Long-Term Effects on Brain and Behavior of Early Treatments with Neuropeptides

G. J. Boer and D. F. Swaab

Netherlands Institute for Brain Research, 1095 KJ Amsterdam, The Netherlands

Ever since Blair-Bell (7) reported the benefits of pituitary extracts for the induction of labour in man, clinical use has been made of the oxytocin (OX) principle of the neural lobe. Originally isolated from the pituitary, and later chemically synthetized, this nonapeptide is routinely used in obstetrics all over the world, not only to promote uterine contractions, but recently also to enhance milk ejection in premature deliveries (42). Oxytocin is a hypothalamic hormone, synthetized like vasopressin (VP) in the large neurons of the hypothalamus and transported via axons to the neurohypophysis for release into the circulation. Immunocytochemistry has not only confirmed the anatomy of this hypothalamo-neurohypophyseal system (HNS), but has also revealed exohypothalamic fibers running from these and other OX- and VP-containing nerve cells into the brain, making synaptic contacts (13). The sites of termination of this putative neurotransmitter system overlay the brain sites at which they exert behavioral actions (56). So when OX is administered around birth, gynaecologists introduce a putative neurotransmitter and psychoactive compound. In general one is becoming more and more reluctant to use compounds influencing neurotransmitters of the amnergic and cholinergic type during early developmental stages, because they may impair fetal brain development and later behavior (see review ref. 53).

OX and VP are not the only peptides that are centrally active and synthetized in the brain. Over the last two decades a list of ‘neuropeptides’ with putative neurotransmitter or neuromodulator actions have even outnumbered the classical aminergic, cholinergic and aminoacid neurotransmitters. They have been tested extensively in laboratory animals both behaviorally and physiologically, and for some
neuropeptides clinical trials in adults have already been performed (see review ref. 55). However, little attention has been paid to their possible effects on brain development. The present paper will deal with the few experimental results in the rat on the effects of neuropeptides in brain development. The outcomes will be placed next to the proposed benefits of the compounds in obstetrics and paediatrics.

**NEUROPEPTIDES**

Many of the currently known neuropeptides were originally only categorized as hormones. Immunocytochemistry, however, revealed extensive peptide hormone containing nerve fiber systems in the brain (see review ref. 48), which suggested direct routes of transportation during behavioral actions. Not only peptidergic fiber systems of the hypothalamic hormones are present in the brain (including OX and VP) but also of peripherally known hormones and peptides, and even peptides not found in mammals so far. Accordingly neuropeptides can be subdivided into four groups (see ref. 48) (a) peptides originally known as hypothalamic hormones, viz., VP, OX, luteinizing hormone releasing hormone (LHRH), somatostatin and thyroid stimulating hormone releasing hormone (TRH), (b) peptides first known as pituitary hormones, viz., the melanocyte stimulating hormones (MSH) and adrenocorticotropic hormone (ACTH), which both appeared to be members of a large family of peptides, the ‘opiomelanocortins’ (e.g., endorphins, enkephalins, dynorphin, lipotropic hormones and their possible derivatives; see ref. 32), (c) peptides not primarily known as hormones, viz., substance P, angiotensin II, neurotensin, vasoactive intestinal peptide (VIP), gastrin, cholecystokinin (CCK), glucagon etc., and (d) peptides first described as non-mammalian peptides, like bombesin, FMRF-amide and hydra head activator.

It should be mentioned, however, that most of these data are obtained immunocytochemically. Antibodies recognize only an antigenic atomic configuration and not the compound as a whole. Immunostaining alone therefore does not provide the proof for the chemical identification of the peptides in the brain. Convincing evidence for the presence in the brain beyond their ‘classical’ hormonal system has been obtained for VP, OX and the opiomelanocortins, whereas substance P has been considered to be brain-derived from the start.

Immunelectronmicroscopy revealed peptide-containing terminals which synapse on other neurons for VP and OX (13), substance P (17) and enkephalin (40),
whereas biochemistry for MSH showed its presence in synaptosome vesicles (2). Several peptides therefore seem to have two distinct functions in adulthood: (a) peripherally, as a hormone and (b) centrally, as a neurotransmitter or neuromodulator. Mentioned already are the HNS and extrahypothalamic fibers for VP and OX. Other clear examples are the dorso-caudal projection of 'opiomelanocortin' immunopositive fibers from the arcuate nucleus together with the existence of ACTH, MSH and endorphin secretory cells of the pituitary and the LHRH-containing extrahypothalamic innervation of the periaqueductal grey together with the pathway to the portal vessels of the median eminence. Such dual functions are known likewise for the monoamines which also obscures the distinction between neuropeptides and the classical neurotransmitters (see also ref. 8).

NEUROPEPTIDES AND BRAIN ONTOGENY

Neuropeptides and their receptors are generally present early in development both in rat and human and always before neurogenesis has largely stopped. Since classical neurotransmitters seem to play a neurohumoral role in brain development (cf. ref. 38) and neuropeptides are putative neurotransmitters or neuromodulators, a similar role has been postulated for neuropeptides (8,47). Though as yet no data are available for all neuropeptides in this respect, abnormalities in some neuropeptide levels indeed seem to lead to disturbances in normal sequence of events in brain development.

Vasopressin

In rat brain radioimmunoassayable VP is present from fetal day 14 onwards (44), whereas the first vasopressin neurons of the hypothalamus stain immunopositively on day 16 and already one day later extrahypothalamic VP fibers show up (14). The HNS is responsive to an osmotic stimulus immediately after birth (44), indicating the mature stage of this system early in development.

Administrations of VP to the pregnant rat (dDAVP, 30) or to neonates within the first week (27) induce a lasting diabetes insipidus (DI), indicating an interference with the development of water regulation systems. Also in the VP-deficient Brattleboro mutant rat neonatal treatment for one month with 8-lysine-VP in oil is reported to enhance the already dramatic diuresis permanently, while this is not found in the heterozygote controls (60). Our own results with Brattleboro neonates given the endogenously lacking
(8-arginine-)VP in oil or Pitressin tannate in a similar dosage did not reproduce these effects. However, one-week continuous endogenous infusion of VP in high concentrations using a newly developed

FIGURE 1 - Long-term and constant release of vasopressin out of microporous Accurel polypropylene tubing. Small 1.6 mm diameter tubing filled with vasopressin in solution (22 μg), heat-sealed at both ends and covered with 45 μm collodion was subcutaneously implanted in a VP-deficient Brattleboro male rat. A constant release of VP brings about normal diuresis for one month, whereas pre-implantation levels were slowly reached thereafter (Boer et al., unpublished results).

![Graph showing urine production over time after implantation.]

TABLE 1. Postnatal vasopressin-induced amplification of diabetes insipidus of vasopressin-deficient Brattleboro rats[a]

<table>
<thead>
<tr>
<th>postnatal age</th>
<th>diuresis (ml/g b.w./day)</th>
<th>control</th>
<th>experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>day 33</td>
<td></td>
<td>1.0±0.05(6)b</td>
<td>1.3±0.05(6)</td>
</tr>
<tr>
<td>34</td>
<td></td>
<td>0.8±0.04</td>
<td>1.4±0.05</td>
</tr>
<tr>
<td>48</td>
<td></td>
<td>0.7±0.06</td>
<td>1.2±0.08</td>
</tr>
<tr>
<td>49c</td>
<td></td>
<td>0.2±0.03</td>
<td>0.4±0.1</td>
</tr>
<tr>
<td>percentage day 48</td>
<td></td>
<td>32±6</td>
<td>36±8</td>
</tr>
</tbody>
</table>

[a] vasopressin treatment was given by a vasopressin/Accurel device subcutaneously implanted on day 5; a continuously decreasing release for one week is obtained this way (from 5 μg to 0.2 ng/day).

[b] average ± SEM (number of animals); all urine data are statistically different, using Student's t-test at 5% significance, percentages are not.

[c] rats received 0.4 U Pitressin tannate.
Accurel(R) technique (Boer et al., submitted; fig. 1) clearly revealed the amplified urine production later on (table 1). Handelmann (27) proposed that kidney receptor systems were down-regulated by the treatment. However, a normal sensitivity of the kidney towards VP was found in the study of Lichardus et al. (30) and ours (table 1), showing that a centrally determined abnormality can be assumed. This might be indicative of an involvement of VP in the development of its own neuronal circuitry related to body water metabolism (41).

Other evidence for a function of VP in brain development came from the Brattleboro rat as well. Life-long absence of VP goes together with impaired brain growth of this mutant. Especially the cerebellum total wet weight, cell content (10) and morphology (Uylings et al., in preparation) are permanently and consistently affected. Recent data on VP substitutions during pregnancy suggest that the supposed trophic role of VP (9) manifests itself in fetal rather than in neonatal (9) brain development (Kragten and Boer, in preparation).

In the human fetal brain the first VP cells were identified by neurophysin immunostaining around the 11th week of pregnancy (23) and by VP staining at week 15 (57) while neurohypophyseal hormone containing fibers in the spinal cord were observed already at week 17 (51).

Applications of VP and analogues in human ontogeny are restricted to hypothalamic diabetes insipidus patients. The few data on children with this disease who score lower in psychological tests (59) support the idea that this peptide might be involved in brain development. Recently the ancestral analogue vasotocin was applied in newborn and prepuberal children to study its effects on REM sleep (39). Apart from the lack of knowledge about possible detrimental effects of this treatment, changes in REM sleep itself causes permanent effects on the brain of the rat (20).

**Oxytocin**

Immunocytochemistry revealed that OX neurons were present in rat hypothalamus after birth only (14), whereas radioimmunoassay could demonstrate low levels in the brain and pituitary already on prenatal day 14 (44). Fetal and maternal OX play a role in inducing and speeding up labor in rat (49) while no placenta barrier for this neuropeptide seems to exist (Swaab and Oosterbaan, unpublished).

In an effort to establish the potencies of the Accurel technique (fig. 1), oxytocin/Accurel/collodion
preparations were recently implanted subcutaneously in Wistar rats at day 16 of pregnancy (i.e., at the moment of onset of the naturally occurring increase in plasma OX) in order to induce labor prematurely. Such an effect has been shown by laborious electro-stimulation of the pituitary stalk (11) or catheter infusion techniques (25). The continuous release of ca. 100 ng OX per day indeed advanced the time of delivery by an expected period of a few hours (median from 16.20 to 13.25 h on day 21; Wilcoxon test 0.05 < P < 0.10).

TABLE 2. Effect of prenatal oxytocin infusion on the first-day body weight of the offspring[a].

<table>
<thead>
<tr>
<th>number of litters[b]</th>
<th>males</th>
<th>females</th>
<th>Student t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>7</td>
<td>6.0±0.05(33)c</td>
<td>5.6±0.04(40)</td>
</tr>
<tr>
<td>oxytocin</td>
<td>9</td>
<td>5.6±0.05(50)</td>
<td>5.3±0.05(56)</td>
</tr>
</tbody>
</table>

[a] subcutaneous implantation of an oxytocin/Accurel/collodion preparation in a day-16 pregnant Wistar rat, which gives a continuous release of approximately 100 ng oxytocin/day in vitro.

[b] average litter size ± SEM: 9.9 ± 1.2 and 10.9 ± 0.8, respectively.

[c] data are in grams and given as an average ± SEM (number of animals born on day 21 of pregnancy, i.e., day 0 postnatally.

TABLE 3. Diuresis of one-month-old rat pups born and/or suckled by oxytocin-infusion-treated females[a]

<table>
<thead>
<tr>
<th>mother prenatal/postnatal[b]</th>
<th>urine production (ml/100 g b.w./day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>8.9±1.3(11)c</td>
</tr>
<tr>
<td>control/oxytocin</td>
<td>5.1±0.6(12)</td>
</tr>
<tr>
<td>oxytocin/control</td>
<td>6.5±1.0(11)</td>
</tr>
<tr>
<td>oxytocin</td>
<td>6.7±0.8(11)</td>
</tr>
</tbody>
</table>

[a] oxytocin ‘infusion’ in the mother throughout the period between day 16 of pregnancy and postnatal day 30 (see legends table 2; in vitro release over that period goes from 100 ng/day to 15 ng/day).

[b] on day 1 ‘oxytocin’ and control mother-born pups were randomly redistributed for cross-breeding and litter size was brought at eight pups (see ref. 10) for condition during rearing.

[c] data are given as an average ± SEM (number of animals).
However, it was noted that pup weight at day 1 postnatally was significantly lower than in the control group (table 2) and secondly diuresis at one month of age was less in those pups that were either born from an 'oxytocin' female or born from a control mother and reared postnatally by an 'oxytocin' mother (table 3; the implant was not removed and acts for months). These preliminary results seem to indicate that even enhanced OX levels of the lactating mother probably via the milk - may interfere with the ontogeny of osmoregulation of the offspring in exactly the opposite way as found for VP (see above). Such a reciprocal effect has also been reported for the permanently changed sensitivity of aortic hormone receptors of rat after a single injection of VP and OX at the day of birth (21).

OX present a few weeks later than VP in human brain (51) is believed to play similar roles in human labor as has been described above for rat (49). Clinical applications in women for the induction of labor or for speeding up the expulsion as well as for stimulating lactation (42) are known, but except for the reported direct detrimental effects to the newborns of oxytocin-mediated delivery (4,15,24) no evaluation can be found in the literature on the possible long-term effects of this peptide.

**Opiomelanocortins**

From day 16 of pregnancy onwards α-MSH, endorphin and enkephalin were found in fetal rat brain (3,8,50). The relatively high concentration of MSH found on day 16 is within the adult range (50), whereas hippocampal endorphin levels of day 16 were even several times higher than that of one month of age (3). Since opiate receptor binding in the brain has been found as early as day 14 of pregnancy (19) early functional systems involving peptides of the opiomelanocortin family seem to be present in the brain.

Fetal MSH is supposed to be related to intrauterine growth. After removal of fetal brain and pituitary, the growth spurt between days 19 and 21 remains absent, while MSH administration to the brain-aspirated rat fetus was (so far) the only brain/pituitary factor that could partially restore body growth (28,50). Moreover, when endogenous fetal α-MSH was neutralized by means of antibodies, body weight and also brain weight diminished while brain DNA remained unchanged, indicating that α-MSH might have a role in nerve cell maturation, rather than in cell multiplication (52). Its trophic action, moreover, is shown in chick embryo, where it restores the
corticosteroid-induced growth inhibition (50).

'Advantageous' effects on later behavior have been described after neonatal administration of MSH, its precursor ACTH and parts of it. Treatment with a-MSH itself improves the performance on learning, memory and attention tasks in male rats (5,6) and a single subcutaneous injection of a long-acting preparation on the third day of life accelerated motor behavior of female rats 2 weeks later (54). Neonatal injection with a MSH 4-9 (ACTH 4-9) also enhanced attention (16), while single neonatal injections of ACTH, ACTH 1-24 or 1-10 were found to accelerate motor behavior (54) and ACTH and ACTH 4-10 treatment between days 2 and 8 increased passive avoidance behavior in adulthood (33,34). These apparently potent organizing effects of MSH and its related peptides fit in with the above-mentioned effects on brain maturation of the endogenous fetal MSH. Other members of the opiomelanocortin family, however, appeared not to be beneficial at all to brain development. Neonatal administration of b-endorphin to the rat causes a permanent insensitivity to temperature stimuli (43) and the well-known deleterious actions of opiates, methadon and naloxone (31,58,61,62) on fetal brain development might also be explained by an action via endogenous opiomelanocortins.

Human fetal pituitary contains MSH containing cells at least by the 15th week of gestation, in relatively larger numbers than ACTH immunopositive cells than in later life (57). This has been interpreted as being indicative of a trophic role of MSH similar to that in the rat during human ontogeny. Antibodies against b-endorphin and ACTH 17-39 stain fetal hypothalamic neurons from the 11th week of pregnancy onwards (12). Additionally, fetal MSH and ACTH are likely to play a role in the onset of or timing of birth in human, but the mechanism is unclear (52).

Neonates actively secrete b-endorphin shortly after birth even in the absence of vaginal delivery, whereas fetal distress enhances this peak release (22), showing a possible involvement in a neonatal adaptive phenomenon. Its clinical use as an analgesic at the time of delivery has already been proposed earlier (36), while also the use of its antagonist naloxone has been recommended for the improvement of fetal heart rate (26). However, against these applications the rat observations of long-term effects on sensitivity to thermal stimuli (43) and maze learning (58) should be set. In human addiction, opiates and methadone (analouges of b-endorphin) seriously impairs fetal brain development and neonatal behavioral responses
From the other neuropeptides mentioned above evidence is available on the early appearance in rat brain of e.g., LHRH, somatostatin, TRH, substance P, VIP, gastrin and CCK, while human data are present for LHRH, TRH and somatostatin (cf. ref. 51). Neonatal administration of TRH enables rats one month later to run faster towards a food reward. Moreover, they were less emotional and more active in an open field and the pineal organ and hypothalamus weighed more (46). No other perinatal application studies are known.

**MODE OF ACTION**

There is general agreement that apart from the genetically determined sequence of events in nerve cell generation, several factors subject to external influences are involved in the functional development of the nervous system. The presence of hormones such as thyroid hormone, sex hormones, growth hormone, insulin, somatomedin as well as the classical neurotransmitters seems to be necessary for normal brain development. Changes in these factors could enhance or reduce the particular events of cell synthesis, cell maturation or neural circuitry formation, which will result in a change in brain organization and so in other behavioral capacities (1,37,38). As is shown above, perinatal changes in neuropeptide balance of the rat also lead to such disturbances, although systematic studies are just starting. Both the fiber route of transport (neurotransmitter) and the blood circulation route (hormonal) of a neuropeptide might be involved in such effects. The latter especially since the blood/brain barrier had not yet developed until the end of the period of neurogenesis (rat; 29).

The preliminary evidence showed that e.g., VP would act as a possible mitogen in brain, as it does for other cell types as well (see ref. 10), but also on the ontogeny of receptor systems (osmoregulation). MSH (and peptides of the opiomelanocortin family) might act as a brain cell maturation factor. This agrees with the accelerating effect of ACTH upon regeneration of the damaged input of peripheral nerve cells (45). However, many more studies— for other peptides as well—are required to identify the particular involvement of individual neuropeptides in brain development. It might be argued that a promising way of doing so is to look following neonatal treatment for long-term effects.
on those functions that are under control of the neuropeptide in adulthood. For instance, changed levels of VP perinatally showed prominent effects on VP-mediated osmoregulation of adults, while for ACTH influences were found in ACTH-provokable grooming behavior (34,54).

ASPECTS OF CLINICAL USE

Trophic effects of neuropeptides on the developing brain may suggest such compounds as valuable tools in correcting developmental disturbances in the brain in the future. Their psychoactive effects have been used more or less successfully in human adults suffering from amnesia, impotence, schizophrenia, depression, pain etc. (see review ref. 55), which makes the threshold for their application during reproductive stages already lower. Detrimental effects of neuropeptides are, however, certainly possible. The use of b-endorphin as an analgetc during delivery (36) should be balanced against the demonstrated disturbance in thermoregulation (43) and the use of VP in mentally retarded children (H. Belmaker, Jerusalem; personal communication) against the induction of diabetes insipidus (see above). For the reasons mentioned above one should in general be very cautious about using neuropeptides during pregnancy and in the developing child. For gynaecologists it will take some time to get used to the idea that even OX can be considered as a psychotrophic drug, but the reduced birth weight and the induction of decreased body water turnover as found in rat as well as the reported neonatal stress at birth (4,15,24) may emphasize its possible long-term effects. Animal investigations may help in predicting if and when obstetrical and paediatric use of neuropeptides may be beneficial or teratogenic, and whether one is relatively better off with other treatments or the use of derivatives. However, careful clinical follow-up will always remain necessary.

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