CHAPTER 16

Neuropeptides and functional neuroteratology

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Introduction

Nowadays it is clear that neurotransmission in the nervous system is brought about by a variety of endogene substances, including nucleotides, acetylcholine, amino acids, catecholamines, serotonin, histamine and neuropeptides. The number of neuropeptides outnumbers by far the other more 'classical' neurotransmitters. Although amino acid and aminergic neurons provide anatomically a prominent and extensive innervation of the central nervous system, the large neuropeptide variety as well as the great number of centrally regulated functions in which they are potently incorporated also make neuropeptides a very important class of neuro-messengers. This might also be illustrated by a recent paper by Zadina et al. (1986), who bibliographed the journal *Peptides* from 1980 to 1985; 40 pages were needed to describe the functions of over 40 neuropeptides. Neuropeptides have been recognized as brain messengers only in recent decades but many of them were previously known to have hormonal effects or to be present in the non-mammalian CNS (cf. Swaab, 1980). Consequently many peptides have dual characteristics, i.e., endocrine and as neurotransmitter or neuromodulator, depending on the site of synthesis and release within the organism.

Both neurotransmitters and hormones have been shown to influence ontogenic processes in the brain and to affect the sequence of neuronal proliferation, migration, maturation, differentia-

tion and the organization of functional circuitries. Since different types of neuron are generated in different brain regions and stages of development (Altman, 1969; Rodier, 1977), the well-defined timing of events in neuronal network formation becomes disturbed and will give rise to an altered organization of the brain at the cellular level. It was therefore regarded as likely (Swaab, 1980) that the potently neuroactive neuropeptides, in their role as hormone or neurotransmitter, might also influence neural development (cf. Boer and Swaab, 1983, 1985).

The present review provides evidence that many neuropeptides actually influence brain development and that abnormalities in the levels of neuropeptide may lead to permanent dysfunction of the nervous system, i.e. to functional neuroteratology. In the present paper particular emphasis will be given to the possible role of vasopressin in normal and abnormal development.

The early presence of neuropeptides

Some neuropeptides can be demonstrated immunochemically early in rat development (Swaab and Terborg, 1981). In fact, peptidergic neurons often show gene expression almost immediately after their birth dates (cf. Whitnall et al., 1985). Data available for catecholamines and serotonin demonstrating the presence of these neurotransmitter compounds prior to the first peak of neurogenesis in which the aminergic and serotonergic neurons are born (cf. Lauder, Chapter 24

TABLE 1

The developmental appearance of several vasopressin systems in the rat brain, exemplifying their separate generation and maturation

Cell bodies	Birth date	Immunodetection cell bodies	Target area	Immunodetection fibers in target areas
SON/PVN (magnocellular)	dpc 13	dpc 16	Neural lobe	dpc 16
PVN (parvocellular)	<dpc 18	Unknown	Median eminence	dpc 19
SCN	dpc 15	dpc 21	OVLT	dpc 21
			PVS	dpc 7
			DMH	> dpc 14
BNST	<dpc 18	Unknown	LS	dpc 10
			LH	dpc 10
LC	dpc 12	Unknown	Unknown	—
Amygdala	<dpc 18	Unknown	Hippocampus	dpc 20
Unknown	—	—	Olfactory bulb	dpc 17
			Amygdala	dpc 18
			Arcuate nucleus	dpc 21

Cell bodies found to be VP-synthesizing following blockade of axonal transport in adults were not investigated during development. They are indicated as unknown for earliest immunocytochemical detection.

Abbreviations: dpc, days postcoitally; dpc, days postnatally; SON, supraoptic nucleus; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; BNST, bed nucleus of the stria terminalis; LC, locus coeruleus; OVLT, organum vasculosum laminae terminalis; PVS, periventricular area of the thalamus; DMH, dorsomedial area of the hypothalamus; LS, lateral septum; LH, lateral habenula.

Data based on Buys et al. (1980), De Vries et al. (1981, 1985), Caffé et al. (1987) and review by Boer (1987).

of this volume) are lacking for the neuropeptides. However, neuropeptides can certainly be demonstrated shortly after this peak. Neuropeptide Y is assayable on gestational day 13 in rat (Foster and Schultzberg, 1984) and somatostatin and substance P on day 14 (McGregor et al., 1982). Vasopressin (VP) and oxytocin (OT) are assayable from fetal day 14 and 17 onwards, respectively (cf. review Boer, 1987). Peptides of the pro-opiomelanocortin family are present on gestational day 15 (Bayon et al., 1979), somatostatin on gestational day 16 (Inagaki et al., 1982), both thyroid stimulating hormone releasing hormone (TRH) and corticotrophin releasing factor (CRF) on day 18 (Conklin et al., 1973; Bugnon et al., 1982) and luteinizing hormone releasing hormone (LHRH) on day 20 (Daikoku et al., 1978). Vaso-intestinal polypeptide (VIP) and bombesin appeared later, i.e., on the day of rat birth (McGregor et al., 1982). However, since neuro-

peptide levels in the brain are generally in the picomole range, further improvement of the sensitivity and detection limit of the immunoassays might reveal even earlier synthesis of these compounds.

Not much is known yet about the ontogenic appearance of neuropeptidergic pathways. The VP neurons are certainly the most well-described in this respect and revealed a rapid maturation process which differed for the various VP cells studied (see review, Boer, 1987). The axons of the magnocellular VP neurons of the hypothalamus, for instance, reveal immunodetection of VP in their neurohemal organ – the neural lobe of the pituitary – on the same fetal day 16 on which the cell bodies were found to stain. The fibers of the parvocellular VP cells of the paraventricular nucleus (PVN), which project neurohemally to the median eminence, appear 1 day later, whereas it takes one or more days (up to 1 week) before

synapses of axonal endings of the extra- and exohypothalamic VP pathways are stainable. The latter exemplifies the fact that several VP-cell-containing nuclei in the brain have their own time-table of development (Table 1).

Notwithstanding the incomplete information about the early immunohistochemical presence of neuropeptide pathways, indications of the early development of the receptor site have become increasingly prominent, due to the rapid developments in the receptor assays for neuropeptides. Brain opiate receptors, as possible target sites for endorphins, were described as being already present on rat fetal day 14 (Clendeninn et al., 1976). More recent studies show somatostatin receptors to be detectable on fetal day 15 (Gonzalez et al., 1987), CRF receptors on day 17 (Insel et al., 1987), binding sites of VP and OT on fetal day 20 and postnatal day 1, respectively (Petracca et al., 1986; Snijdewint et al., 1987), cholecystokinin (CCK) receptors not later than rat birth (Hays et al., 1981) and brain LHRH receptors on postnatal day 6 (Reubi et al., 1986). One may even speculate that, due to technical improvements, in the near future ontogenic studies of several other neuropeptide binding sites in the brain will reveal an even earlier appearance. Anyhow, one can say that the early presence of neuropeptide receptors makes them accessible for agonistic and antagonistic action during the early phase of maturation (cf. Csaba, 1986).

For several neuropeptides a possible temporary activation has been described during normal brain ontogeny on either the pre- or the postsynaptic site. For instance, the levels of VP, α -melanocyte stimulating hormone (α -MSH), β -endorphin and somatostatin are (regionally) at certain periods of development higher than later on or in adulthood (Bayon et al., 1979; Boer et al., 1980b; Buijs et al., 1980; Inagaki et al., 1982; Bugnon et al., 1982; Alessi and Quinlan, 1985). For enkephalin it has even been shown that immunoreactivity is present only in the germinative cells of the cerebellum and not in adult cells (Zagon et al., 1985). Sometimes binding sites also

appeared to be expressed only transiently. This is seen for VP binding in the cingulate cortex (Petracca et al., 1985) and lateral reticular nucleus of the rat (Fig. 1), for OT binding in the dorsal hippocampus and parietal and cingulate cortex (Snijdewint et al., unpublished observations), and for somatostatin receptors in the cerebellum (Gonzalez et al., 1987). The latter observation is intriguing, since additionally no peptidergic synapses are involved, and one might think of a temporarily neurotrophic action of neuropeptides in a non-neurotransmitter fashion. Such a mechanism is also supposed for the aminergic neurotransmitters (cf. Lauder, Chapter 24 of this volume).

Approaches to reveal a peptidergic role in brain development

Research with steroid and thyroid hormones has shown that manipulation of the endocrine environment of the developing rat can have effects on body growth as well as organizational effects on the nervous system (De Kloet et al., Chapter 8, and Vaccari, Chapter 6 of this volume). The same holds for catecholamine neurotransmitters (cf. Patel and Lewis, Chapter 25, and Kostrzewa, Chapter 26). The approach to unravel a similar role for neuropeptides in brain development has followed the same line of investigation, including its neuroteratological aspects. On the one hand, cell generation and maturation are measured in relation to early alterations in the availability of neuropeptides, on the other hand organizational effects on the neuropeptidergic system and its target sites are studied.

The approach of looking for general growth effects is also justified by the trophic actions of certain neuropeptides. VP, for instance, has a mitogenic action on several cell systems, and may interfere with a variety of metabolic pathways (see review, Boer and Swaab, 1985) and with the release of other hormonal systems (cf. Hedge and Huffman, 1987) that might in turn affect brain growth. A recent report showed that VP may also

Fig. 1. Autoradiography of [^3H]vasopressin binding in the lateral reticular nucleus (arrow heads) of the medulla oblongata of a day 20 fetal rat, a binding which disappeared after postnatal day 15. Autoradiogram of transversal brain section. CER, cerebellum (cf. Snijder et al., 1987).

promote neuritic growth of cultured embryonic neurons (Brinton and Gruener, 1987). α -MSH and ACTH (adrenocorticotrophic hormone) also have potent trophic effects on nervous cells. α -MSH-related compounds are particularly potent in inducing central (Swaab et al., 1977) and peripheral nerve cell maturation (Frischer et al., 1985), as well as in regenerating of peripheral (Strand and Kung, 1980; Bijlsma et al., 1983a,b) and central nervous system lesions (Veldhuis et al., 1985). VIP probably has a role in mediating a neuron-glia-neuron interaction which influences the trophic regulation of neuronal survival (Brenneman et al., 1987). Endogene opiates such as Met-enkephalin also seem to be involved in nerve cell proliferation but rather by an inhibitory mechanism (Zagon and McLaughlin, 1987; see also below).

Organizational effects are determined by testing the aberrations on the anatomical, biochemical and behavioral level. Receptor imprinting processes appeared to be an important aspect of such effects (Csaba, 1986; Miller and Friedhoff, Chapter 31) and the usual strategy is to test the mature animal for perturbations in the physiological systems, including behavior, in which the neuropeptide is known to play a role (Handelmann, Chapter 32). It goes without saying that if a particular peptidergic system is affected by early

manipulation, such changes might have consequences for interrelated systems (cf. Bakke et al., 1978; Lichtensteiger and Schlumpf, 1985).

The levels of neuropeptides may be increased by treatment of the pregnant female, fetus or neonate with the native peptide and decreased by the use of antisera. Congeners of certain peptides (e.g. VP and OT, α -MSH and ACTH) and other compounds with agonist or antagonist activity are also useful in developmental studies. As will be shown below, the effect of imbalance of neuropeptide levels has its vulnerable period for long-lasting effects. In addition, the timing of the exposure to neuropeptide changes also appears to be critical for the type of anomaly induced (see below). Changes in the offspring induced by maternal treatment with neuropeptides can rather be interpreted as indirect effects, since neuropeptides do not efficiently pass the placental barrier (e.g. Oosterbaan et al., 1985a).

Long-lasting effects upon early neuropeptide changes

This section will only summarize long-lasting effects of neuropeptides, since several reviews are available providing a profound insight into this rapidly broadening field with clinical implications (cf. Boer and Swaab, 1983, 1985; Zagon and McLaughlin, 1983; Handelmann, 1985). Moreover, it will focus on the functional *neuro*-teratological aspects, i.e. on brain impairments that have a long-term character (Table 2).

Endogene opiates. Studies on the developmental effects of opiates provided the first examples of peptide neuroteratology because of the occurrence of narcotic use by pregnant women. Most investigations, however, concern the manipulation of the early perinatal opiate environment in the rat by the use of opiate agonists (methadone, morphine, heroin) or antagonists (naloxone, naltrexone) rather than direct alterations in endogene opiates such as Met-enkephalin and β -endorphin.

In general, the studies on *prenatal*, i.e. mater-

TABLE 2

Brain functional teratology of neuropeptides in rats

	Treatment	Effects	References
β -Endorphin $\uparrow$	Pups	Hyperanalgesia Decreased β -endorphin content Reduced opiate receptors Socio-sexual behavior influenced Reduced body weight	Sandman et al. (1979) Zadina and Kastin (1986) Moldow et al. (1981) Zadina and Kastin (1986) Meyerson (1985) Zadina et al. (1985)
	Mother	Opiate receptors increased	Zadina et al. (1985)
Met-enkephalin $\uparrow$	Pups	Facilitation maze performance Decreased neurogenesis Hyperanalgesia	Kastin et al. (1980) Zagon and McLaughlin (1987) Handelmann (Chapter 32)
α -MSH $\uparrow$	Pups	Improved learning, memory and attention tasks	Nyakas (1975) Nyakas et al. (1981) Champney et al. (1976) Beckwith et al. (1977a,b)
		Accelerated motor behavior Advances eye opening	V.d. Helm-Hylkema (1973) V.d. Helm-Hylkema and De Wied (1973)
		Reduced feedback of MSH on accelerated motor behavior	Lichtensteiger and Schlumpf (1985)
		Improved T-maze learning	Acker et al. (1985)
α -MSH $\downarrow$	Antibody into fetus	Disturbed brain cell maturation	Swaab et al. (1977)
VP $\uparrow$	Mother	Impaired memory in brightness discrimination test	Tinius et al. (1987)
		Impaired memory retrieval in passive avoidance test (males)	
VP $\downarrow$	Genetic deficiency (Brattleboro rat)	Disturbed brain growth	Boer et al. (1982)
			Greer et al. (1982)
		Earlier eye opening	Boer et al. (1980a)
		Changed catecholamine innervation	Versteeg et al. (1978)
		'Memory' deficits	cf. Van Wimersma Greidanus (1982)
		Impaired temperament-related tests	cf. Gash et al. (1987)
	Antibody in pups	Hyperresponsiveness	Warren and Gash (1983)
		Hyperanalgesia	Bodnar et al. (1980)
		etc. etc.	
		Hyperanalgesia	Moratella et al. (1986)
		Impaired memory in retrieval in passive avoidance test	Moratella et al. (1987)
		Impaired acquisition in active avoidance test	
VT $\uparrow$	Pups	Disturbed brain growth	Goldstein and Rodica (1986)
		Increased REM sleep	

TABLE 2 (continued)

Brain functional teratology of neuropeptides in rats

	Treatment	Effects	References
CRF ↑	Pups	Earlier eye opening	Zadina and Kastin (1986)
		Reduced body weight	
		Increased rearing	Zadina et al. (1985)
		Body temperature regulation affected	
		Increased exploration open field	Insel et al. (1987)
		Better recovery following stress	
CCK ↑	Mother	Eye opening delayed	Telegdy et al. (1986)
		Impaired active avoidance test	
		Impaired memory retrieval in passive avoidance test	
		Decreased rearing in open field	
		Altered monoamine levels	
Substance P ↑	Pups	Increased brain substance P receptors	Handelmann et al. (1987)
		Reduced pain threshold	Handelmann et al. (1984)
TRH ↑	Pups	Increased weight hypothalamus	Stratton et al. (1976)
		Impaired T-maze learning	

Abbreviations: α -MSH, α -melanocyte stimulating hormone; VP, vasopressin; VT, vasotocin; CRF, corticotrophin releasing factor; CCK, cholecystokinin; TRH, thyroid hormone releasing hormone.

nal, treatment with opiates such as morphine or methadone showed retardation of somatic and neurobiological development (cf. Zagon and McLaughlin, 1983) and altered the [3 H]Met-enkephalin and [3 H]naloxone binding in the nervous system of the offspring (Tsang and Ng, 1980; Kirby, 1983). On the other hand, there is a report showing that morphine delayed normal cell death in the ciliary ganglion of the chick embryo (Meriney et al., 1985). Maternal naloxone, an opiate antagonist, appeared to evoke opposite effects, i.e., it accelerated post-weaning growth and postnatal behavioral development of the offspring in many tests (Vorhees, 1981). However, it induced long-term impairments of learning behavior. Prenatal treatment of rat mothers with β -endorphin also decreased somatic growth and temporarily reduced [3 H]naloxone binding in the brain (Zadina et al., 1985). In the latter study one might wonder whether β -endorphin has a direct effect on the developing fetus, since no aber-

rations were found in a tail flick test measuring analgesic responsiveness known to be affected by maternal treatment with morphine (Steele and Johannesson, 1975; cf. also Zagon and McLaughlin, 1983).

Postnatal exposure to the endogenously present opiates β -endorphin or Met-enkephalin has been performed in a limited number of studies. Neonatal β -endorphin administration was shown to (a) cause long-term elevation in the threshold for thermal pain (Sandman et al., 1979; Zadina and Kastin, 1986), (b) lower concentrations of β -endorphin in the brain (Moldow et al., 1981), (c) alter the responsiveness to β -endorphin in socio-sexual behavior tests (Meyerson, 1985) and (d) reduce the number of brain [3 H]naloxone (μ) and [3 H]DADLE (δ) opiate receptors (Zadina et al., 1985; Zadina and Kastin, 1986), whereas no changes were observed in the somatic development (Meyerson, 1985; Zadina and Kastin, 1986). Neonatal Met-enkephalin treat-

Fig. 2. Influence of a single injection of naltrexone on cell proliferation in the cerebellar external granular layer of a 6-day-old rat. Naltrexone (NTX) blocking the opiate receptor either shortly (1 mg/kg) or for 24 h (50 mg/kg) had temporally related effects on the incorporation of [3 H]thymidine into the DNA of the cells. The high dose stimulated incorporation by preventing the inhibitory role of endogenous opiates, whereas the low dose does so only transiently followed by a period of decreased incorporation. The latter may be induced by short-term receptor down-regulation (from Zagon and McLaughlin, 1987).

ment (a) decreased neurogenesis in the cerebellum (Zagon and McLaughlin, 1987), (b) facilitated later performance in maze tasks (Kastin et al., 1980) and (c) elevated pain threshold as well (Handelmann, Chapter 32). Studies using opiate agonists or antagonists revealed a lot of additional information. Zagon and McLaughlin (1987) demonstrated that opioid receptor blockade in the developing brain increased cell division, indicating that the endogenous opiate system takes part in the orchestration of neurogenesis (Fig. 2). At the same time, maturation of neurons increased, as seen by increased dendritic growth and spine formation (Hauser et al., 1987). The opiate mu receptors are probably not involved in the inhibitory action on neurogenesis of endogenous opiates

(Zagon and McLaughlin, 1986), since the specific mu antagonist β -funaltrexamine failed to stimulate brain growth as did naltrexone. However, upon neonatal application it blocks later analgesia like naltrexone, indicating that lasting changes in mu receptors are probably involved. Methadone and morphine treatment of rat pups similarly affected the pain threshold in adulthood (Zagon and McLaughlin, 1982; Handelmann and Dow-Edwards, 1985) but Handelmann and Quirion (1983) were not able to relate this directly to mu or delta opiate receptor changes in brain regions involved in this behavior.

It is clear that opiate agonists and antagonists affect rat development in a variety of behavioral measures (cf. Zagon and McLaughlin, 1983, 1985), and that the involved manipulation of the endogenous opiate environment has severe and lasting functional teratological effects both pre- and postnatally. This is clearly reflected, e.g. following perinatal administration of methadone, when severe deficits in the neurochemistry of the biogenic amine systems are observed (Slotkin et al., 1979, 1982 and Chapter 17). It seems that dividing cells of germinal zones of the nervous system may be controlled by endogenous opiates (Zagon and McLaughlin, 1987), while organizational effects on the function of the opiate system also occur.

ACTH and its fragments. Several trophic actions of ACTH, α -MSH (*N*-acetyl-ACTH-(1-13)-amide) and other fragments such as ACTH-(4-10), ACTH-(4-9), etc., have been reported (see above). None of these compounds has ever been shown to affect postnatal somatic growth, but α -MSH antibodies inhibit prenatal body and brain growth (Swaab et al., 1976, 1977). When these antibodies were injected subcutaneously into rat fetuses, the size of the brain but not the number of cells (total DNA content) was affected, suggesting a maturation role for endogenous α -MSH activity. No other anatomical, neurochemical or functional change in adulthood due to prenatal (or maternal) treatment with ACTH-derived compounds has been reported. In

a study by Peruzović and Milković (1986), however, following maternal treatment with metopirone, which causes fetal adrenal hyperplasia by inhibition of corticosterone synthesis and increases ACTH, a facilitation of two-way avoidance behavior was found in adulthood. Brain growth data were not reported in this study.

The latter result is in line with early postnatal exposure studies. Generally, treatment with ACTH, α -MSH or derivatives seems to improve performance in learning, memory and attention tasks. Early enhanced levels of ACTH result in improved acquisition of passive avoidance behavior, an effect opposite to that found after corticosterone treatment (Nyakas, 1975). Consequently, it was supposed that ACTH possessed an extra-adrenal influence on the development of this behavior. Similarly, early administration of α -MSH facilitated a lever-press learning task, and improved two-way active avoidance and brightness discrimination performance (Beckwith et al., 1977a), while females but not males exhibited greater body contact in adulthood (Beckwith et al., 1977b). Studies with early postnatal exposure to α -MSH, ACTH, ACTH-(1-24) and ACTH-(1-10) showed an acceleration of motor behavior of rat (Van der Helm-Hylkema, 1973; Saint-Côme et al., 1982), whereas treatment with ACTH-(4-10) (Nyakas et al., 1981; Acker et al., 1985), and ACTH-(4-9) (Champney et al., 1981) and its analogue Org 2766 (Acker et al., 1985) showed later improvement of passive avoidance behavior, T-maze learning and attention tasks.

Suppression of the α -MSH serum peak in 5-6-day-old rat pups impaired the development of the α -MSH feedback on the tubero-hypophyseal dopamine neurons (Lichtensteiger and Schlumpf, 1985). These dopamine neurons remained unresponsive in adulthood, possibly due to affected α -MSH receptor number in this circuitry.

So far, it may be concluded that ACTH-related peptides facilitate fetal brain growth by a mechanism unrelated to nerve cell proliferation but comparable with its trophic action on nerve fiber

regeneration (cf. Bijlsma et al., 1983a,b; Edwards et al., 1986; Gispen et al., 1987) or its facilitation action on motor development (see above). The latter is brought about by a more rapid maturation of the neuromuscular junction (Frischer et al., 1985) and one may speculate that a more rapid maturation of synapse formation in the brain by neonatal ACTH compounds may underlie the organizational changes revealed by permanently changed behavior of adult rats.

Vasopressin and congeners. Although VP has frequently been described as a mitogen (see above), it failed to act this way when given to the

Fig. 3. Effect on brain growth by a postnatally applied vasopressin antagonist. The 3-week treatment with twice daily $2.5 \mu\text{g}$ dP[Tyr(Me)²]VP, a central antagonist of vasopressin (VP) had affected body and brain weight of Wistar pups killed on day 32. Brains of treated pups are too small for their body size since the data points were below the (dashed) reference line of the allometric brain/body relationship. Thus, brain development was particularly affected. Co-treatment of VP in the same dose was not able to counteract the growth impairments, which possibly indicates a non-competitive effect of the antagonist (from Snijdwint and Boer, 1988a). ●, controls; △, treated with dP[Tyr(Me)²]VP; ▲ treated with VP + dP[Tyr(Me)²]VP.

rat in its perinatal period. On the contrary – at high dosages (daily μg quantities) – it may disrupt somatic growth (Oosterbaan et al., 1985b; Snijdwint and Boer, 1986) possibly by its vasoconstrictive action. Nevertheless, the genetic absence of VP in the Brattleboro rat (Boer et al., 1982) or postnatal treatment with a centrally acting VP antagonist in Wistar rat (Snijdwint and Boer, 1988a; Fig. 3) results in disturbed brain growth. Decreased availability of VP may therefore be disruptive for nervous system ontogeny. The neuropeptides structurally related to VP, such as OT and [Lys⁸]VP (LVP), did not result in severe alterations of postnatal brain growth (Boer and Kruisbrink, 1984; Snijdwint and Boer, 1986), but the precursor peptide vasotocin (VT) in 0.1 ng amounts daily given the first week neonatally induced a remarkable decrease in brain weight and total brain lipid and galactolipids at the end of the treatment on day 8 as well as a reduction in active sleep (Goldstein and Rodica, 1986). Similar effects were found in kittens but not following application of VP, OT or LVP (Goldstein, 1984). Pharmacological evidence is given that these effects by VT on brain maturation are mediated by GABA mechanisms (Table 3),

TABLE 3

Vasotocin (VT) effects on early brain development of rat: mediation by a GABA mechanism

	REM sleep (% total)	Brain weight (g)	Total lipids (% weight)
–	67	0.78	8.0
VT	92*	0.60*	5.9*
VT + picrotoxin	64	0.75	8.2
VT + valproic acid	100*	0.61*	4.1*
Valproic acid	86*	0.81	6.1*

Daily i.p. treatment of neonatal rat between days 2 and 8 with 100 pg VT and/or 50 ng picrotoxin (GABA antagonist) or 1 ng valproic acid (GABA agonist). Assays were performed immediately after the treatment period. Asterisk indicates statistical significant difference from control treatment. Data summarized from Goldstein and Rodica (1986).

but their permanent character has not been investigated so far.

Abnormalities in adult behavior of VP-deficient Brattleboro rats and other strains in which the mutation was transferred by cross-breeding (cf. reviews Van Wimersma-Greidanus, 1982; Gash et al., 1987) point to a possible role for VP in the organization of the central nervous system, the more so since many of these abnormalities could not be restored by VP supplementation (cf. review Gash et al., 1987). However, some behavioral deficits in, for example passive avoidance behavior (De Wied et al., 1975; cf. Baily and Weiss, 1979; Carey and Miller, 1982) are not consistently found among several investigators (see further Gash et al., 1987). The age at which the later tests were performed might be of influence (Van Haaren et al., 1986), but it might also well be that the problematic breeding of these animals and the special care they need because of their severe polyurea have resulted in Brattleboro rat stocks with different behavioral patterns. Environment, handling and previous behavioral tests interfering with functional and chemical development of the brain may also play a role but are too often ignored (cf. Uphouse et al., 1983). Herman et al. (1986) have indeed reported avoidance responses to be significantly different between VP-deficient Brattleboro rat groups obtained from various animal suppliers (including even the results seen for the Long-Evans controls).

Recently administration of VP to pregnant rat has been shown to improve performance in a brightness discrimination test of their offspring (Ermisch et al., 1986; Tinius et al., 1987), but to impair memory retrieval of a 25-day-retention passive avoidance response at 2 months of age (Tinius et al., 1987). Early postnatal treatment with VP had no influence on a 1-day-retention passive avoidance test nor on open-field behavior at 2 months of age (Boer et al., unpublished observation). Interestingly, a single injection with antiserum to VP in 2-day-old rat pups also impaired this passive avoidance response at

TABLE 4

Controversies in the change of passive avoidance behavior of adult rats in which the vasopressin levels have been manipulated neonatally

Rat strain	VP treatment		Behavioral testing		Reference
	Dosage	Period	Age	Outcome	
Sprague-Dawley	1 μ g/24 h by osmotic minipump s.c.	Gestational day 13–19	2 months	Latency of test trial shortened (25-day retention)	Tinius et al. (1987)
Wistar	5 μ g twice daily injections s.c.	Postnatal day 1–9	2 months	No effect (1-day retention)	Boer et al. (unpublished data)
Wistar	100 μ l purified immunoglobulin of VP antiserum s.c.	Postnatal day 2	3–4 months	Latency of test trial shortened (1-day retention)	Moratella et al. (1987)

3–4 months (as well as the acquisition in a 2-way active avoidance test; Moratella et al., 1987). The outcomes of the passive avoidance test in these investigations have so far been difficult to match, since the same defect, i.e., shortened latency for entering in the post-shock trial, was found following opposite changes of neonatal VP levels (Table 4). An effect of the age of testing, the test protocol and the difference in dosage might be involved. However, one could also imagine that during the subsequent stages in the long maturation process of the various VP neuronal subsystems (Table 1), different manipulations of the VP levels may have altered the organization of the neuronal system involved differently but with the same behavioral outcome. Moratella et al. (1986, 1987) additionally described their neonatally VP antiserum-treated rats to be hyperanalgesic and hypertensive, and pointed out that the latter lasting change in blood pressure might have modified the pathways for pain control and memory processes. Whether VP receptor changes are involved in such changes still has to be established (see below).

Other neuropeptides. Developmental and neuroteratological studies on other endogene neu-

ropeptides are very limited. Zadina et al. (1985) reported on the effects of maternally or postnatally administered CRF in rat. CRF-treated pups showed the most dramatic changes, including decreased body weight, accelerated eye opening and, in adulthood, increased open-field rearing behavior and altered temperature control.

Another single study reported brain developmental changes upon perinatal CCK exposure (Telegdy et al., 1986). Both the sulfated and the non-sulfated forms were applied maternally and postnatally. Body growth and active and passive avoidance behavior appeared to be affected only after the prenatal maternal treatment of the non-sulfated CCK and more clearly so in males. Additionally, regional monoamine transmitter levels were changed, yet in a sex-independent way.

Postnatal substance P treatment has been shown to affect later pain threshold, and increased substance P binding capacity in various, but not all, brain areas (Handelmann et al., 1984, 1987). Prenatal treatment with Tyr-MIF-1, an endogene neuropeptide with opiate-modulatory effects, increased postnatal body weight gain (Zadina et al., 1985). Finally, thyrotropin-releasing hormone (TRH) appears to facilitate brain

growth, in particular of the hypothalamus, when given in the first neonatal week, but impaired later T-maze learning (Stratton et al., 1976).

In conclusion, neuropeptides join the growing group of neuroactive compounds that play a role in brain development (Table 2). Or, in other words, imbalances of these peptides during the strict time-table of events in brain ontogeny might lead to permanent anomalies in the anatomical and neurochemical organization, which will indubitably underlie the behavioral changes seen in adulthood. These observations support the hypothesis proposed by Swaab (1980) that any compound which acts on the adult nervous system would have long-term effects on brain growth and maturation when given at some vulnerable point in early life.

Vasopressin's influences explored in the Brattleboro rat

VP has a great variety of functions both peripherally and centrally. Nowadays it is even known that VP is not only synthesized in the brain, but also – though at low levels – in peripheral organs such as adrenal, testis, ovary and thymus. The time course of maturation of all these VP sources is not well-known (cf. Boer, 1987) but various VP neuron groups are known to develop at different times (Table 1). The same is possibly true for the receptors in target sites located either centrally or peripherally. So, following increased or decreased availability of VP during development one might have to investigate functional teratogenicity of different regulatory systems. The hereditary VP-deficient Brattleboro rat has been chosen as a model to unravel the role of VP in brain development, the rationale being that the functional changes in these animals will be due to the absence of VP from the onset of life (cf. Boer, 1985).

As mentioned above, the Brattleboro rat exhibits impaired body and brain growth, and the particular etiology of stunted brain growth seems to support VP's role in brain cell generation.

Based on regional DNA and [^3H]thymidine incorporation studies, the main effects on post-natal brain cell mitosis exist in the cerebellum (Boer et al., 1982; Boer and Patel, 1983). Attempts to correct these deficits by long-term neonatal VP supplementation have failed so far (Wright and Kutscher, 1977; Boer et al., 1984a). No effects on the growth of the Brattleboro offspring were seen upon long-lasting VP treatment of pregnant Brattleboro mothers (Boer, 1985; Snijdwint et al., 1985). The latter studies were initiated as brain and cerebellar growth deficits were already evident at birth (Boer et al., 1984b). However, partial recovery was observed when LVP, instead of VP, was used as a therapy. Seven-fold-enhanced blood levels of VP in pregnant Wistar rats failed to induce passage to the fetal compartment (Oosterbaan et al., 1985a). Moreover, such high maternal VP levels reduced fetal growth. The results with LVP are therefore not easy to explain. Placental transfer might be different or the action of LVP might have its own specificity (this was also found for the ancestor peptide of both, i.e., VT; Goldstein, 1984).

Evidence that VP is a missing factor in brain cell proliferation of Brattleboro rat is therefore still incomplete. Nutritional and several other humoral and trophic factors (thyroid hormones, corticosterone, growth hormone, insulin-like growth factor) were largely ruled out as playing a role in the stunting of Brattleboro growth, but it is not possible to eliminate other still-unrecognized factors which might be related to the VP deficiency (cf. Boer, 1985, 1987). The continuously 2-fold-enhanced OT levels in these rats could be such a detrimental factor, since these levels are not sensitive to neonatal VP treatment (Boer et al., 1988) whereas maternal OT has already been shown to have some transient inhibitory effects on early body growth of Wistar rats (Boer and Kruisbrink, 1984).

In view of the mitogenic actions of VP in non-neuronal cells in vitro and in vivo (cf. review Boer, 1985), there are a few peculiar findings related to [^3H]thymidine incorporation into the

brain DNA of young Brattleboro rats which could be VP-dependent but have not yet been investigated as such. The tissue availability and breakdown of [^3H]thymidine is enhanced (Boer and Patel, 1984; Snijdwint and Boer, 1988b) and the fate of DNA labelled on day 11 postnatally suggested enhanced cell death in the cerebellum, olfactory bulb and medulla oblongata (Fig. 4).

Another interesting aspect in the Brattleboro rat brain concerns the setting of VP receptors. These are undoubtedly present and appear as early and in the same brain regions as seen in controls (Petracca et al., 1986; Ravid et al., 1987; Snijdwint et al., 1987, and in preparation). In

Fig. 4. Changes of brain cell turnover in young rats of the Brattleboro strain. On day 11 postnatally a subcutaneous injection of [^3H]thymidine was given to label the dividing brain cells. On days 12, 25 and 39, the [^3H]DNA content of several brain regions was determined and plotted here as percentage of day 12 data in order to compare VP-deficient homozygote (----) Brattleboro rats with their control heterozygote (—) strain mates. Relative [^3H]DNA levels of day-25 VP-deficient rats were always higher due to increased tissue availability of [^3H]thymidine on day 12; the steeper slopes between day 25 and 39 indicate more cell loss, or enhanced cell turnover for the VP-deficient Brattleboro rat (Snijdwint and Boer, 1988b). ●, cerebellum; ▲, medulla oblongata; ■, olfactory.

adults, however, the number of [^3H]VP binding sites is increased and receptor affinity is lowered (Shewey and Dorsa, 1986). This up-regulation of receptor number is also seen in liver and adenohypophysis, but not in kidney (Koch and Lutz-Bucher, 1985; Shewey and Dorsa, 1986). Opposite changes, i.e. lasting down-regulation of [^3H]VP binding sites of kidney, have been reported upon neonatal exposure of normal rat to VP (Handelmann and Sayson, 1984). VP receptors therefore seem to be subject to receptor imprinting (Csaba, 1986) which could explain the organizational effects of perinatal VP on several types of behavior (see before).

So far the VP-deficient Brattleboro rat has only been partially useful for exploring the role of VP in development. Additional perinatal VP treatment studies in both the Brattleboro rat (supplementation for VP defect) and normal rat (enhanced VP action) as well as antagonist and antibody treatment studies (decreased VP action) have therefore to be performed to further establish growth-related and organizational mechanisms. With respect to supplementation of VP in the Brattleboro rat, the question can be raised whether exogenously applied peptide can really replace the physiological function of VP cells. It might be better to supplement VP cells by using fetal graft techniques (Boer et al., 1985). Another approach might be to selectively kill the VP neurons in developing normal rat. Monoclonal antibodies to VP were recently shown to penetrate VP neurons (Burlet et al., 1987). A covalently coupled cytotoxin might this way be targeted to eliminate (particular) VP cells and the early and later consequences for brain and behavioral parameters could be investigated.

Clinical significance of rat perinatal neuropeptide studies

The question might be raised to what extent neuropeptides might also affect nervous system development in human. The data reviewed above on long-lasting effects of early neuropeptide exposure

were usually obtained by the administration of relatively high dosages to the rat mother or to the pups. Many of these studies were moreover not primarily meant to investigate functional neuroteratological effects, but rather were initiated to substantiate the role of a neuropeptide in a particular physiological process of the organism. On the other hand, there are many situations in which human serum neuropeptide levels become considerably increased under physiological, pathological and environmental conditions. For instance, at birth, maternal and newborn plasma levels of VP, OT, ACTH and β -endorphin become very high (Csontos et al., 1979; Facchinetti et al., 1982; Oosterbaan, 1985), and smoking and stress are also potent releasers of neuropeptides (cf. Seyler et al., 1986; Weinstock et al., Chapter 21). It goes without saying that opiate addiction during pregnancy also leads to dramatic activity changes in the endogenous opioid systems (Zagon and McLaughlin, 1983). Finally, trials on the therapeutic use of neuropeptides have started and have shown that they can enhance endogenous peptides considerably above physiological levels. To mention only a few examples of the clinical use of neuropeptides during human development: OT is in use to initiate birth in late human pregnancy (Cerutti et al., 1979) and to promote lactation (Ruis et al., 1982), β -endorphin is used as an analgesic at the time of delivery (Oyama et al., 1980), VP and its analogues are administered to mentally retarded children (Andersen et al., 1979) and children with learning disorders (Eisenberg et al., 1984) or brain trauma (Wit et al., 1986), angiotensin is used in the early diagnosis of hypertensive disorders in pregnancy (Oney and Kaulhausen, 1982), and growth-hormone releasing hormone (GHRH) to treat GH-deficient children (Smith et al., 1986). Detrimental effects of enhanced neuropeptide levels might also be expected, because of the often greater sensitivity of human beings than of rat to teratological effects (Swaab et al., Chapter 1), a generally lower clearance rate of peptides in human (Lauson, 1974), an immature blood-brain barrier in young children

(Saunders, 1977), and the transfer of neuropeptides from mother to offspring via milk intake (pig; Weström et al., 1987a) followed by intestinal transmission into the circulation, a pathway which is still present during development (rat; Weström et al., 1987b).

Although neuropeptide receptors have not been recognized in rat fetal stages before the first peak of neurogenesis (see before), Friedler (1974) reported that deleterious effects might even be transferred by the sperm or occur at the stage of the egg-cell. Morphine treatment of male or female rats prior to mating resulted in hyperanalgesia in their offspring, and the rate of weight gain was found to be reduced, even in a second generation (cf. Sonderegger et al., 1979). Thus, functional neuroteratological effects of neuropeptide exposure may have transgenerational aspects (see review, Campbell and Perkins, Chapter 33 of this volume), which should make us even more aware of the possibilities and stimulate us to carry out thorough basic and clinical studies on functional neuroteratology also for the class of behaviorally active neuropeptides.

Summary

Neuropeptides form a relatively recently recognized putative class of centrally active neurotransmitters which formerly were only known as mammalian or non-mammalian hormones. These compounds are very potent in inducing behavioral changes in mature mammals and in their variety they outnumber the other (classical) neurotransmitters. Neuropeptides are synthesized early in brain development and binding sites appear in the same period. Imbalances in hormonal as well as aminergic neurotransmitter levels in ontogeny have shown to be deleterious for brain development, both anatomically, neurochemically and functionally. Because neuropeptides have hormonal as well as neurotransmitter characteristics and because many of them are present early in ontogeny, it was reasonable to suppose that such compounds play a role in brain ontogeny as well.

Moreover, several neuropeptides have been found to have trophic actions in a variety of cell proliferation or regeneration studies. The present review summarizes the growing evidence that changes in the neuropeptidergic environment of the perinatal rat can indeed result in long-lasting or life-long alterations in the nervous system, and consequently in behavioral changes. This supports the hypothesis (Swaab, 1980) that any chemical which can act functionally on the adult brain will affect brain organization when given at certain vulnerable stages of development.

Perinatal exposure to neuropeptides, even when these are given to the mother and therefore probably do not reach the fetus in large amounts, may affect neural cell proliferation and maturation on the one hand, and may have organizational effects on the developing neuropeptidergic system on the other hand. Neuropeptides may possibly alter the homeostatic set-point for their receptor synthesis, which is seen as an ontogenetic imprinting mechanism that has also been shown for peptide hormonal (Csaba, 1986) and catecholamine receptor systems (Miller and Friedhoff, Chapter 31). Such effects would also explain why often particularly those behavioral aspects are affected by the perinatally applied neuropeptide in which the compound has a regulatory or modulatory role in adulthood (cf. Handelsmann, Chapter 32).

Neuropeptides and their agonist and antagonist analogues are therefore potential neuroteratogens which upon perinatal use can alter the brain in a subtle but long-lasting way. This aspect should also alert clinicians who intend to use these compounds therapeutically in pregnant mothers and young children.

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Discussion

R. Balazs: It is interesting that the negative effect of vasotocin on brain growth seems to be mediated through GABA, as it has been reported that in certain parts of neuron systems GABA has a trophic influence on nerve cells. This brings me to a more general point concerning trophic effects. These seem to be maturation-dependent, and therefore in vivo, when cells may be at different development stages, the effects can be easily masked.

G. J. Boer: Goldstein and Rodica (1986) describe their effects in terms of total brain weight and tissue parameters. So regional differences, which could be indicative for your point, were not reported.

E. R. De Kloet: Does your observation on the presence of the vasopressin (VP) and oxytocin (OT) mRNA as well as the presence of VP and OT immunoreactivity in the fetal brain imply that processing of the precursors for these peptides occurs in the same way as in the adult brain? In other words,

are VP and OT found in the brain already bioactive around birth?

G. J. Boer: There is no reason to believe that processing is different. However, there is evidence that the maturation of the processing process for the OT precursor is delayed. Immunocytochemistry revealed the fetal presence of both VP and OT precursor, as well as of VP, but OT staining is only visible around rat birth. VP is not only present very early in the cell nuclei of the hypothalamus, but is also present extrahypothalamically as well as in the pituitary. Moreover, it can be released from the fetal pituitary in vitro, and is present in serum of newborns (cf. Boer, 1987). It can, therefore, be bioactive if any binding site is at hand.

T. D. Yih: Were there abnormal fetuses obtained in the study in which the Brattleboro rat was treated with vasopressin (VP) during pregnancy? According to Jost (1951) vasopressin includes peculiar defects in rats, e.g. absence or reduction of digits of the extremities.

G. J. Boer: In these studies VP and lysine-VP were given in a dosage that cured the polyurea of the VP-deficient Brattleboro mothers, that is given as near-physiological levels. No abnormalities of the kind you mentioned were seen. However, we know from our own studies that 10 times higher dosage rates with VP in normal rat induce fetal growth reduction (Oosterbaan et al., 1985).

J. Del Rio: I would like to make a comment on the similar deficits in passive avoidance behavior found in rats after perinatal administration of either vasopressin or vasopressin antiserum. It is possible to think that vasopressin has a biphasic effect on memory processes and a facilitating or disrupting effect may depend on the dose. In the latter case, a high enough dose of vasopressin could perhaps induce a deleterious effect on specific receptors for this neuropeptide.

G. J. Boer: Although I immediately agree with a stage- and dose-dependency of the vasopressin effects, it does not explain why lasting behavioral changes occur in the same direction when neonatal occupation of vasopressin receptors was either low or high. However, one might speculate that the underlying neurochemical changes are different and that changes in other vasopressin-related central functions might reveal more about this.

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