Do you regard the fact that many of the vasopressin-transmitting fibres seem to terminate next to CSF spaces as confirmatory evidence that the CSF system is an extracirculatory portal system for transmission of hormones or transmitters from one area of the CSF system to another, or do you think that the central effects of vasopressin are mediated entirely within the brain substance?

Swaab: We think that CSF transport is not important for the central effects of vasopressin and oxytocin in the adult. It might be a transport system in fetal life. In adult life the central effects of these peptides can be explained by the extrahypothalamic fibres and terminals. The peptides in the CSF (Dogterom et al 1977) are probably peptides that have been released by extrahypothalamic pathways in certain brain areas and are being transported back to the blood.

Liggins: Is there any evidence for neuropeptides other than those for oxytocin and vasopressin? And what do you think is the functional significance of neuropeptides?

Swaab: The only suggestions and few observations I know are that neuropeptides may have effects on lipolysis, and be hypo- or hyperglycaemic, natriuretic and amino acid level-depressing (Rudman et al 1975, Trygstad et al 1975, Robinson et al 1975). It does not seem logical, anyhow, to make such a large molecule in order to produce only nine active amino acids and discard the rest. In relation to knowledge of the opiomelanocortin family, one might also expect that neuropeptides or split products of them will have biological functions. But final proof of function is still lacking.

Stahlman: Many of these peptides are common to brain, gut and, now, fetal lung. Many have also been found in lung tumours in the adult. We have begun to look for them in the 'K' cells of human fetal lung from eight weeks to term. Wharton et al (1978) first demonstrated bombesin in fetal lung, and this peptide is readily found throughout fetal life. We have also found VIP (vasoactive intestinal peptide), which Said has shown in extracts of adult human lung and in lungs of other species (Said & Mutt 1977). We find it in neuroepithelial cells by horseradish peroxidase binding techniques. Said has demonstrated VIP by immunofluorescence, surrounding blood vessels and bronchi (Dey & Said 1980). Bombesin and VIP both have physiological effects on the fetal or newborn lung. In the newborn lung in lambs, bombesin increases airway resistance when infused intravenously, and VIP lowers pulmonary vascular resistance. It has been suggested that paracrine cells operate locally to regulate local matching of ventilation and perfusion.

Dawes: Professor Blakemore, your view as an experimental neurophysiologist is slightly different from that of some of the other participants. Have you any comments on the widespread availability of these substances?

Blakemore: I think it's appropriate to distinguish between the development of transmitter mechanisms as such and of neuromodulators. There seems to be more and more evidence that substances which act as localized transmitters at some sites can act, either in the nervous system or elsewhere in the body, as modulators, as growth factors, as agents with other functions. The fact that certain substances are present in the fetus doesn't prove that they are functionally active in the same capacity that they have in the adult.

Swaab: That is true, but I think I showed some evidence that oxytocin and vasopressin may well be active in brain development, central functions and behaviour.

Blakemore: It depends what you mean by brain development. Do you mean the promotion of growth and connections in the brain, or the propagation of impulses through the fetal nervous system?

Swaab: I have no information on impulse propagation. So far as brain development is concerned, I am thinking along the lines of stimulatory effects on cell division. Rozengurt et al (1979) showed the enhancement of mitotic activity in cultured mouse 3T3 fibroblasts by vasopressin. Conversely, the unpublished work of G. J. Boer, in collaboration with Dr A. J. Patel and Dr R. Balázs, shows retarded DNA synthesis in the cerebral cortex, cerebellum and olfactory bulb in the homozygous Brattleboro rat.

Blakemore: It is an interesting concept that the developing nervous system goes through two major phases, one in which it grows and forms connections without impulses being present; and a second in which there are impulses. There could be completely different rules for the mechanisms of construction of the nervous system during these two phases. When axons grow out towards their target cells, the mechanism of guidance obviously cannot depend on the presence of connections between the target cells and the growing axons; but the refinement of local connections, and perhaps the modulation of synaptic efficiency, once axons have reached their targets, might well depend on the traffic of impulses through those channels.

Swaab: It may well be that synapse formation is not dependent on impulses (see p 169). Ultrastructural investigations indicate that the function of the visual system does not so much influence the number of synapses but rather the presynaptic grid and the number of synaptic vesicles (Müller et al 1981, Vrensen & Nunes Cardozo 1981).

Blakemore: Is it possible that the same substance could act at one phase in the development of the neuron as a general factor regulating growth, or a chemospecific factor for guiding growth, and at later stages as a transmitter?

Swaab: That is what I believe for vasopressin, that in the developing brain

it is released into the CSF, acting in a general way on the CNS by changing the rate of formation of nerve cells, and then in adulthood it goes by extrahypothalamic pathways, acting in a fairly precise way on certain functions like osmotic and temperature regulation, blood pressure regulation, and behaviour, depending on the site of termination of the fibres (Swaab 1980).

Johnston: I would like to add that a revolution appears imminent in neuropharmacology because of Hökfelt's demonstration that the same neuron can contain both a classical neurotransmitter, such as serotonin or dopamine, and a peptide. That is a revision of the rule that one neuron can contain only one transmitter substance. For instance, dopamine neurons projecting from the mesencephalon up to the forebrain contain dopamine and cholecystikinin immunoreactivity (Hökfelt et al 1980). We shall now have to re-study the peripheral and central autonomic nervous system for the co-release of these two substances. In some of these neurons the co-release of a peptide and a conventional neurotransmitter may allow modulation of the effect of the transmitter that is released at the same synapse.

Swaab: The proof for the existence of co-transmitters depends so far only on immunoelectron microscopy, and hence on the specificity of that technique. It is at present hard to say what one is actually staining by means of this technique. The antibody is specific for an antigenic site and not for the compound as such. The combination of amino acids which makes up the antigenic site of the peptide might also be present in other compounds in the cell. Cross-reactivity is a serious problem in light and even more in immunoelectron microscopy (Swaab et al 1977, Pool et al 1981). It has been difficult to prove the specificity of vasopressin staining, and it was in fact only possible using the Brattleboro rat. So, because of the pitfalls in the technique, we have no final proof of the existence of co-transmitters yet. In addition, does the presence of a given compound in a synapse prove that it is acting as a transmitter, or not?

REFERENCES

- Azukizawa M, Murata Y, Ikenoue T, Martin CB, Hershman JM 1976 Effect of thyrotropin-releasing hormone on secretion of thyrotropin, prolactin, thyroxine, and triiodothyronine in pregnant and fetal rhesus monkeys. *J Clin Endocrinol Metab* 43:1020-1028
- Chard T, Hudson CN, Edwards CRW, Boyd NRH 1971 Release of oxytocin and vasopressin by the human foetus during labour. *Nature (Lond)* 234:352-353
- Clarke G, Wood P, Merrick L, Lincoln DW 1979 Opiate inhibition of peptide release from the neurohumoral terminals of hypothalamic neurones. *Nature (Lond)* 282:746-748
- Croskerry PG, Smith GK 1975 Prolongation of gestation by growth hormone: a confounding factor in the assessment of its perinatal action. *Science (Wash DC)* 189:648-650

- De Vries GJ, Buijs RM, Swaab D 1981 Ontogeny of the vasopressinergic neurons of the suprachiasmatic nucleus and their extrahypothalamic projections in the rat brain. Presence of a sex difference in the lateral septum. *Brain Res* 218:67-78
- Dey RD, Said SI 1980 Immunocytochemical localization of VIP-immunoreactive nerves in bronchial walls and pulmonary vessels. *Fed Proc* 39:1062 (abstr 4171)
- Dogterom J, Van Wimersma Greidanus TJB, Swaab DF 1977 Evidence for the release of vasopressin and oxytocin into cerebrospinal fluid: measurements in plasma and CSF of intact and hypophysectomized rats. *Neuroendocrinology* 24:108-118
- Gluckman PD, Marti-Henneberg C, Leisti S, Kaplan SL, Grumbach MM 1980 Alpha melanocyte stimulating hormone inhibits prolactin secretion in fetal and infant lambs. *Life Sci* 27:1429-1433
- Hökfelt T, Lundberg JM, Schultzberg M, Johansson O, Ljungdahl A, Rehfeld JF 1980 Coexistence of peptides and putative transmitters in neurons. In: Costa E, Trabucchi M (eds) *Neural peptides and neuronal communication*. Raven Press, New York
- Jacobson CD, Gorski RA 1981 Neurogenesis of the sexually dimorphic nucleus of the preoptic area in the rat. *J Comp Neurol* 196:519-529
- Lundborg P, Engel J 1978 Neurochemical brain changes associated with behavioural disturbances after early treatment with psychotropic drugs. In: Vernadakis A et al (eds) *Maturation of neurotransmission*. Karger, Basel (6th Meeting Int Soc Neurochemistry 1977), p 226-235
- Martin R, Voigt KH 1981 Enkephalins co-exist with oxytocin and vasopressin in nerve terminals of rat neurohypophysis. *Nature (Lond)* 289:502-504
- Morris R, Salt TE, Sofroniew MV, Hill RG 1980 Actions of microiontophoretically applied oxytocin, and immunohistochemical localization of oxytocin, vasopressin and neurophysin in the rat caudal medulla. *Neurosci Lett* 18:163-168
- Müller L, Pattisellano A, Vrensen G 1981 The postnatal development of the presynaptic grid in the visual cortex of rabbits and the effect of dark-rearing. *Brain Res* 205:39-48
- Pool C, Buijs R, Swaab DF, Boer G, van Leeuwen F 1981 On the way to a specific immunocytochemical localization. In: Cuello AC (ed) *Neuroimmunocytochemistry*. Wiley, New York and London (IBRO Handbook Series: Methods in neurosciences) in press
- Robinson AG, Michelis MF, Warms PC et al 1975 Biologic activity of neurophysin: natriuresis. *Ann NY Acad Sci* 248:317-323
- Rozengurt E, Legg A, Pettican P 1979 Vasopressin stimulation of mouse 3T3 cell growth. *Proc Natl Acad Sci USA* 76:1284-1287
- Rudman D, Chawla RK, Khatra BS et al 1975 Observations on the lipolytic and melanotropic properties of neurophysin proteins. *Ann NY Acad Sci* 248:324-335
- Said SI, Mutt V 1977 Relationship of spasmogenic and smooth muscle relaxant peptides from normal lung to other vasoactive compounds. *Nature (Lond)* 265:84-86
- Sara VR, Lazarus L 1974 Prenatal action of growth hormone on brain and behaviour. *Nature (Lond)* 250:257-258
- Slotkin TA, Whitmore WL, Salvaggio M, Seidler FJ 1979 Perinatal methadone addiction affects brain synaptic development of biogenic amine systems in the rat. *Life Sci* 24:1223-1230
- Swaab DF 1980 Neurohypophysial hormones and their distribution in the brain. In: De Weid D, Van Keep PA (eds) *Hormones and the brain*. MTP Press, Lancaster, p 37-100
- Swaab DF, Pool CW, Van Leeuwen FW 1977 Can specificity ever be proved in immunocytochemical staining? *J Histochem Cytochem* 25:388-391
- Tappaz ML, Aguera M, Belin MF et al 1981 GABA markers in the hypothalamic median eminence. In: Costa et al (eds) *GABA and benzodiazepine receptors*. Raven Press, New York, p 229-236
- Thomas AL, Jack PMB, Manns JG, Nathanielsz PW 1975 Effect of synthetic thyrotropin releasing hormone on thyrotropin and prolactin concentrations in the peripheral plasma of the pregnant ewe, lamb fetus and neonatal lamb. *Biol Neonate* 26:109-116

- Trygstad O, Foss I, Sletten K 1975 Metabolic activities of human neurophysins. *Ann NY Acad Sci* 248:304-316
- Van Oyen JGM 1981 Reactions to novel and aversive stimuli in female and male rats. Dissertation, University of Utrecht. Rodopi, Amsterdam
- Vrensen G, Nunes Cardozo J 1981 Changes in size and shape of synaptic connections after visual training—an ultrastructural approach to plasticity. *Brain Res*, in press
- Wharton J, Polak JM, Bloom SR, Ghatei MA, Solcia E, Brown MR, Pearse AGE 1978 Bombesin-like immunoreactivity in the lung. *Nature (Lond)* 273:769-770
- Willumsen NBS, Bie P 1969 Tissue to plasma ratios of radioactivity in the rat hypothalamo-neurohypophyseal system after intravenous injection of ^3H -lysine-vasopressin and ^3H -mannitol. *Acta Endocrinol* 60:389-400