Editorial Review

Neuropeptides and brain development
Current perils and future potential

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Introduction
The booming animal experimental literature in the last few years concerning central effects of neuropeptides presently is being followed by a rapidly increasing number of clinical trials with such compounds. Neuropeptides have been administered with varying amounts of success, to patients for their supposed effects on memory, attention, impotence, schizophrenia and pain, to mention just a few. In addition, clinical observations on the effects of peptides in pregnant women and in children have recently been reported in the literature. Deserving of special mention are apart from (a) the routine use in obstetrics of the neuropeptide oxytocin, (b) naloxone (an opiate antagonist) administered in order to return flat fetal heart rate to normal, (c) β-endorphin that has been injected intrathecally during delivery in order to provide analgesia, (d) vasotocin that was tried out on newborns and pre-pubertal children for the purpose of studying its effect upon REM sleep, and (e) vasopressin that is currently being given to mentally retarded children. Since there is a considerable amount of experimental data showing that peptides, when given during certain stages of development, may cause permanent abnormalities of the central nervous system, it is amazing how little attention is generally paid in clinical studies to the question of possible long-term side-effects which these substances might have on the developing brain.

The presence of neuropeptides in the developing brain
The large number of neuropeptides which have been identified so far, can be subdivided into the following four historically determined groups (Swaab, 1982): (1) those peptides, which were originally known as hypothalamic hormones, produced by neurons which were more recently shown to innervate not only the hypophysis and median eminence but extrahypothalamic areas as well (viz., vasopressin, oxytocin, LHRH (luteinizing hormone releasing hormone), somatostatin and TRH (thyroid stimulating hormone releasing hormone)); (2) those peptides which were first known as pituitary hormones but which later turned out to be produced by a variety of
neurons as well (viz., the peptides of the opiomelanocortin family); (3) peptides from which formerly no neuroendocrine action was known (e.g. substance P, angiotensin II, neurotensin, VIP (vasoactive intestinal peptide) but which recently turned out to be released into portal capillaries as well); (4) peptides which were first described as being typically “non-mammalian peptides” but which appear now to be present also in the mammalian brain, such as the hydra head activator peptide, the amphibian skin peptide bombesin, pancreatic polypeptide, and the moluscan cardioexcitatory peptide FMRF-amide.

Peptides of these groups have been demonstrated within the developing rat brain, in some cases as early as at fetal day 14 (e.g. vasopressin) and in all cases well before postnatal day 24, the time at which neurogenesis has virtually ceased. In the human fetal brain peptidergic cells are to be found from 5 weeks of gestation onwards (Swaab & Ter Borg, 1981). Improvements of techniques used will almost certainly shift their detection to even earlier stages of development. However, since most of the currently available data are based solely on immunocytochemical methods, with which the criterion specificity is difficult to fulfil, it is by no means yet certain that all of the stained compounds are in fact identical to the corresponding adult peptides.

**Transport of neuropeptides into the fetal brain**

Neuropeptides which are produced by the fetal brain could act on the developing brain via the following three different routes:

(a) The classical neuroendocrine way of peptide release, i.e. into the bloodstream most probably takes place already in the fetus. The neural lobe of the pituitary is innervated between day 17 and 18 of rat pregnancy by vasopressinergic and oxytocinergic fibres, and LHRH-containing fibres are present in the external zone of the rat median eminence at day 20.5 of gestation. The fetal neurohypophysial hormones oxytocin and vasopressin are, respectively, supposed to enhance uterine contractions (and thus to accelerate the course of labour) and to redistribute the fetal blood flow during labour so that the most important organs (heart, brain, adrenal) are favoured (Swaab & Ter Borg, 1981). Unlike in the adult situation, neuropeptides which are released into the bloodstream might also return to the fetal brain and exert an effective action upon its maturation because the blood–brain barrier is not yet fully developed (Johanson, 1980).

(b) Peptide influences upon CNS development can also be mediated via the peptidergic fibres that innervate other brain areas. Thus, vasopressin-containing fibres are present within the olfactory bulbs by fetal day 17 in the rat, and 2 days later in the amygdala (Boer, Buijs, Swaab & De Vries, 1980; Buijs, Velis & Swaab, 1980), while neurohypophysial hormone-containing fibres were observed in the human spinal cord already at 17 weeks of gestation. In the human fetal cortex. TRH and somatostatin have been found as early as 8–10 weeks of pregnancy (Swaab & Ter Borg, 1981).

(c) Vasopressin-containing fibres in the fetal and neonatal rat brain are seen protruding into the third and lateral ventricles, suggesting that the cerebrospinal fluid too might serve as a peptide avenue for transport during development (Boer et al., 1980; Buijs et al., 1980).

Although the extrahypothalamic nerve pathways are currently considered to be the preferential route for endogenous central peptide effects in the adult, the two
alternative routes (a) and (c) might also be important for the developing brain. It is not yet known whether or not maternal neuropeptides that cross the placenta (which has been suggested by us for oxytocin and by others for TRH: Azukizawa, Murata, Ikenoue, Martin & Hershman, 1976) are involved in normal brain development but this route is surely of importance for mediating effects of exogenously administered peptides.

**Central effects and functions of neuropeptides in development**

For its normal development neurons are dependent on a large number of chemical factors from outside as well as inside the central nervous system, such as thyroid hormones, gonadal hormones and a wide range of neurotransmitters (Patel & Lewis, 1982; Patel & Balázs, 1980). In fact, the developing brain appears to be affected by precisely those factors which are important for brain function in adult life. Since neuropeptides are presently considered to be potent centrally active compounds, and even as putative neurotransmitters (Swaab, 1982), they may be suspected of permanently influencing CNS growth processes when present during early development (Fig. 1). This is illustrated by the fact that prenatal or neonatal administration of vasopressin or its analogues interferes with the development of osmoregulation in the rat (Boer & Swaab, 1983). Furthermore neonatal TRH administration results in faster running towards a food reward and leads to an increase

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**Fig. 1** Neuropeptide involvement in the development of the central nervous system.
Fig. 2  Sex-dimorphism in the vasopressinergic innervation of the lateral septum (ls) of the adult rat brain. The lower panels of higher magnification even more clearly show the denser fiber density in males (lv: lateral ventricle; photographs from G. J. de Vries).
Fig. 3 Cerebellar morphology in the absence of vasopressin throughout life. The midsagittal view of the vermis of cerebellum are of six-month-old adult Brattleboro rats, being either heterozygous (het, control) or homozygote (hom) for recessive hereditary absence of vasopressin. Apart from the reduced size and cell content, morphologically most remarkable is the absence of lobulus VId (arrowhead) in the hom Brattleboro rat (Preparation from H. B. M. Uylings).
in pineal and hypothalamic weight (Stratton, Gibson, Kolar & Kastin, 1976). Neonatal administration of α-MSH or its analogues alters later social behavior and attention mechanisms, and also accelerates motor behavior development. Neonatally administered ACTH and its analogues all accelerate eye opening and the development of motor behavior (Beckwith, O'Quinn, Petro, Kastin & Sandman, 1977a; Beckwith, Sandman, Hothersall & Kastin, 1977b; Van der Helm-Hylkema, 1973; Van der Helm-Hylkema & De Wied, 1976).

In this connection it is important to point out that many of these effects have been reported to be sex-dependent. Sexual dimorphism of the brain is often denied a priori and, indeed, there is at present no morphological evidence on record for sexual dimorphism within the human brain. However, many convincing demonstrations of sex-linked differences in brain morphology and function in other species are on record, and these may constitute the morphological basis of sex-dependent peptidergic effects. Sex-linked differences within the brain are present in the rat quite early in development, also as regards peptidergic systems. For instance, a sex difference was found from postnatal day 12 onwards in the vasopressinergic innervation of the lateral septum (Fig. 2) and the lateral habenula (De Vries, Buijs & Swaab, 1981). Recent observations reveal, moreover, that this difference – the male rats having denser innervation than the female rats – can be manipulated by the administration of gonadal steroids. Also effects of peptides have been reported to be sex-dependent. Neonatal administration of α-MSH causes increased body contact in the open field in adulthood only in male rats, and the improvement of learning, memory and attention was also found only in males (Beckwith et al., 1977a,b). Neonatally administrated ACTH had more effect on the advancement of eye opening in females and caused more ambulation and less grooming in female rats, whereas in males no effect was found (Van der Helm-Hylkema, 1973; Van der Helm-Hylkema & De Wied, 1976).

The central effects of exogenously administered peptides might, in principle, be interpreted as ‘pharmacological side-effects’ instead of being indicative for physiological functions of neuropeptides during development. At present there are only a few observations which suggest a function of peptides in normal brain development. In the first place, a protracted brain development was found in the Brattleboro mutant rat (which is incapable of synthesizing vasopressin). This disturbance leads to permanent changes in cerebellar cell number (Boer et al., 1980; Boer, Van Rheenen-Verberg & Uylings, 1982) and structure (Fig. 3). Current experiments are being directed towards restoration of cerebellar development by vasopressin substitution, in order to substantiate the possibility of a direct involvement of this peptide. The second observation is that α-MSH antibodies, injected subcutaneously into 19 days-old rat fetuses, retarded brain maturation as measured two days later (Swaab, Boer & Visser, 1978). A third piece of evidence comes from observations that the opiate antagonist naloxone leads to a permanent insensitivity to temperature and to accelerated early development when administered neonatally (Sandman, McGirvem, Berka, Walker, Coy & Kastin, 1979; Vorhees, 1981).

**Long-term continuous administration of peptides**

A major problem in studies on the possible effects of peptides in long-term processes such as brain development is their very short biological half life. Injection of peptides
Fig. 4 Vasopressin supplementation of the deficient Brattleboro rat by means of the Accurel technique. Subcutaneous implantation immediately brings about a reduction of the diuresis to control levels, which did not return to the pre-operative value before day 80 (solid line). Vasopressin was generated in a rather constant manner during one month, as shown by the urine excretion (dotted line). (From unpublished work of G. J. Boer, J. Kruisbrink & H. M. L. van Pelt-Heerschap.)

may therefore result, on the one hand, in toxic effects at the peak of release immediately following injection, and on the other hand, in very short effective exposure times. The use of long-acting analogues and of suspensions in oil or gels may mitigate these 'saw-tooth' fluctuations, but a satisfactory constant peptide level cannot be attained in this manner. Moreover, analogues may have different effects from those of the genuine peptides. The commercially available osmotic minipumps are excellent for constant release over prolonged periods of time, but their size makes implantation impossible in, for instance, the fetal or neonatal rat. Recently, however, we have developed a matrix for continuous release of peptides which seems to solve these problems. A small-sized microporous Accurel® polypropylene tubing, filled with vasopressin and enclosed by a semipermeable membrane, can be either implanted or injected (even into the fetal rat) which provides a constant release of vasopressin for at least 30 days (Kruisbrink & Boer, 1982) (Fig. 4). The general applicability of this procedure for neuropeptide research is now under investigation.

Trophic effects of neuropeptides and their clinical use

The trophic effects of a number of neuropeptides on the developing brain (see above), the stimulation of nerve regeneration by ACTH (Strand & Kung, 1980), and the mitogenic effect of vasopressin in vivo and in vitro (Boer et al., 1980, 1982) suggests that neuropeptide research can provide us with valuable tools for correcting certain developmental disturbances of the nervous system. On the other hand, detrimental effects of exogenous neuropeptides on brain development are also a real possibility which is a cause of concern resulting from their clinical use. Against the advantages of β-endorphin as an analgesic at the time of delivery (Oyama, Matsuki, Taneichi, Ling & Guillemain, 1980) and the use of naloxone to regulate fetal heart rate (Goodlin, 1981), we should set the animal experimental observations of long-term effects upon the sensitivity to thermal stimuli (Sandman et al., 1979) and impaired maze learning (Vorhees, 1981). The clinicians applying vasopressin or their analogues to children in order to improve their effects upon sleep (Pavel, Goldstein, Petrescu & Popa, 1981),
should be made aware of the permanent effects which such compounds can have in developing organisms (Boer & Swaab, 1983). It will probably take gynaecologists some time to get used to the idea that even oxytocin can be considered as a transmitter, and thus as a 'psychotropic drug' that may be detrimental to the child (Liston & Campbell, 1974; Swaab & Ter Borg, 1981; Boer & Swaab, 1983) via a direct action on the developing brain. All in all it therefore seems to be high time to start paying more attention to the long-term effects of this new class of centrally acting compounds. Systematic investigations now of the possible functional teratological effects of neuropeptides may prevent some unpleasant surprises for our children.

References


