Influence of Gonadotropic Hormones on the Hypothalamic Neurosecretory Activity in the Rat

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Abstract

The present work deals with the relationship between gonadotropic hormones and neurosecretory activity. For this purpose, the oestrous cycle was studied during elevated vasopressin blood levels due to thirst. In addition, the neurosecretory activity in the magnocellular supraoptic and paraventricular nuclei (SON and PVN) was determined after administration of oestrogens and gonadotropic hormones. The parameter for neurosecretory activity in this investigation was the distribution of the Golgi-apparatus specific enzyme, thiamine diphosphate-phosphohydrolase (TPP-ase), as measured by a semi-quantitative histochemical method.

Key words

Hypothalamic neurosecretion
Gonadotropic hormones
Oestrogens

During water deprivation, no increase in the amount of pro-oestrous and oestrous smears was found. After injection of oestrogens in ovariectomized rats, the neurosecretory activity decreased, while a reactivation was observed after gonadotropic hormone (HMG, HCG, NIH-LH, NIH-FSH) administration. This reactivation was not observed after prolactin (NIH) administration. The changes in neurosecretory activity found under conditions that cause alterations in the gonadotropic-hormone and oestrogen levels are, therefore, due to the influence of FSH and LH on the neurosecretory activity.

The cells of the supraoptic and paraventricular nuclei (SON and PVN) synthesize the posterior lobe hormones [for a review see HARRIS and DONOVAN, 1966]. Although these magnocellular nuclei are not considered to be concerned in anterior lobe regulation [OLIVECRONA, 1957; SZENTÁGOTHAI *et al.*, 1968], they are affected by gonadectomy, during the course of the oestrous cycle, and during light-induced persistent oestrus [SWAAB and JONGKIND, 1970; SWAAB, 1970]. Under these conditions a parallelism was observed between high neurosecretory activity and elevated levels of gonadotropic hormones, while no direct relationship was found between oestrogen levels and

neurosecretory activity [SWAAB and JONGKIND, 1970; SWAAB, 1970]. The connection between the level of gonadotropic hormones and neurosecretory activity might be caused by a stimulatory action of the neural lobe hormones on gonadotropic hormone release [for a review see MARTINI, 1966]. However, this connection might also result from a stimulatory effect of the gonadotropic hormones on the hypothalamo-neurohypophyseal system (HNS). To distinguish between these two possibilities, gonadal function during thirsting was followed by means of vaginal smears, while on the other hand, the neurosecretory activity of the SON and PVN was determined after administration of oestrogens and gonadotropic hormones. 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; SWAAB and JONGKIND, 1970; SWAAB, 1970].

Materials and Methods

General Procedure

Virgin female Wistar rats (approximately 190 g) 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. The different experimental groups were chosen at random.

Osmotic Stress and Oestrous Cycle

For this study, 20 rats were used. The stage of the oestrous cycle was determined daily by examination of vaginal smears, 4 stages being distinguished: dioestrus, pro-oestrus, oestrus, and metoestrus [LONG and EVANS, 1922]. Starting on day 0 of water deprivation (see fig. 1), 10 animals with free access to food received no water for 6 days, while 10 control animals received both chow and tap water *ad libitum*. The difference between the number of the three groups of vaginal smears (see fig. 1) during water deprivation (1–6 days) and those in the control group during the same period was tested by means of the χ^2 -test [DE JONGE, 1963a].

Injection of Oestrogens, HMG, and HCG

For this experiment, 25 rats were ovariectomized [see SWAAB and JONGKIND, 1970] 14 days prior to sacrifice and divided into 5 groups of 5 animals each. Starting on the day of surgery, group I received arachis oil (0.1 ml d.d., s.c.), group II was injected with 0.4 μ g oestradiol benzoate (O.B.) (Dimenformon; 0.1 ml d.d., s.c.), while groups III, IV, and V received 2.0 μ g O.B. (0.1 ml d.d., s.c.); O.B. dilutions were made with arachis oil.

During the last 2 days prior to sacrifice, group IV received HMG (Humegon; 6 I.U. d.d., s.c.) and group V received HCG (Pregnyl; 6 I.U. d.d., s.c.), while groups I, II and III were injected with the same volume of the injection vehicle only. All injections were given once daily, at 8.30 a.m., and the animals were killed 2 h after the last hormone injection.

Injection of FSH and LH

In this series, 30 rats were ovariectomized 14 days prior to sacrifice. Starting on the day of surgery, all animals received 2 μ g O.B. d.d. during the entire experimental period (see above). They were divided into 6 groups of 5 animals each. During the last 2 days prior to sacrifice, one group received 100 μ g FSH d.d., a second group was injected with 2 μ g FSH d.d., a third group received 100 μ g LH d.d., and a fourth group 2 μ g LH d.d.; two control groups of 5 rats each received the injection vehicle only. All hormone doses, expressed in amounts equivalent to NIH-FSH or LH-S-1 preparations, were injected s.c., once daily, at 8.30 a.m. in 0.5 ml of a physiological saline solution (Ringer). The animals were killed 2 h after the third gonadotropic hormone injection. The preparations used in this study (ovine NIH-FSH-S-7 and NIH-LH-S-15) were kindly provided by the Endocrinology Study Section, NIH.

Injection of Prolactin

For this study, 10 rats were ovariectomized 14 days prior to sacrifice. Starting on the day of surgery, all animals received 2 μ g of O.B. d.d. during the entire experimental period (see above). During the last 3 days, 5 rats received 200 μ g prolactin (NIH-P-S-9 containing 30.30 I.U./mg), s.c., twice daily, at 8.30 a.m. and 5 p.m. The injections were given in 0.5 ml of a Ringer solution; the control group of 5 rats received the injection vehicle only. The animals were killed 2 h after the seventh gonadotropic hormone injection. Prolactin was kindly provided by the Endocrinology Study Section, NIH.

Histochemical Method and Counting Procedure

Sections of glyoxal-fixed hypothalami were cut in a cryostat and stained for TPP-ase according to a method described previously [SWAAB and JONGKIND, 1970]. The distribution of TPP-ase (percentage of hits) in the SON and the magnocellular part of the PVN was determined by means of a HENNIG ocular [HENNIG, 1957]. For every animal, 16 sections of the SON and 8 of the PVN were each counted 4 times by turning the ocular over 90°. The results of these countings were expressed as the percentage of positive hits in the respective neurosecretory nucleus. The differences between the hit-countings in the sections of the experimental groups were tested by Student's t-test, using the mean percentage of hits and mean variance of each group [DE JONGE, 1963b], and significant differences ($p < 0.05$) were distribution free checked by the Wilcoxon test [see SWAAB and JONGKIND, 1970; SWAAB, 1970].

Results

Osmotic Stress and Oestrous Cycle (fig. 1)

During the 6-day period of water deprivation, no significant changes were found in the number of the three groups of vaginal smears (fig. 1) as compared with the number of these smears in the control group during the same period ($0.25 > \alpha > 0.10$).

Fig. 1. Stages of the oestrous cycle from 10 individual animals 4 days before and during 6 days of water deprivation (left) and from 10 control animals (right). Each square represents a single day; ▨ = pro-oestrus; ■ = oestrus; □ = di- or metoestrus.

Injection of Oestrogens, HMG, and HCG (fig. 2)

Both doses of oestrogens used in the present investigation (0.4 and 2 μ g d.d.) induced vaginal cornification in the ovariectomized rats. After administration of 0.4 μ g O.B. to the ovariectomized animals, a small but significant decrease in TPP-ase distribution was found in the SON as compared with the ovariectomized controls ($p < 0.05$). After injection of 2 μ g O.B., a large decrease in distribution was observed in this nucleus ($p < 0.001$). In the PVN, the decrease after 0.4 μ g O.B. treatment was not significant ($p < 0.20$); however, injection of 2 μ g O.B. caused a large decrease ($p < 0.001$). After administration of HCG or HMG to the oestrogen-primed animals, a significant increase in TPP-ase distribution was seen in both neurosecretory nuclei ($p < 0.001$).

Injection of FSH and LH (fig. 3 and 4)

A significant increase in TPP-ase distribution was seen after administration of 100 μ g d.d. FSH, as well as of 100 μ g d.d. LH, in both neurosecretory nuclei as compared with the control group ($p < 0.001$). No difference was observed between the effects caused by FSH or LH, either in the SON ($p < 0.10$) or in the PVN ($p < 0.30$).

After injection of 2 μ g of FSH or LH, no increase in TPP-ase distribution was found in either the SON or the PVN ($p > 0.5$).

Fig. 2a and b. Percentage of TPP-ase-produced lead-sulphide positive hits in the SON (a) and PVN (b) of individual rats, 14 days after ovariectomy (OV-E). The different groups were treated with oestradiol-benzoate (OB), Humegon (HMG), or Pregnyl (HCG). Each point represents the mean percentage of hits in 1 animal. The vertical lines indicate the standard error of the mean (SEM).

Fig. 3. Percentage of TPP-ase-produced lead-sulphide positive hits in the SON and PVN of individual rats, 14 days after ovariectomy (OV-E). All animals were oestradiol-primed (2 μ g OB d.d.); 2 experimental groups received 100 μ g NIH-FSH or LH d.d. for 2 days. Each point represents the mean percentage of hits in 1 animal. The vertical lines indicate the SEM.

Injection of Prolactin (fig. 5)

No difference in TPP-ase distribution was observed between the control group and prolactin-treated animals, either in the SON or in the PVN ($p > 0.80$).

Discussion

Although the blood level of ADH increases approximately 10-fold as early as after 24 h of water deprivation [HELLER and ŠTULC, 1960], no increase in the amount of pro-oestrous or oestrous smears was found during the 6-day thirsting period (fig. 1). Moreover, injection of the other posterior lobe hormone, oxytocin, does not change the oestrous cycle in either the rat [KANAZAWA *et al.*, 1961] or the guinea pig [DONOVAN, 1961]. Consequently, it is unlikely that a stimulatory effect of the posterior lobe hormones on gonadotropic hormone secretion or release could explain the relationship [SWAAB and JONGKIND, 1970; SWAAB, 1970] between HNS-activity and gonadotropic hormones in the rat. The alternative explanation for this relationship was

Fig. 4a and b. Percentage of TPP-ase-produced lead-sulphide positive hits in the SON (a) and PVN (b) of individual rats, 14 days after ovariectomy (OV-E). All animals were oestradiol-primed (2 µg OB d.d.); 2 experimental groups received 2 µg NIH-FSH or LH d.d. for 2 days. Each point represents the mean percentage of hits in 1 animal. The vertical lines indicate the SEM.

a stimulatory effect of gonadotropic hormones on the synthetic activity of the SON and PVN. The stimulatory action of gonadotropic hormones on neurosecretory activity, as was observed in the present study (fig. 2 and 3), agrees with the increase in nucleolar diameter in the SON after gonadotropic-hormone treatment [IFFT, 1966], as well as with the humoral action of the anterior pituitary on the neurosecretory nuclei postulated by BODIAN and MAREN [1951]. According to the specification of the National Institute of Health, each of the purified LH and FSH preparations is contaminated less than 2% (in our case 2 µg d.d.) with the other gonadotropic hormone. Since 2 µg FSH or LH d.d. did not produce a rise of neurosecretory activity in the SON

Fig. 5. Percentage of TPP-ase-produced lead-sulphide positive hits in the SON and PVN of individual rats, 14 days after ovariectomy (OV-E). All animals were oestradiol-primed ($2 \mu\text{g}$ OB d.d.); the experimental group received $200 \mu\text{g}$ prolactin 2 d.d. for 3 days. Each point represents the mean percentage of hits in 1 animal. The vertical lines indicate the SEM.

or PVN (fig. 4), the stimulatory effect of these preparations (fig. 3) cannot be caused by contamination with the other gonadotropic hormone. Consequently, it can be stated that both LH and FSH are able to stimulate the neurosecretory activity.

No change in neurosecretory activity was found after administration of prolactin (fig. 5), although the total amount injected causes an almost 2-fold rise in ovarian progesterone production [HASHIMOTO and WIEST, 1969] and was nearly 5 times more than that used in the LH and FSH experiment (fig. 3). Therefore, the high neurosecretory activity that occurs during mid-pregnancy [SWAAB and JONGKIND, 1970] could not be explained by a stimulation of this activity by the high level of serum luteotropic hormone observed during this period [MATTHIES, 1965; COHEN and GALA, 1969].

In addition, the decrease in neurosecretory activity found after oestrogen administration in ovariectomized rats can be explained by the stimulating effect of gonadotropins on this activity. The increase in serum LH occurring after ovariectomy [GAY and MIDGLEY, 1969] is inhibited by $0.4 \mu\text{g}$ oestradiol benzoate d.d. [RAMIREZ and McCANN, 1964; PARLOW, 1964], while the increase in serum FSH [PARLOW, 1964] after ovariectomy is inhibited by administration of $2.0 \mu\text{g}$ oestradiol [PARLOW, 1964].

Few data are available in the literature concerning the physiological effect of the different doses of gonadotropins that induce increased neurosecretory activity. In hypophysectomized rats, administration of 100 μg NIH-FSH-S-1 d.d. for 3 days causes the formation of medium-sized follicles and partial repair of the interstitial ovarian tissue [LOSTROH and JOHNSON, 1966]. In addition, doses of 200–250 μg NIH-LH or FSH have been used to increase ovarian hormone production [HORI *et al.*, 1968; HASHIMOTO and WUEST, 1969]. To induce ovulation in hypophysectomized rats by means of small doses of LH, it was necessary to employ a pretreatment schedule of 131 μg NIH-FSH-S-1 d.d. for 4 days [MALVEN and SAWYER, 1966]. The amount of NIH-FSH and LH we injected are comparable to the doses mentioned above. The 6 I.U. HMG administered in the present study contains about 200 μg NIH-FSH-S-1 [MADLENER and ESSE-LINK, personal communication], while the amount of HCG injected (6 I.U.) was approximately three times the dose necessary to induce ovulation in hypophysectomized rats [WELSCHEN, 1970]. Since endogenous plasma levels of gonadotropins rise approximately 50-fold after gonadectomy [GAY and MIDGLEY, 1969] and during pro-oestrus [MONROE *et al.*, 1969], the doses of exogenous gonadotropins given in the present study, to explain the neurosecretory-gonadotropin relationship found under these conditions, can be considered to be within the physiological range.

The neurosecretory activity of the SON and PVN is known to be correlated with posterior lobe hormone synthesis and release during water deprivation [see SWAAB, 1970]. Whether the increased neurosecretory activity induced by gonadotropic hormones is linked to an increased release of posterior lobe hormones is still unknown, owing to the lack of data on blood levels of these hormones. However, the changes in water diuresis, parallel with the changes in neurohypophysial vasopressin content during the oestrous cycle [HELLER, 1961], are an indication of a direct connection between neurosecretory activity and release of the posterior lobe hormones under these conditions.

The physiological meaning of the unilateral connection between the HNS and gonadotropins is not yet clear. The increase in neurosecretory activity starting during late pro-oestrus points, however, to a possible function of the posterior lobe hormones in reproduction, e.g., in sperm- or egg-transport. In agreement with the concept of HARRIS [1947] is the recent finding that oxytocin influences the trans-

port of spermatozoa [MORTON, 1969]. Pressure recordings in the female genital tract demonstrated that the myometrium is very sensitive to vasopressin and the tuba to oxytocin [COUTINHO, 1968]. This author therefore assumes that vasopressin may facilitate sperm migration into the tuba or egg passage in the direction of the uterus. The finding that conception is not prevented by destruction of the posterior lobe [e.g., CROSS, 1966] does not contradict such a facilitatory role of these posterior lobe hormones in fertilization, since the posterior lobe possesses the capacity to recover functionally after destruction [FITZPATRICK, 1966; SLOPER, 1966; KAWASHIMA, and TAKASUGI 1968].

No data are available regarding a possible pathological effect of the increased neurosecretory activity due to a stimulatory action of gonadotropic hormones. This mechanism may, however, be present during gestosis (toxaemia) of pregnancy, since increased serum anti-diuretic activity [HAWKER, 1957; HOLEČEK *et al.*, 1969] combined with increased gonadotropic hormone levels and excretion are found during this condition [SMITH and SMITH, 1939; LORRAINE and MATTHEWS, 1950].

Determination of the neurosecretory activity in the SON by means of a cytochemical method [JONGKIND, 1969] instead of the semi-quantitative histochemical method served to confirm the results of the present study [SWAAB, JONGKIND and ARKENBOUT, in preparation].

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