The Hypothalamo-Neurohypophysial System and Reproduction

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INTRODUCTION

The hypothalamo-neurohypophysial system (HNS) consists of the magnocellular supraoptic and paraventricular nuclei (SON and PVN), the hypothalamo-neurohypophysial tract (HNT), and the posterior lobe of the pituitary. This system synthesizes, transports and releases the posterior lobe hormones and their carrier-proteins (see Chapter by Jongkind, in this volume).

The HNS is involved in the regulation of diuresis. Consequently, many studies on the HNS have concerned the activation of this system by means of osmotic stimuli such as thirsting (*e.g.*, Jongkind, 1967, 1969; Jongkind and Swaab, 1967; Swaab, 1970). In addition, a relationship seems to exist between this osmotically sensitive HNS and reproductive physiology. The present paper deals therefore with the HNS, its afferent pathways and peripheral effects during pregnancy, parturition, lactation, the oestrous cycle and mating.

Moreover, attention will be paid to the dual nature of stimuli that affect the HNS, and to some methodological problems in the study of this system.

A. PREGNANCY

The nature of the *afferent stimuli* activating the HNS during pregnancy is not known. Changes in gonadotropic hormone levels may however be concerned in this activation (see Swaab, 1970).

The HNS and peripheral effects

Enzymatic studies showed an undulating course in the synthesizing activity of posterior lobe hormones in the SON and PVN during the course of pregnancy. This activity rises in both of these neurosecretory nuclei to a peak at about mid-pregnancy, after which it gradually decreases again until parturition (Swaab and Jongkind, 1970a; Swaab, 1970). The neurosecretory material (NSM) increases in the HNS during the course of gestation (Malandra, 1956; Vazquez, 1970). During the second half of

pregnancy the pituicytes and their nucleoli increase in size and show more mitoses (Malandra, 1956).

In spite of these signs of HNS-activation, no changes were observed in the vasopressin/oxytocin ratio nor in the content of these hormones in the rat's posterior lobe during pregnancy (Acher, 1956; Heller, 1959). Unfortunately, no reliable data are available on blood levels of posterior lobe hormones during the course of gestation, while it is also unknown whether the HNS plays a role in the physiology of pregnancy.

B. PARTURITION

Maternal HNS

Mechanical stimulation of the female genital tract liberates oxytocin via a neuro-hormonal reflex (Ferguson, 1941). The neural component of this "Ferguson reflex" appears from its blockade by lesioning the neural afferent pathway (see below), while the humoral component is obvious both from cross-circulation experiments (Debackere *et al.*, 1961) and from the long latency time (40–60 sec) between genital stimulation and peripheral effects (Richard, 1970).

Afferent pathways to the HNS

The major afferent fibers from the female genital tract run via the pelvic and pudendal nerves (Cross, 1966). Stimulation of mechanoreceptors in the uterus induces action potential discharge in its efferent nerves (Bower, 1959). The stimuli from the female genital tract are transmitted to the HNS via a spinal pathway that is mainly contralateral and diffusely distributed in the ventral and lateral fasciculi (Richard, 1970).

The HNS and peripheral effects

During parturition signs of increased production and release of posterior lobe hormones are observed. Enzymatic studies showed an increased synthesis of posterior lobe hormones in the SON and PVN (Swaab, 1970; Swaab and Jongkind, 1970a).

Stimulation of the female genital tract causes an increased firing rate of PVN cells (Brooks *et al.*, 1966) and increased electrical activity in the HNT (Richard, 1970). These electrophysiological events are accompanied by a release of oxytocin (Roberts and Share, 1968, 1970) and of vasopressin (Kühn and Peeters, 1971), and by an increase of uterine pressure waves (Hindson *et al.*, 1968, 1969).

That the Ferguson reflex is indeed an essential part of the mechanism of labor is a conclusion based partly on the signs of activation of this reflex arc that accompany parturition and partly on experiments which interfere with this arc and also influence parturition. Parturition is accompanied by a release of oxytocin and vasopressin as appears from their decreased posterior lobe levels (Fuchs and Saito, 1971) com-

bined with the increased blood levels of oxytocin (Folley, 1969; Haldar, 1970) and of vasopressin (Cobo *et al.*, 1968; Coutinho, 1970) during the expulsive stage of labour. Moreover, expulsion of a litter is preceded by strong oxytocin-like responses of the uterus (Fuchs and Poblete, 1970). Administration of oxytocin close to term advances the development of synchronous pressure waves that favour expulsion (Fuchs and Poblete, 1970) and induces labour (*e.g.*, Fuchs and Poblete, 1970; Fuchs and Saito, 1971). Direct evidence for a role of the Ferguson reflex in labour is obvious from the induction of labour by stimulation of the HNS (Cross, 1958; Lincoln, 1970) and from the occurrence of dystocia after blocking the reflex arc at the level of the spinal cord (Fitzpatrick, 1969; Beyer and Mena, 1970) or of the HNS (see Ginsburg, 1968). Moreover, labour is postponed by inhibition of oxytocin release (Fuchs, 1969; Fuchs *et al.*, 1967).

A role in the initiation of labour has been ascribed to many factors, *e.g.*, (1) the relative growth rates of uterus and conceptus or, (2) the release of a progesterone block on myometrial activity (Csapo, 1969), (3) the increased oestrogen production (Yoshinaga *et al.*, 1969) near term (*e.g.*, Csapo, 1969; Fuchs and Poblete, 1970), (4) the resistance of the cervix passage (Fuchs and Poblete, 1970), and (5) foetal factors (Liggins, 1969). Also (6) oxytocin has been mentioned frequently in this context (*e.g.*, Fuchs, 1966; Porter and Schofield, 1966; Schofield, 1969).

High spinal cord lesions induce dystocia, but the initiation of labour still occurs within the normal time (Beyer and Mena, 1970). This shows that the initiation of parturition is independent of neural stimuli from the genital tract. Oxytocin will therefore not trigger parturition via the Ferguson reflex, which must consequently be only concerned with acceleration of the expulsive stage. High oxytocin blood levels are in fact observed only in the second stage of labour (*e.g.*, Folley, 1969), which makes oxytocin release by whatever mechanism very improbable as the cause of the initiation of labour, although not impossible according to Fitzpatrick (1969).

Progesterone affects the uterus not only directly by inhibiting its spontaneous pressure waves (Csapo, 1969), but also indirectly by suppressing the Ferguson reflex. Oxytocin release (Roberts and Share, 1970) and uterine responses (Hindson *et al.*, 1969) induced by stimulation of the female genital tract are centrally (Fuchs, 1966; Hindson *et al.*, 1969) inhibited by progesterone. Withdrawal of the progesterone block will therefore not only influence labour via a direct action upon the myometrium, but also via disinhibition of the Ferguson reflex.

In contradistinction to oxytocin release, the liberation of vasopressin during parturition is probably only a consequence of labour pains (Cobo, 1968; Cobo *et al.*, 1968; Fuchs and Saito, 1971) or of emotional stress (Haldar, 1970). Teleologically, however, vasopressin release can be of importance because it would protect the mother from hypotension and excessive fluid loss (Coutinho, 1970).

Apart from its role in parturition, the Ferguson reflex has been applied since ancient times in cattle raising. The release of oxytocin in response to genital tract stimulation has been and is still being used in many parts of the world to assist the milking process (Folley, 1969).

Foetal HNS

Immediately after delivery, the vasopressin level is much higher in newborns' cord blood than in maternal blood during or after delivery (Hoppenstein *et al.*, 1968). High levels of oxytocin have recently been demonstrated in newborns' cord plasma (Chard *et al.*, 1970). Their finding that the arterial level was higher than the venous one suggests that oxytocin is produced by the foetus. Moreover, cord samples taken from forceps-delivered newborns were nearly all negative. These findings, combined with the observation that initiation of parturition failed to occur following lesion of the pituitary gland or stalk (Liggins *et al.*, 1967), suggests that the foetal HNS may play a role in delivery. Further work is needed to test this possibility.

C. LACTATION

Milk is liberated from the mammary gland partly by passive withdrawal, and partly by an active process. The HNS is involved in the latter as part of a neurohormonal reflex arc by which milk is forcibly ejected from the gland in response to oxytocin release (Bisset, 1968; Bisset *et al.*, 1970; Cleverley and Folley, 1970). The existence of a neural component of this milk ejection reflex arc is obvious from its blockade after lesioning the afferent pathway (see the next subchapter). The hormonal component is demonstrated by the finding that in rat, during suckling of the mammary glands cranial to the level of a spinal cord section, milk ejection also appears in the anaesthetic glands caudal to this section (Ingelbrecht, 1935). The long latency (40–50 sec) between suckling and milk ejection (Folley, 1969) is also to be expected for a humoral factor.

Afferent pathways to the HNS

Suckling activates the segmental mammary nerves via pressure and temperature sensitive nerve endings (Findlay, 1966, 1968).

The spinal tracts for the milk ejection reflex are mainly ipsilateral, but the exact localization differs appreciably in various species. In the ewe, the spinocervico-thalamic tract is probably involved. This is situated in the dorsal tract at thoracic levels and ventrally at cervical levels (Richard *et al.*, 1970). The spinal part of the reflex arc is situated in the dorsal tract in the goat (Popovici, 1963) and ventrolateral in the rabbit (Mena and Beyer, 1968). Tindal and co-workers (1967, 1968, 1969) showed by electrical stimulation studies that, in the mesencephalon, the milk ejection pathway is situated in the lateral tegmentum. In the diencephalon the pathway bifurcates into (1) a dorsal path situated close to the extreme rostral central grey, and (2) a ventral path ascending through the subthalamus. Both paths reunite in the posterior hypothalamus. This is a pathway for the preferential release of oxytocin, and not important for vasopressin release (Tindal *et al.*, 1968). The importance of these tracts for the milk ejection reflex needs, however, still to be confirmed by means of lesions made in them.

A release of oxytocin without activation of the HNS via the milk ejection reflex arc is possible in cows if the reflex becomes conditioned to stimuli associated with the milking routine (Cleverley, 1968; Cleverley and Folley, 1970) and in women by the sound of a baby crying (Newton, 1961, cited by Ginsburg, 1968).

In some species (*e.g.*, sheep and goats) milk ejection can still occur after complete denervation of the udder and without any oxytocin release, possibly through direct mechanical stimulation of the myoepithelium of the mammary gland (see Cleverley and Folley, 1970).

The HNS and peripheral effects

During lactation, the production and release of posterior lobe hormones and also the rate of axonal flow in the HNT are enhanced.

The increased production appears from the increased nucleolar size of the SON and PVN cells (Flament-Durand, 1967) and from enzymatic parameters for this production (Swaab and Jongkind, 1970a, b; Swaab, 1970; Swaab *et al.*, 1971).

An enhancement of axonal transport appears from the observed quicker carriage of ^{35}S -cysteine along the HNT (Flament-Durand, 1967). According to Jones and Pickering (1970), however, vasopressin and oxytocin in rat are synthesized and transported to the posterior lobe within 2 h, whereas ^{35}S -cysteine appears in the posterior lobe only after 9 h (Flament-Durand, 1967). The latter method of labelling is, therefore, probably not specific for the posterior lobe hormones. Recent studies did not confirm an increased transport rate during lactation (Norström and Sjöstrand, 1971).

An activation of the pituicytes in the posterior lobe is obvious from their increased size and vacuolisation, and from their increased number of mitoses (Malandra, 1956).

Stimulation of the nipples induces a rise in firing rate of PVN cells (Brooks *et al.*, 1966) and an increased release of posterior lobe hormones. This release is obvious from the decreasing amount of NSM-containing vesicles found in the posterior lobe during suckling (Monroe and Scott, 1966), combined with increased blood levels of oxytocin that appear in short bursts (Folley, 1969; Fox and Knaggs, 1969; Bisset *et al.*, 1970; Cleverley and Folley, 1970). The amount of oxytocin released depends upon the number of pups suckling simultaneously (Fuchs and Wagner, 1963).

The controversy (see Ginsburg, 1968) whether oxytocin release during suckling is accompanied by a release of vasopressin or not (Cobo *et al.*, 1967; Bisset *et al.*, 1970) may be explained by a slight antidiuretic activity of oxytocin itself and by the vasopressin release caused by emotional stress (Bisset *et al.*, 1970). Although such a possible vasopressin release would not be essential for milk ejection (Bisset *et al.*, 1970), it could be considered as a physiological reaction to loss of fluid (Coutinho, 1970).

The released oxytocin gives rise to alveolar myoepithelium contraction (Linzell, 1955) resulting in milk ejection (see Bisset, 1968). Milk ejection in response to oxytocin in rat already occurs 12 h before parturition (Deis, 1968).

The reflex arc described has proved to be essential for milk ejection in most species by stimulation and destruction of its various parts. Milk ejection was observed after

stimulation of the nipples (*e.g.* Folley, 1969; Brooks *et al.*, 1966; Richard *et al.*, 1970), stimulation of the afferent pathways in the brain (Tindal *et al.*, 1967, 1968, 1969), and direct stimulation of the SON and PVN (Aulsebrook and Holland, 1969; Cleverley *et al.*, 1968; Bisset *et al.*, 1969) or of the HNT (Brooks *et al.*, 1966; Bisset *et al.*, 1969). Suppression of the milk ejection reflex was found after anaesthesia of the teats or section of the mammary nerves (see Findlay, 1968), section of the spinal cord (Ingelbrecht, 1935; Mena and Beyer, 1968; Beyer and Mena, 1970), blocking (Oba *et al.*, 1971) of a cholinergic mechanism (Bisset, 1968) in the PVN, lesions made in the HNT (for reviews see Benson and Fitzpatrick, 1966; Cross, 1966), section of the stalk (Desclin, 1969), ablation of the posterior pituitary (see Benson and Fitzpatrick, 1966), and administration of oxytocin antibodies in the mother (Kumaresan *et al.*, 1971).

D. OESTROUS CYCLE

During the oestrus cycle, changes in posterior lobe hormone synthesizing activity were observed in the SON and PVN, both nuclei showing an increase during early oestrus (see below).

Afferent pathways to the HNS

In order to detect the possible hormonal stimuli inducing cyclic changes in the SON and PVN (Swaab, 1970), we studied the hormone synthesizing activity by means of enzymatic parameters in both of these neurosecretory nuclei during conditions that cause a change in gonadotropic or sex hormone levels. As appeared from the high activity in the SON and PVN during light-induced persistent oestrus, during early oestrus, and after gonadectomy, a parallelism exists between posterior lobe hormone synthesis in the HNS and blood levels of gonadotropic hormones (Swaab and Jongkind, 1970a, b; Swaab, 1970). The increase occurring in this synthetic activity after ovariectomy was inhibited by oestradiol benzoate. In ovariectomized oestrogen-primed rats, HCG (Pregnyl), HMG (Humegon), NIH-LH¹ and NIH-FSH¹ all caused an increase in posterior lobe hormone synthesizing activity. From these findings it could be concluded that the hormonal stimuli for the SON and PVN were LH and FSH (Swaab, 1970; Swaab and Jongkind, 1970a, b; 1971; Swaab *et al.*, 1971).

It was shown recently that gonadotropic hormones affect the neurosecretory nuclei directly (Swaab, unpublished observations). Cocoa butter pellets (approximately 300 µg) (Hirono *et al.*, 1970) containing 1 µg NIH-FSH-S8¹, placed within 50 µm of the SON, stimulated synthetic activity in the SON, as was indicated by the increased distribution of TPP-ase (Fig. 1) in this nucleus. No change was found in TPP-ase distribution after these implantations either in the same SON in sections

¹ NIH-LH and FSH were kindly provided by the Endocrinology Study Section, N.I.H., U.S.A.

Fig. 1. Distribution of thiamine diphosphate-phosphohydrolase (TPP-ase) determined according to Swaab and Jongkind (1970, 1971) by a semi-quantitative histochemical method. The percentage of TPP-ase-produced lead sulphide hits was counted in the SON of individual male (200 g) rats 2 days after implantation of NIH-FSH-S8¹ adjacent to the SON. In these series 4 groups of 5 rats were used: (1) unilateral implantation of 1.0 µg FSH (1.0 µg), (2) implantation of 0.1 µg FSH (0.1 µg), (3) implantation of cocoa butter only (CO) and (4) animals that did not receive any treatment (BL). The distribution of TPP-ase was determined in each animal in 8 sections through the SON: at the site of implantation (I), at the corresponding place in the SON of the contralateral side (C), on the site of implantation but at least 160 µm remote from the implant (IC), and at the corresponding place on the contralateral side (CC). At these 4 localizations a significant difference ($P < 0.001$) between the 4 groups was observed only after implantation of 1 µg of FSH.

situated at least 160 μm from the implant, or in this nucleus after implants containing cocoa butter only, or in the contralateral SON during any of these conditions. These data show that the receptors for gonadotropic hormones must be situated within the SON.

HNS and peripheral effects

Using the same enzymatic parameters for neurosecretory activity, we found a high hormone synthetic activity in the SON and PVN during early oestrus, and a low activity during di- and metoestrus (Swaab, 1970; Swaab and Jongkind, 1970a; Swaab *et al.*, 1971). Earlier studies using nuclear measurements (Smollich, 1969), changing amounts of NSM within cell bodies (Belajev *et al.*, 1967) or autoradiographic observations (Belajev *et al.*, 1967) also showed cyclic changes in activity of the neurosecretory nuclei.

These results could, however, not be interpreted as reflecting to hyperactivity of the HNS, since no correlation exists between these parameters and hormone production (see Swaab, 1970).

Parallel to the increased activity in the SON and PVN described in our work, the vasopressin and oxytocin content in the posterior lobe increases during pro-oestrus and oestrus (Heller, 1959; König and Böttcher, 1966). Blood levels of these hormones during the course of the oestrous cycle are not known.

According to the observations discussed above, the relation between the HNS and gonads (see, *e.g.*, Zambrano and De Robertis, 1968; Stutinsky, 1970; Korfsmeier, 1970; Gabe *et al.*, 1968) can be explained by the direct stimulating action of gonadotropic hormones upon the neurosecretory nuclei.

This mechanism thus provides an increased production of posterior lobe hormones during the fertile period of the oestrus cycle, a stage during which these hormones may play a role either in sperm or egg transport (see next subchapter).

E. MATING

Female HNS

G. W. Harris (1947) suggested that stimuli associated with coitus might result in a release of oxytocin that causes an increase in uterine activity and, in this way, influences sperm transport.

Afferent pathways to the HNS

As was shown in the subchapter on parturition, stimulation of the female genital tract induces posterior lobe hormone release. A similar mechanism was supposed to stimulate the HNS during mating (*e.g.*, Cross, 1966). It was shown recently (McNeilly and Folley, 1970), however, that oxytocin is frequently released in the female goat,

even before intromission. Consequently, it does not seem that mechanical stimulation of the female genital tract is necessary for oxytocin release during coitus. Although at present a mechanism similar to "conditioning" of the milk ejection reflex (see subchapter on lactation) cannot be excluded, the work of McNeilly and Folley (1970) points more to auditory, visual or olfactory stimuli as afferents for HNS stimulation.

HNS and peripheral effects

No data are available, either in female or in male individuals, on activity changes in the SON or PVN in connection with coitus.

Peripheral effects, *i.e.*, milk ejection, antidiuresis (Harris and Pickles, 1953) and pressure changes in the female genital tract (Michael and Reinke, 1970; Fox *et al.*, 1970), point to a release of posterior lobe hormones during mating. Moreover, increased oxytocin blood levels have been shown during this condition in human females (Fox and Knaggs, 1969) and in goats (McNeilly and Folley, 1970). Human blood values demonstrated a prolonged release in connection with mating (McNeilly and Folley, 1970), which is in contrast to the spurt-like release during milk ejection (see Lactation). In various species, spermatozoa ascend faster than can be accounted for by their flagellate activity (*e.g.* Fox *et al.*, 1970). The search for a physiological role of posterior lobe hormone release during mating has been concentrated, therefore, on the promotion of sperm transport by oxytocin (*e.g.* Mroueh, 1967; Morton, 1969). However, Coutinho (1970) showed that during the fertile period of the human menstrual cycle the uterus is refractory to oxytocin, whereas the fallopian tubes are very sensitive to this hormone during the entire menstrual cycle. This suggests that, at least in the human, oxytocin could not be of physiological importance in facilitation of sperm transport following uterine stimulation. Vasopressin, on the other hand, appeared to be a very potent myometrial stimulant. On the basis of sensitivity to posterior lobe hormones, therefore, vasopressin is more likely to stimulate the uterus and so facilitate the initial sperm ascent, whereas oxytocin might facilitate either sperm migration into the tubes or egg passage towards the uterus (Coutinho, 1970).

A sharp fall in uterine pressure, possibly causing sperm to be sucked inwards, has been reported following female orgasm (Fox *et al.*, 1970). It seems of interest to investigate whether this pressure fall is caused by a release of vasopressin, since administration of this hormone can produce a similar pressure drop (Coutinho, 1970).

Partly as a result of methodological problems (see below), it is not known whether destruction of the HNS impairs fertility. Until this kind of experiment has been carried out, no definite role in the physiology of reproduction can be attributed to the release of the posterior lobe hormones during mating.

Male HNS

On an average, similar posterior lobe contents of oxytocin and of vasopressin are found in female and male individuals (see Fuchs and Saito, 1971), despite the fact that

no role can yet be assigned to oxytocin in the male. Certain data point, however, to a relationship between this hormone and sperm transport in the male.

Afferent pathways to the HNS

The increased blood levels of oxytocin found already prior to coitus (see below) indicate a release mechanism of posterior lobe hormones comparable to that in the female (see Female HNS).

The HNS and peripheral effects

A release of posterior lobe hormones in relation to coitus appears from demonstrations of changes in urine flow and electrolytes balance, increased oxytocin levels in jugular blood of a male donkey immediate before coitus (see Fitzpatrick, 1966), and high oxytocin levels in blood from bulls immediately before and after service (Bereznev, 1963; cited by Knight and Lindsay, 1970). Fox and Knaggs (1969) however, failed to find any oxytocin activity in human male blood taken either shortly before or just after coitus.

Administration of oxytocin immediately before ejaculation increases the volume of semen expelled and the number of spermatozoa per ejaculate (Fjellström *et al.*, 1968; Knight and Lindsay, 1970), but this effect declines during long-term administration and spermatogenesis becomes impaired (Knight and Lindsay, 1970). The influence of posterior lobe hormones on the contractive activity of the vas deferens, ductus epididymis (Melin, 1970) and seminiferous tubules (Neimie and Korman, 1965) can explain the immediate stimulatory effects of oxytocin. Nevertheless, for the same reasons as were given for the female HNS, the physiological meaning of these findings remain doubtful. This also applies to the higher motivation reported in the male for copulation after oxytocin administration (Melin and Kihlström, 1963; Fjellström *et al.*, 1968).

F. DUAL QUALITY OF STIMULI AFFECTING THE HNS

The factors that activate the HNS can be grouped into neural and hormonal sources of stimulation.

Neural stimuli can reach the HNS via either short (Fig. 2b) or long (Fig. 2a) pathways. Osmoreceptors which are situated in the immediate perinuclear zone of the SON (Hayward and Vincent, 1970) activate the SON neurones via short neural pathways (Fig. 2b). Factors affecting the HNS via long neural pathways (Fig. 2a) are, *e.g.*, suckling (see Lactation), genital stimuli (see Parturition) stimuli from peripheral osmotic receptors (Haberich, 1968), and pain (Covey and Moran, 1966; Ginsburg, 1968).

Fig. 2. The HNS is activated via long (a) and short (b) neural pathways and by hormonal (c) stimuli.

Hormonal stimuli (Fig. 2c) for the HNS are, *e.g.*, gonadectomy, early oestrus, light-induced persistent oestrus, and administration of gonadotropic hormones. These stimuli can all be explained by a direct stimulatory effect of gonadotropic hormones upon the hormone synthesizing activity of the neurosecretory cells (Swaab, 1970; Swaab and Jongkind, 1970a, b; 1971).

The activation of the magnocellular nuclei by these various kinds of stimuli does not involve activation of different cells within these nuclei. Rather the same cells become activated irrespective of the kind of stimulus (Swaab, 1970; Swaab and Jongkind, 1970b).

The subdivision into stimuli that affect the HNS by respectively neural and hormonal mechanisms reflects the dual quality of neurosecretory cells showing characteristics of neurones as well as of endocrine cells (*e.g.*, Scharrer, 1967; Stutinsky, 1970; Sawyer, 1970).

G. SOME METHODOLOGICAL PROBLEMS IN THE STUDY OF THE HNS

In the study of the physiological role of the HNS during the various reproductive stages it would be of great importance to be able to make observations following elimination of the HNS. *Lesion* studies involving the HNS are problematical however.

HNS function recovers soon after posterior lobectomy (Kawashima and Takasugi, 1968) or after stalk sectioning (Fendler *et al.*, 1970; Rodriguez and Dellmann, 1970), since the upper end of the stalk becomes reorganized into a "miniature neurohypophysis". Moreover, a large portion of PVN cells may not even innervate the neural lobe (Cross *et al.*, 1969). In addition, since islands of magnocellular elements are situated between the SON and PVN (Bodian and Maren, 1951) and even within the optic chiasm (Swaab, unpublished observations), elimination of all neurosecretory

cells is impossible without extensively damaging the rest of the hypothalamus. These ectopic cells possess the cytological characteristics of neurosecretory cells (Bodian and Maren, 1951; Sloper, 1966), and are also activated by the same stimuli as the other SON and PVN cells (Swaab, unpublished observations). Selective elimination of HNS function will, therefore, only be possible by blocking the peripheral effects of the posterior lobe hormones, *e.g.*, by means of specific antibodies (Kumaresan *et al.*, 1971) or antagonists (Bisset and Clark, 1968; Datta and Chaudhury, 1968; Rudinger and Krejčí, 1968).

Physiological and clinical studies desperately need specific and sensitive *assays for posterior lobe hormones*. Since the posterior lobe content of these hormones are of little value in the study of the HNS (Swaab, 1970), blood level values have to be determined. Although bioassays are insufficient for the measurement of basal levels of vasopressin or oxytocin which amount to less than 1 μ U/ml plasma, however, radioimmunoassay techniques in combination with concentration and extraction procedures might solve this problem (*e.g.*, Chard *et al.*, 1970; Robertson *et al.*, 1970).

Anaesthesia interferes seriously with brain metabolism (*e.g.*, Folbergrová *et al.*, 1970). With respect to the HNS, this influence is reflected in increasing blood levels of vasopressin (Ginsburg, 1968), ultramicroscopical alterations (Daniel and Lederis, 1966), changes in animal behaviour correlated with suppression of the spontaneous firing and osmosensitivity of SON cells (Hayward and Vincent, 1970). Apart from the trained animals used by these last authors, electrophysiological studies of the hypothalamic units using telemetric techniques are promising in this connection and are currently being carried out in this Institute. Using this procedure together with newly developed techniques for stopping brain metabolism within 2 sec (see below), we were able to correlate hypothalamic unit activity with brain substrate levels during anoxia (Swaab and Boer, in preparation).

A great obstacle in the *biochemical* investigation of the HNS is its position deep in the basal region of the brain. Due to this fact, the metabolism in this area can be stopped in the rat only after 1.5 min following decapitation using the normal freezing procedure (Swaab, 1971). This is not quick enough for the maintenance of hypothalamic *in vivo* levels of many substrates. This problem has now been solved by the development of an operative procedure which allows the hypothalamic metabolism to be stopped within 2 sec after death. By means of this technique it could be demonstrated that no clear differences exist in the rat between cortex and hypothalamus with respect to glycolytic substrate utilization or energy consumption (Swaab and Boer, in preparation). Since the hypothalamus is subdivided into small areas having specialized functions (*e.g.*, Szenthaghai *et al.*, 1968), biochemical studies on the hypothalamus require the use of microchemical techniques (Jongkind, 1967, 1969, 1971; Swaab, 1970; Swaab *et al.*, 1971). The techniques described above, in combination with known ultramicrochemical procedures (Lowry and Passonneau, 1971) make such studies possible at present, even on biologically labile substrates in the hypothalamus.

CONCLUSIONS

Because of their known action upon contractile elements, research on the function of the HNS hormones in reproduction has concentrated on transport processes, *i.e.*, milk ejection, parturition and sperm or egg transport. Activation of the HNS has indeed been observed during these conditions as well as during pregnancy and the oestrus cycle. Yet, a definite role for the HNS in reproductive stages has been well-established only for parturition and lactation. Thanks to new methodological developments, however, the question will probably be settled in coming years with respect to sperm and egg transport.

Although the hypothalamic parvocellular releasing-factor producing system and the magnocellular HNS are anatomically entirely separated (*e.g.*, Szentagothai *et al.*, 1968; Akmayev, 1969), they are functionally strongly related as was demonstrated during the oestrus cycle. This relation between anterior and posterior lobe function could be of importance for the optimal regulation of mechanisms that are necessary for the preservation of the species.

The different parameters used in various research disciplines to study hypothalamic synthesis of neurohormones can be compared by their application on the HNS during many physiological stages of hyperactivity. Since hyperactivity can, moreover, be produced experimentally via neural or hormonal stimuli, this system is an especially suitable model system for research on hypothalamic function.

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DISCUSSION

KNOWLES: This is a nice way for showing the possible importance of the posterior pituitary system in reproduction.

BERN: A methodological question: could you say something about your TPP-ase method and its reliability as a measure of synthetic activity of these neurones?

SWAAB: We compared our TPP-ase method with a number of other parameters during many experimental conditions to compare the increased synthetic activity of the magnocellular nuclei. These methods are: posterior lobe hormone determinations, ultramicroscopical signs of activation and changes in cell body volume and nucleolar volume. Measurements of the nucleolar diameter are the most reliable parameter for activation of this system up to now.

SLOPER: Could you give some details about the technique. I am particularly interested in the standardization of the sections.

SWAAB: The standardization of section thickness is no problem in our studies. We are using two techniques which give comparable results: a histochemical one and a microchemical one. For the microchemical procedure we are using frozen-dried cryostat sections. The supraoptic nucleus is dissected out under the microscope, weighed on a quartz-fibre balance, and incubated in a small (5 μ l) volume of a medium containing TPP. The phosphate that has been split off by TPP-ase is fluorimetrically determined by means of an enzymatic phosphate mixture. So this is a real quantitative method in which section thickness is of no importance. The second procedure is a histochemical technique, which is based on a random hit procedure. By means of a Hennig ocular we count the percentage of points (hits) that are in contact with a TPP-ase stained Golgi apparatus. This may be regarded as the mathematical basis for the determination of the size of the Golgi-apparatus. The Golgi-apparatus increases during activation of the neurosecretory system.

FINDLAY: As far as Liggin's work is concerned I think it is true to say that all that work was intended to implicate the role in parturition of the fetal anterior pituitary and particularly ACTH and cortisol rather than the fetal posterior pituitary.

SWAAB: Indeed. However, pregnancy appeared also to be prolonged after pituitary stalk section.

FINDLAY: Several people have worked on cervical stimulation in human. Some of these studies seem to indicate that many effects of stimulation of the genital tract are not mediated through the posterior pituitary but through spinal reflexes, and possibly even through direct spread of activity through the myometrium.

SWAAB: Yes, indeed. However, as I showed by the combined results of stimulation and lesion studies, there has to be an effect mediated through the HNS too.

FINDLAY: I think that the method of a reversible lesion would be a very helpful one in the kind of experiments you are performing. If you would make a lesion at the right time by placing cooling

probes in the paraventricular nucleus you could observe the interference with parturition. By reversing the cooling you could observe whether the parturition would proceed.

SWAAB: This may indeed be a nice procedure. It would however give the same problems as the normal type of lesions, since it would be necessary to cool a large area before you have cooled all neurosecretory cells. So you will cool also fibres, vessels and neurones located in other areas. I think that administration of specific antibodies to oxytocin and vasopressin is a more promising approach in this field.

STOECKHART: From your FSH implantation experiments you draw the conclusion that FSH acts directly on the supraoptic nucleus. From the section you showed, it was clear that the implantation caused a rather large lesion in this area. Doesn't the lesion activate the supraoptic nucleus?

SWAAB: Control implantations that cause lesions of the same size do not activate the supraoptic nucleus.

STOECKHART: Are you sure that FSH is not acting on an afferent input of the supraoptic nucleus?

SWAAB: I don't think so. When implants are placed directly under the supraoptic nucleus they cause an activation too. If you look at the activity about 160 μ remote from the implant, it will not be possible any more to demonstrate an activation of the same nucleus. This finding agrees with the concept of a local action on the supraoptic nucleus.

DE WIED: As you know the supraoptic-neurohypophyseal tract is activated during stress. I would like to ask you, can you place this into the system you are using?

FINDLAY: Aulsebrook and Holland published a number of papers on the central control of ADH and oxytocin release. They believe that there are two opposing systems and that the ADH-system will be active in flight, fright and fight and that the oxytocin-system will be active in resting conditions.

SWAAB: There is indeed a close relationship between the HNS and the adrenal system. This relationship has, however still to be elaborated.

DREIFUSS: Neurophysin determinations by Dr. Legros show a relation between reproductive activity and neurophysin in blood. During pregnancy there is a steady increase of neurophysin levels in the human, there is a further increase during labour, but there is no increase during lactation. Oestradiol has been shown to increase neurophysin levels in blood of men and women. As far as the question of foetal oxytocin and vasopressin is concerned I have seen results indicating that blood neurophysin levels are higher in children that are born after 8 months of pregnancy than after 9 months. – I have a question about another problem: how do you manage to determine the enzyme activity only a few seconds after decapitation?

SWAAB: The method is based on the quick isolation of the rat brain via a trepanation made one week previously. During this period a plastic roof is placed over the trepanation that can be removed without anaesthesia before the experiment. The brain is removed by means of a spoon via the trepanation and frozen in liquid nitrogen-cooled freon.

TER HAAR: Might it be possible to put a small catheter into the brain so that you could freeze the supraoptic nucleus fast?

SWAAB: If you will freeze tissue by means of a cooling medium via a tube, the tube will be blocked by the frozen tissue around it.

TER HAAR: Could you explain the biochemical relationship between TPP-ase and oxytocin or vasopressin?

SWAAB: Not yet.

SLOPER: What other enzymes did you study by means of the cytochemical technique?

SWAAB: This work has been done by Dr. Jongkind. Perhaps he wants to comment on this point.

JONGKIND: By means of the microchemical procedure we found in the stimulated supraoptic nucleus a slight increase in glucose-6-phosphate dehydrogenase activity. Golgi apparatus enzymes increased about 40% already after 3 days of water deprivation.

SLOPER: What other Golgi-apparatus enzymes did you study?

JONGKIND: UDP-phosphohydrolase, GDP-phosphohydrolase and IDP-phosphohydrolase, which showed about the same increase as TPP-ase.