The Hypothalamic Neurosecretory Activity During the Oestrous Cycle, Pregnancy, Parturition, Lactation, and Persistent Oestrus, and After Gonadectomy, in the Rat

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Summary

The neurosecretory activity in the magnocellular supra-optic and paraventricular nuclei (SON and PVN) of the rat was studied during the course of pregnancy, parturition, and lactation, during the oestrous cycle and persistent oestrus, and following gonadectomy.

The parameter for neurosecretory activity in this investigation is the distribution of the Golgi-apparatus specific enzyme, thiamine diphosphate-phosphohydrolase (TPP-ase), as measured by a semi-quantitative histochemical method.

The SON and PVN react simultaneously under all the experimental conditions mentioned above. A peak in neurosecretory activity occurs at about mid-pregnancy in both magnocellular nuclei. A high neurosecretory activity is seen during and shortly after parturition and during lactation. A rapid rise in neurosecretory activity occurs in the SON and PVN as early as two weeks after gonadectomy. During the course of the oestrous cycle, high neurosecretory activity is seen during oestrus. During light-induced persistent oestrus, a high neurosecretory activity is seen in both nuclei.

The existence of a close relationship between the blood level of gonadotropic hormones and the neurosecretory activity in the SON and PVN is discussed.

Key words

Hypothalamic neurosecretion
Oestrous cycle
Persistent oestrus
Pregnancy
Parturition
Lactation
Gonadectomy

The magnocellular supraoptic and paraventricular nuclei (SON and PVN) synthesize the neurohypophyseal hormones vasopressin and oxytocin (for a recent review see SLOPER, 1966). Much is known about the physiological stimuli that release these hormones from the neurohypophysis, e.g., suckling, parturition, and osmotic stress [for review see HELLER and GINSBURG, 1966]. During the oestrous cycle, the content of vasopressin and oxytocin in the pituitary seems to fluctuate [HELLER, 1957]. Little is known, however, about the hormone production during any of these conditions.

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The present investigation deals with the determination of the synthetic, also termed neurosecretory, activity of the SON and PVN during pregnancy, parturition, and lactation. In addition, the neurosecretory activity of these nuclei has been determined under conditions that cause the gonadotropic hormone levels to change, *i.e.*, during the oestrous cycle, during light-induced persistent oestrus, and after gonadectomy. As an enzymatic parameter for neurosecretory activity, the Golgi-specific enzyme, thiamine diphosphate phosphohydrolase (TPP-ase), was determined in the two neurosecretory nuclei by a semi-quantitative histochemical method [JONGKIND and SWAAB, 1967].

Material and Methods

General Procedure

Wistar rats (approximately 2.5 months old) were kept in individual cages at 25°C and exposed to 12 h light daily (from 7 a.m. to 7 p.m.). They received tap water and standard chow *ad libitum*. The animals were killed between 10 and 11 a.m. by decapitation, and the hypothalamic areas were excised within 4 min. These were then fixed for 24 h in 4% glyoxal and washed for at least 24 h [SABATINI *et al.*, 1963].

Sections were cut at 16 μ on a cryostat, and placed on albuminized slides. The slides from control and experimental animals were incubated together for a period of 30 min at 4°C in a TPP-ase medium [JONGKIND and SWAAB, 1967]. The lead-phosphate deposits in the sections were transformed into lead-sulphide by 10% ammonium sulphide. The sections were then dehydrated in alcohol and mounted in malinol (Chroma-Gesellschaft).

The distribution of TPP-ase in the SON and the magnocellular part of the PVN [BODIAN and MAREN, 1951] was determined by means of a HENNIG ocular [HENNIG, 1957], without knowing whether the animal belonged to an experimental or a control group. For every animal, 16 sections of the SON and 8 of the PVN were each counted 4 times by turning the ocular over 90°. Hits with bloodvessels or nucleoli were not counted. The results were expressed as the percentage of positive hits per nucleus. The distribution of the hit-countings within the experimental groups, comprising the countings of the animals receiving a given experimental treatment, was normal. The differences between the hit-countings in the experimental groups were tested by Student's *t*-test, using the mean percentage and mean variance of each group, and significant differences were distribution free checked by the Wilcoxon test.

Oestrous Cycle

For this study, 19 virgin rats with a cycle length of 4–5 days were used. The length of the oestrous cycle of each animal was determined by examination of vaginal smears stained with Giemsa (BD11) during 9 days prior to sacrifice. The exact vaginal stage at the time of sacrifice was determined by counting the percentage of each of the three cell types in the vaginal smear, supplemented with data on the uterine weight [LONG and EVANS, 1922; RAMIREZ and McCANN, 1964]. Seven stages were distinguished; the first and second day of dioestrus (D₁, D₂), prooestrus (P), the transition period between prooestrus and oestrus (P-O), early and late oestrus (O₁, O₂), and metoestrus (M).

Ovariectomy and Oestrous Cycle

Using Hypnorm anaesthesia (0.1 ml/100 g, i.m.), 5 female rats were ovariectomized and 10 others were subjected to sham-ovariectomy. From the latter group, 5 animals were killed during early oestrus (characterized by cornified smears together with heavy, distended uteri) and the remaining 5 during metoestrus, together with the ovariectomized rats, 2 weeks after the operation.

Castration

Under Hypnorm anaesthesia, 5 male rats were castrated and 5 others were sham operated. All animals were killed 2 weeks after operation.

Pregnancy, Parturition, Lactation

In this series, 46 prooestrous female rats were mated overnight with males of the same strain, the day on which spermatozoa were found in the vaginal smear being taken as the first day of pregnancy. Groups of animals were decapitated either during the course of pregnancy, during and 1 h after parturition, or during lactation, the day of parturition being regarded as day 0 of lactation; 7 control animals were killed during metoestrus.

Light-induced Persistent Oestrus

Female rats were exposed to continuous light for 3 weeks; 5 animals that were in persistent oestrus for at least 5 days were then killed, together with 5 controls in metoestrus that had been exposed to the 12-h light rhythm.

Results

Oestrous Cycle

Changes were observed in the distribution of TPP-ase in the SON and PVN during the oestrous cycle. These changes consisted of differences in the abundance of lead-sulphide deposits in the magnocellular perikarya. When measured by the Hennig ocular, the area covered by the lead deposits in the SON and PVN showed small values for the dioestrous and metoestrous stages and greater values during the oestrous stages (fig. 1a, b).

Ovariectomy and Oestrous Cycle

The distribution of TPP-ase in both nuclei (fig. 2) during early oestrus as well as after ovariectomy was significantly greater as compared with the metoestrous controls ($p < 0.001$).

Castration

The increase in TPP-ase distribution after castration of male rats (fig. 3) was highly significant ($p < 0.001$) in both neurosecretory nuclei.

Pregnancy, Parturition, and Lactation

During pregnancy, parturition, and lactation, the TPP-ase distribution was seen to follow an undulating course for both the SON

Fig. 1a and b. The percentage of TPP-ase-produced lead sulphide positive hits in the magnocellular nuclei of individual rats during the course of the oestrous cycle (a: SON; b: PVN). Each point represents the mean percentage of one animal. The vertical lines indicate the standard error of the mean (SEM). The stages are: early and late dioestrus (D1, D2), prooestrus (P), transition between prooestrus and oestrus (P-O), early and late oestrus (O1, O2), and metoestrus (M).

Fig. 2a and b. The percentage of TPP-ase-produced lead sulphide hits in the magnocellular nuclei of individual rats during metoestrus (M), early oestrus (O₁), and 14 days after ovariectomy (OV-E) (a: SON; b: PVN). Each point represents the mean percentage of one animal. The vertical lines indicate the SEM.

Fig. 3a and b. The percentage of TPP-ase-produced lead sulphide positive hits in individual male control rats (C) and 14 days after castration (CAS) (a: SON; b: PVN). Each point represents the mean percentage of one animal. The vertical lines indicate the SEM.

(fig. 4a) and PVN (fig. 4b). High values occurred at about mid-pregnancy, both during and shortly after parturition, and during lactation (figs. 7 and 8).

As can be observed from figure 5, there was a large distribution of TPP-ase in both nuclei after 7 days of pregnancy ($p < 0.001$ between 7 days and controls), and a decrease in distribution between days 7 and 21 ($p < 0.001$). The distribution after 21 days of pregnancy was, however, still greater than in the metoestrous controls, both in the SON ($p < 0.001$) as well as in the PVN ($p < 0.05$). The increase in

Fig. 4a and b. The percentage of TPP-ase-produced lead sulphide positive hits in individual rats as follows: metoestrous controls (0 days), during the course of pregnancy, during and 1 h after parturition, and during lactation (a: SON; b: PVN). Each point represents the mean percentage of one animal. The vertical lines indicate the SEM.

distribution between day 21 and parturition was highly significant in both nuclei ($p < 0.001$). The parturition values did not differ from those recorded during 10 days of lactation.

Persistent Oestrus

The distribution of TPP-ase during the light-induced persistent oestrus (fig. 6) was significantly greater in both nuclei as compared with the metoestrous controls ($p < 0.001$).

Fig. 5a and b. The percentage of TPP-ase-produced lead sulphide positive hits in individual rats during metoestrus (M), at days 7 and 21 of pregnancy, at parturition, and at day 10 of lactation (a: SON; b: PVN). Each point represents the mean percentage of one animal. The vertical lines indicate the SEM.

Discussion

Our study of the distribution of TPP-ase in the neurosecretory nuclei was based upon the finding that both the enzyme distribution and the enzyme activity serve as a measure of the neurosecretory activity in these nuclei [JONGKIND and SWAAB, 1967, 1968; JONGKIND, 1969], since both parallel other signs of hyperactivity [see ENESTRÖM, 1967]. Although determination of the enzyme *activity* and of the enzyme *distribution* yields the same results in animals subjected to

osmotic stress, there is a principal difference between the two methods. An increase in TPP-ase *activity* as determined by the cytochemical method [JONGKIND, 1969] may be caused by an increase in the enzyme activity at the same place in the cells, by an increase in the distribution of this enzyme through the cytoplasm, or by a combination of both possibilities. Since differences in the density of the deposits are not taken into account in the semiquantitative-histochemical method, only changes in the enzyme *distribution* are determined in this way.

An increase in the distribution of the TPP-ase-generated deposits may be caused by an increase in the number of Golgi apparatus, by an increase in the size of the Golgi apparatus, or by a relative increase in the size of the Golgi apparatus in the area counted, owing to shrinkage of the cells. In the SON and PVN, however, every cell shows a TPP-ase-marked Golgi apparatus. Moreover, hyperactivity in these nuclei is paralleled by hypertrophy of the neuronal cell bodies [ENESTRÖM, 1967; FLAMENT-DURAND, 1967; ZAMBRANO and DE ROBERTIS, 1968], so that the increase in the hit-countings is not caused by a relative increase in size of the Golgi apparatus. Consequently, the increase in the distribution of the deposits does not result from an increase in the number, but from an increase in the size of the Golgi apparatus.

Although TPP-ase may serve as an indicator for neurosecretory activity, its actual function in the cell is still unknown. This enzyme is present on the membranes of the Golgi apparatus and in the small granules that are in close contact with this apparatus; but it is absent

Fig. 6a and b. The percentage of TPP-ase-produced lead sulphide positive hits in individual rats during metoestrus (M) and during light-induced persistent oestrus (LL.) (a: SON; b: PVN). Each point represents the mean percentage of one animal. The vertical lines indicate the SEM.

Fig. 7a and b. TPP-ase activity in cells of the SON in a control animal (a) and during lactation (b). $\times 830$.

Fig. 8a and b. TPP-ase activity in cells of the PVN in a control animal (a) and during lactation (b). $\times 830$.

in mature neurosecretory vesicles [OSINCHAK, 1964]. This phenomenon points to a role in the formation of these vesicles. Besides the Golgi apparatus, only blood vessels and nucleoli become stained in this method. Since both structures can easily be distinguished from the Golgi apparatus, they were never included during the hit-counting procedure.

The Hennig ocular enables semi-quantitative measurement of the TPP-ase-produced lead sulphide deposits. Since the reliability of this method depends on the size of the area occupied by the deposits [HENNIG, 1957], it is very suitable for the cell-rich SON. The sensitivity of the method is considerably lower in the PVN because, there, the cell population is 20% less dense [SWAAB, unpublished].

During all the experimental conditions studied in the present work, both magnocellular nuclei reacted in the same way. This simultaneous

reaction had previously been shown after osmotic stress [HILLARP, 1949; JONGKIND and SWAAB, 1967], during lactation [FLAMENT-DURAND, 1967] and after gonadectomy [IFFT, 1964; ZAMBRANO and DE ROBERTIS, 1968; FLERKÓ, 1968]. Since the SON and PVN are phylogenetically related [NIIMI *et al.*, 1963], this identical reaction is comprehensible. In mammals, however, the SON produces mainly vasopressin and the PVN mainly oxytocin [LEDERIS, 1962]. It seems, therefore, that during the conditions investigated the production of both hormones is increased. Nevertheless, an exact interpretation of the simultaneous reaction of the SON and PVN is impossible in the absence of data concerning the levels of both posterior lobe hormones in these nuclei, in the posterior pituitary, and in the blood, during the experimental conditions investigated.

The high neurosecretory activity at mid-pregnancy precedes both the morphological signs of hyperactivity in the nuclei and the increase of neurosecretory material in the posterior lobe that starts during the second half of pregnancy [MALANDRA, 1956]. The high neurosecretory activity that occurs during and shortly after parturition does not find its expression in morphological signs of hyperactivity in the SON and PVN [MALANDRA, 1956], but is accompanied by an increased release of posterior lobe hormones, as indicated by a depletion of these hormones from the posterior lobe of the rat [ACHER *et al.*, 1956] and an increased level of oxytocin in the blood of women and some large mammals [for review see FITZPATRICK, 1966]. Since dystocia may occur following supraoptico-hypophyseal tract lesions in the rat [GALE and McCANN, 1961], it is quite possible that the magnocellular nuclei play a role in the mechanism of parturition also in this species. The high neurosecretory activity in the SON and PVN during lactation agrees with the results reported by FLAMENT-DURAND [1967], who showed an increase in nuclear and nucleolar size as well as an increased cystein-S³⁵ incorporation during this condition.

The neurosecretory hyperactivity in the SON and PVN during oestrus is accompanied by an increase of the concentration of vasopressin and oxytocin in the pituitary gland [HELLER, 1957]. Exposure of rats to continuous light induces a persistent oestrus [LAWTON and SCHWARTZ, 1967]. The high neurosecretory activity observed during this condition is in conformity with the increase in nuclear and nucleolar size, and the increased cystein-S³⁵ incorporation found in the SON by FLAMENT-DURAND [1967]; this author, however, found no significant differences with caryometric measurements in the PVN.

The increase in neurosecretory activity of the SON and PVN after *gonadectomy* agrees with data reported in the literature, *e.g.*, an increase in nucleolar size at 20 days after ovariectomy [IFFT, 1964] and ultrastructural signs of hyperactivation one month after gonadectomy [ZAMBRANO and DE ROBERTIS, 1968]. It is also possible that the decrease in nuclear size observed in the SON and PVN at 60 days after gonadectomy is still another sign of hyperactivation [FLERKÓ, 1968]. However, the fact that an increase in nuclear size occurs within the same nuclei when hyperactivation is caused by osmotic stress [ENESTRÖM, 1967] underlines the difficulty in interpreting data concerning changes in nuclear diameter.

The direct cause of hyperactivity following gonadectomy is not yet clear. ZAMBRANO and DE ROBERTIS [1968] have proposed that the stimulation of the SON and PVN after ovariectomy is due to the decreased level of sex hormones, as illustrated by the finding that the ultrastructural signs of activation after ovariectomy disappear again when high doses (200 $\mu\text{g/day}$) of oestradiol are injected. However, this explanation is unlikely, since during early oestrus and persistent oestrus, the high neurosecretory activity is accompanied by a high blood level of oestrogens [RAMIREZ and McCANN, 1964; NEGRO-VILAR *et al.*, 1968].

The high neurosecretory activity observed during oestrus, after gonadectomy, and during light-induced persistent oestrus is, however, accompanied in all cases by a high blood level of gonadotropic hormones [RAMIREZ and McCANN, 1964; McCLINTOCK and SCHWARTZ, 1968; GAY and MIDGLEY, 1969; PARLOW, 1964; NEGRO-VILAR *et al.*, 1968; LAWTON and SCHWARTZ, 1967]. Whether a close relationship exists between the level of gonadotropic hormones and the neurosecretory activity of the magnocellular nuclei is now under investigation.

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