Quantitative Histochemical Study on the Influence of Lactation and Changing Levels of Gonadotropic Hormones on Rat Supraoptic Nucleus

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ABSTRACT. The activity of thiamine diphosphate phosphohydrolase (TPP-ase) was measured by a quantitative histochemical method in a histologically pure supraoptic nucleus (SON) and an adjacent area of the anterior hypothalamus (AH). During lactation, after castration, during early estrus and after LH administration, an in-

creased TPP-ase activity (57–86%) was found in the SON, while no changes were observed in the AH. These results are indicative of an increased synthetic activity in the SON. Consequently, the activity of TPP-ase in the SON can be used as a parameter for posterior lobe hormone production. (*Endocrinology* 89: 1123, 1971)

THE SUPRAOPTIC and paraventricular nuclei (SON and PVN) synthesize the posterior lobe hormones, vasopressin and oxytocin (for a review see ref. 1). In these nuclei the Golgi apparatus responds to osmotic activation by enlargement and changes in organization (2–4). In line with these morphological findings the spatial distribution of thiamine diphosphate phosphohydrolase (TPP-ase)—the marker enzyme of the Golgi apparatus (5, 6)—increased in the magnocellular nuclei (7). Moreover, an increased TPP-ase distribution was shown during other stimuli for the SON and PVN, *i.e.*, during midpregnancy, parturition, lactation, after gonadectomy, during early estrus and light-induced persistent estrus, and after administration of gonadotropic hormones (8–10).

In the present study the quantitative histochemical method of Lowry (11) was applied, since the localizing enzyme histochemical procedure only registers enzyme *distribution*, which does not implicate changes in enzyme *activity*. Both procedures have been used during osmotic activation of the SON, and showed the same results (7, 12). To compare both procedures also during other circumstances that activate the neurosecretory nuclei (8–10), the present study deals with the determination of TPP-ase activity during lactation, after castration, during early estrus and after gonadotropic hormone administration.

Materials and Methods

Wister rats (T.N.O., Zeist, approximately 200 g) were kept in individual cages at 25 C and exposed

to 12 hr light daily (from 7 AM to 7 PM). They received tap water and standard chow (Hope Farms)

Seven animals were killed at day 10 of *lactation* together with 7 control animals during *metestrus*. Using Hypnorm anesthesia (0.1 ml/100 g body wt, im), 7 male rats were *castrated* and 7 others were sham operated. All animals were killed 2 weeks after operation. Seven female rats were killed during *metestrus* together with 8 animals in *early estrus* (characterized by cornified smears together with heavy, distended uteri). Fourteen female rats were ovariectomized, using Hypnorm anesthesia. Starting on the day of operation all animals received 2 µg estradiol benzoate (Dimenformon) daily (0.1 ml, sc) during the entire experimental period. During the last 2 days before sacrifice 7 animals received 150 µg LH¹ 2 times daily (NIH-LH-S15). The animals were killed 2 hr after the fifth gonadotropic hormone injection.

All animals were killed at 10:30 AM. The TPP-ase activity was determined (see Fig. 1) in dissected pieces of SON (approximately 0.3 µg) and adjacent anterior hypothalamus (AH, approximately 2µg), as has been described by Jongkind (12).

Enzyme activities are expressed in moles of substrate hydrolyzed/kg dry weight of tissue during 1 hr of incubation at 30 C (MKH units).

The results of 3 separate TPP-ase determinations in the SON during the particular experimental condition were combined according to De Jonge (13). The significance of differences between experimental and control groups was calculated using the Student *t*-test, while these results were distribution free checked by the Wilcoxon test.

¹ LH was kindly provided by the Endocrinology Study Section, NIH.

FIG. 1. Principle of the quantitative histochemical procedure for TPP-ase determination. The outlined part of this figure represents the phosphate determination mixture of Schulz *et al.* (14) containing glycogen, TPN and the 3 enzymes. Phosphate is transferred stoichiometrically into TPNH. Abbreviations used: G1P = glucose-1-phosphate, G6P = glucose-6-phosphate, G6PD = glucose-6-phosphate dehydrogenase, Pi = inorganic phosphate, 6PG = 6-phosphogluconolactone, TP = thiamine monophosphate, TPN, TPNH = oxidized and reduced forms of triphosphopyridine nucleotide, TPP = thiamine pyrophosphate.

Results

During *lactation* (Table 1), the average increase in TPP-ase activity as compared with the

TABLE 1. Influence of lactation on TPP-ase activity in SON

	Controls	Lact.
SON 1	4.0 (0.63)	6.8 (1.29)
2	3.2 (0.45)	6.5 (1.33)
3	3.7 (0.37)	6.0 (0.45)
Mean (1, 2, 3)	3.6 (0.27)	6.4 (0.58)
AH	0.55 (0.111)	0.59 (0.033)

TPP-ase activity and in parentheses the standard error of the mean (SEM) in the SON and AH of 7 metestrous control rats and of 7 animals at day 10 of lactation (Lact.). Three separate determinations in the SON and one in the AH. Enzyme activity is expressed as moles of substrate/kg dry wt/hr (MKH).

SON of metestrous controls was 77% ($p < 0.001$). There was no difference in TPP-ase activity between the AH of these 2 groups of animals ($p > 0.50$).

After *castration* (Table 2), the average increase in TPP-ase activity in the SON was 86% as compared with the controls ($p < 0.001$). No difference in TPP-ase activity was found in the AH of these 2 groups ($p > 0.20$).

During *early estrus* (Table 3), the mean TPP-ase activity in the SON was 58% higher as compared to the animals in *metestrus* ($p < 0.0025$). No difference was found between the

TABLE 2. Influence of castration on TPP-ase activity in SON

	Controls	Castr.
SON 1	3.7 (0.92)	8.7 (1.15)
2	3.8 (1.02)	6.9 (1.45)
3	3.5 (0.43)	4.6 (0.34)
Mean (1, 2, 3)	3.6 (0.30)	6.7 (0.41)
AH	0.56 (0.068)	0.40 (0.049)

TPP-ase activity (and SEM) in MKH units in the SON and AH of 7 sham-operated male control rats and of 7 rats 14 days after castration (Castr.).

TABLE 3. Influence of estrous cycle on TPP-ase activity in SON

	Metestrus	Early estrus
SON 1	3.7 (0.19)	5.4 (0.50)
2	2.3 (0.60)	3.7 (0.60)
3	3.3 (0.51)	5.8 (0.87)
Mean (1, 2, 3)	3.1 (0.36)	5.0 (0.56)
AH	0.84 (0.126)	0.81 (0.129)

TPP-ase activity (and SEM) in MKH units in the SON and AH of 7 animals during metestrus and of 8 animals during early estrus.

enzyme activities in the AH during both stages ($p > 0.5$).

In the SON of LH-treated animals (Table 4),

TABLE 4. Influence of LH on TPP-ase activity in SON

	Controls	LH
SON 1	3.8 (0.86)	6.1 (1.31)
2	4.3 (0.35)	7.4 (0.51)
3	3.8 (0.33)	5.2 (0.43)
Mean (1, 2, 3)	3.97 (0.377)	6.25 (0.565)
AH	0.49 (0.023)	0.43 (0.045)

TPP-ase activity (and SEM) in MKH units in the SON and AH of 14-day ovariectomized and estradiol benzoate (2 μ g O.B. d.d.)-primed animals. The 7 experimental animals received 150 μ g NIH-LH 2 d.d. for 2 days (LH), the 7 control rats receiving the injection vehicle only.

TPP-ase activity was *ca.* 57% higher as compared with the control rats ($p < 0.001$). No difference in TPP-ase activity was found in the AH of both groups ($p > 0.5$).

Discussion

In the literature various parameters for posterior lobe hormone-synthesizing activity are mentioned. With the exception of nucleolar size, these parameters (*e.g.*, nuclear size, RNA- or neurosecretory-material content per nerve cell body) appeared, however, not to be suitable

as indicators for the synthesizing activity (9). A localizing enzyme histochemical parameter for the hormone synthesizing activity, based on the determination of the distribution of the Golgi-apparatus enzyme TPP-ase during water deprivation, was introduced to avoid the laborious nucleolar measurements (7). This parameter also paralleled the changes in nucleolar size during lactation (8, 9), after gonadectomy (8, 9), during persistent estrus (8, 9) and after administration of gonadotropic hormones (9, 11). Moreover, an increased distribution of TPP-ase during osmotic activation (7), parturition and lactation (8, 9) went together with an increased production of posterior lobe hormones (for review see ref. 9). In the localizing enzyme histochemical procedure, however, not only the enzyme activity itself may contribute to the localization of the reaction product, but also lead catalyzed hydrolysis of the substrate and enzyme activators (15, 16). Moreover, differences in density of the deposits are not taken into account in this procedure (8, 9). Consequently, before an increased enzyme activity during hyperactivity of the neurosecretory nuclei is proved, quantitative histochemical data confirming the localizing enzyme histochemical results are necessary. The increased TPP-ase activity during lactation, after castration during early estrus and after injection of gonadotropic hormones confirms the earlier localizing enzyme histochemical results (8-10). Since, moreover, these conditions are known to increase the posterior lobe hormone synthesizing activity (8-10), it is also permissible to use TPP-ase activity as a parameter for the hormone production.

The localizing enzyme histochemical procedure has the advantage of being more sensitive, less difficult and less time consuming, and produces, moreover, a sharp localization (11). The quantitative histochemical procedure yields greater specificity and better quantification (11). Since both procedures have some advantages, it was considered of importance to compare and combine the results obtained by both techniques with each other.

The rise in TPP-ase activity during lactation and early estrus in the SON, observed in the present investigation, is comparable to the rise in activity found after 6 days of water deprivation (12). Consequently, it is possible to use physiological stimuli instead of the strong artificial stimulation by means of water deprivation for the study of the hormone-synthesizing activity of the SON.

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