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THYROTROPIN-RELEASING HORMONE AFFECTS THE OXYTOCIN, VASOPRESSIN AND PROLACTIN RELEASE IN FEMALE RATS DURING MIDLACTATION: RELATION TO SUCKLING *

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The effect of thyrotropin-releasing hormone (TRH; 200 ng i.c.v.) on oxytocin (OT), vasopressin (AVP) and prolactin (PRL) release was estimated in female Wistar rats during midlactation. The hypothalamo-neurohypophysial radioimmunoassayed OT and AVP storage as well as blood plasma level of both neurohypophysial hormones and PRL in females suckled or not suckled have been studied.

I.c.v. administration of TRH increased AVP content both in the hypothalamus and neurohypophysis of suckled females; however, plasma AVP level did not change. TRH increased the hypothalamic as well as neurohypophysial OT content during suckling. Simultaneously, TRH inhibited OT release into the blood plasma. On the contrary, in not suckled females TRH increased OT plasma concentration.

I.c.v. TRH raised the PRL concentration in plasma of lactating but, at the moment, not suckled females. On the contrary, i.c.v. TRH injection into females just suckled was followed by a decrease in PRL plasma level.

TRH probably acts in the central nervous system as an inhibitory neuromodulating factor for the vasopressin release. Also, it cannot be excluded that TRH — otherwise known to enhance the PRL release — suppresses the oxytocin-prolactin positive feedback mechanism when activated temporarily by suckling.

Key words: *thyrotropin-releasing hormone, oxytocin, vasopressin, prolactin, lactation*

INTRODUCTION

There are a few reports concerning some effects of TRH on the hypothalamo-neurohypophysial system. TRH was shown to increase the level of blood plasma vasopressin and oxytocin in the rabbit (1, 2). On the contrary, TRH decreased plasma vasopressin in man (3). In the rat, some authors could not show any changes in plasma vasopressin following i.v. TRH treatment (4, 5)

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but several reports from this laboratory support the view that TRH increases the hypothalamo-neurophypophysial vasopressin content and decreases that of oxytocin in the dehydrated, salt-loaded or haemorrhaged male rats (6—10).

In female rats, periodic high frequency bursts of action potentials evoked in oxytocinergic neurons of the hypothalamic paraventricular and supraoptic nuclei were noted during suckling (11) or parturition (12). In lactating females, this is related to pulsatile oxytocin release from the neurohypophysis and to consequent milk ejection. Moreover, suckling induces in lactating rats a rapid increase of prolactin release (13). Oxytocin is known to be involved in the regulation of suckling- (14) and mating-induced (15) prolactin secretion, most probably, at the lactotroph level (16).

It may be hypothesized that in lactating females the concomitant release of both oxytocin and TRH in posterior pituitary acts in a synergistic manner: oxytocin by its direct influence on the milk ejection while TRH being active indirectly, i.e., by increased release of prolactin (17). However, studies on possible role for TRH in mechanisms of the oxytocin or vasopressin release in female rats during midlactation seem to be lacking so far.

This study reports the effect of TRH, administered intracerebroventricularly to lactating female rats (not suckled or suckled at the time of experiment) on the oxytocin (OT) and vasopressin (AVP) content in the hypothalamo-neurohypophysial system. In the blood plasma the levels of OT, AVP and prolactin (PRL) were evaluated.

MATERIAL AND METHODS

Animals

The experiments were performed on 112 primiparous Wistar female rats in midlactation, of the body weight 250±24 g (mean±S.D.). They were kept at a temperature of about +22°C and in regulated light-dark conditions (artifficial illumination from 6.00 a.m. to 8.00 p.m.). The animals received standard pelleted food and had free access to tap water.

Experimental design and procedure

At 8th—12th day of lactation, each animal was separated overnight from all pups. On the following morning, the females were anesthesized by an intraperitoneal (i.p.) injection of 10% urethane solution (1.0 g/kg body weight) and the intracerebroventricular cannula for chronic injection was implanted. The animals were immobilized in a simple stereotaxic apparatus as recommended by Noble *et al.* (18); a small hole was drilled in the skull (1.5—2.0 mm laterally and 1.5—2.0 mm posteriorly to the crossing of the sagittal and coronal sutures). A simple stainless steel cannula (inner diameter 0.5 mm) was inserted into the left cerebral ventricle; its tip was 0.4 mm below the dorsal skull surface. The cannula was fixed to the skull with dental cement. At the end of

surgery the rats were given an intravenous (i.v.) injection of propranolol solution (250 μg/kg b.w.) in order to preserve the milk-ejection reflex in anaesthesized rats (19).

In a separate experimental group, the effectiveness of i.c.v. injections was verified by injecting of 10 μl 0.25 per cent trypan blue solution (one rat injected with trypan blue solution for every eight animals injected with drug solution or 0.15 M sodium chloride) and was found to be quite satisfactory, i.e., the dye was distributed in an uniform manner within all cerebral ventricles.

Experimental series I

Not less than one hour after end of surgery, the animals were injected intracerebroventricularly (i.c.v.) as follows: group A — 10 μl of vehicle (i.e., 0.15 M sodium chloride solution); group B — thyrotropin-releasing hormone (TRH; synthesized by Institute of Chemistry, University of Gdańsk, Poland) dissolved in sterile saline and administered at a dose of 200 ng (i.e., 10 μl of solution). The syringe was connected with the i.c.v. cannula by a flexible catheter. A 50 μl Hamilton syringe with plunger pushed by a microscrew was used. The duration of i.c.v. injections was about 15—20 sec. 2—3 min following TRH or vehicle injection (time "0") in subgroups II—IV (see below) 8—10 pups were allowed to suck the mother female for up to 15 min.

In groups A and B four further subgroups were set up: I — females isolated from their litters 14—15 hrs before the experiment and decapitated at time "0" (no suckling); II—IV — females whose pups were given suck for 5, 10 or 15 min, respectively.

The females of subgroups I—IV were decapitated at time "0", "5", "10", or „15", respectively. Mixed arterial-venous blood from the trunk was collected in heparinized test tubes, centrifuged for 5 min at +4°C (relative centrifugal force ca 650 G, i.e., 6380 m/sec^2), the plasma was removed and stored in a freezer at −23°C until extracted. The hormones (AVP and OT) were extracted from plasma using C18 Sep Pak columns (Waters, Associate Ltd., Northwick, U.K.); the recoveries of hormones during extraction procedure were > 75% and therefore values were not corrected for procedure losses.

The procedure of the preparation of the neurohypophysial and hypothalamic extracts was described in detail earlier (9).

Experimental series II

Immediately after cannulation of the left lateral cerebral ventricle, the left external jugular vein was cannulated to collect the blood samples. Then, the heparin solution (0.05 ml/100 g b.w.) was intravenously injected.

The rats were then divided into four groups and injected i.c.v. as follows: group A (females not just suckled) — with 10 μl of vehicle (0.15 M sodium chloride solution); group B (females not just suckled) — with TRH dissolved in sterile saline at a dose of 200 ng (i.e., 10 μl of solution), injected at a rate of 10 μl per 15—20 sec; group C (females just suckled; see below) — with 10 μl of vehicle as animals of group A; group D (females just suckled) — with TRH solution as animals of group B.

In each group five further subgroups were set up: I — "0" minute: collection of 1.0 ml blood sample from the left external jugular vein and immediate (i.e., following 10 sec at the latest) i.c.v. injection of vehicle or solution of TRH; II — V: similar collection of 1.0 ml blood at "5 min", "10 min", "15 min" and "30 min" after i.c.v. injections, respectively. The collected blood was each time centrifuged at once for 2 minutes (temperature +4°C; relative centrifugal force about 650 G, i.e., 6380 m/sec^2), the plasma removed and preserved at −23°C in sealed glass vials until radioimmunoassayed. Each sediment (mainly red cells) was resuspended in an equal volume of 7% solution of Dextran 70000 (Dekstran 70000, Polfa, Kutno, lot No 01290) and reinjected i.v., into the same donor animal, before the next blood sample was collected.

Radioimmunoassay (RIA) of vasopressin, oxytocin and prolactin

Anti-AVP (serum No 1228/1987-08-24) and anti-OT (serum No 1232/1988-02-03) antibodies were raised in rabbits by Dr. Monika Orłowska-Majdak (Department of Physiology, Institute of Physiology and Biochemistry, Medical University of Lodz). Characteristics of AVP and OT antisera were described earlier (9).

Iodination of arginine vasopressin and oxytocin.

Arginine vasopressin [(Arg^8)-Vasopressin, Peninsula Lab., lot No 032179] as well as oxytocin (Peninsula Lab., lot No 027179) were iodinated with ^{125}I using the chloramine-T method (20). Unreacted iodide was removed by mixing the reaction mixture with Amberlite (Ion Exchange Resin, type CG-400). Further purification was carried out on a column with 0.05 M/l acetic acid. Labeled AVP and OT were identified in the third peak by their ability to bind to the corresponding antibodies (21). The effectiveness of the iodination procedure was 70—90%. The top or the 1-st descending portion of this peak was used as the tracer in RIA. Labeled hormones retained their antibody bindability for up to four weeks.

The assay kit for determination of PRL was obtained from Amersham International (Little Chalfont, U.K.; Rat prolactin (rPRL) [^{125}I] assay-system; lot 57A). As evaluated by the producer, cross reaction with rat PRL for anti-PRL antibodies was 100%; with rat PRL (NIH-RP2) was 98.6%; with rat TSH (NIH-RP2) was $< 0.14\%$; with rat LH (NIH-RP3) was $< 0.07\%$; with rat GH (NIH-RP2) was 0.07%; with rat FSH was $< 0.07\%$ and with rat ACTH was $< 0.007\%$. The sensitivity of anti-PRL antiserum was 0.07 ng per tube with intra-assay coefficient of variation of 3.2%.

All specimens from individual experiments were measured in duplicate in the same assay.

Statistical evaluation of the results.

The vasopressin and oxytocin content was finally expressed in nanograms for whole hypothalamus or neurointermediate lobe and in picograms per millilitre of plasma. The prolactin concentration was expressed in nanograms per millilitre of blood plasma. Values are reported as the mean $\pm$ standard error of the mean (S.E.M.). Data were calculated by analysis of variance (ANOVA); if ANOVA revealed significant effects, post hoc analyses were done using D-Duncan test ($p < 0.05$ was considered to be statistically significant).

RESULTS

Experimental series I

The TRH influence on the vasopressin content in the hypothalamus, neurohypophysis and blood plasma level of female rats in midlactation (Fig. 1).

The hypothalamic vasopressin content (*Fig. 1A*) in rats treated with vehicle remaind unchanged from time "0" (no suction) up to the end of the experiment (i.e., in females suckled over 5, 10 and 15 min). The single i.c.v. injection of TRH increased the hypothalamic vasopressin content in females suckled for 5 min (subgroup A-II versus B-II: $p < 0.05$).

In females treated i.c.v. with 0.15 M NaCl the neurohypophysial vasopressin content (*Fig. 1B*) increased up to 10-th minute of suckling. Following 15 min of suckling, the neurohypophysial vasopressin content somewhat decreased. I.c.v. TRH increased the neurohypophysial vasopressin content in females suckled over 5, 10 and 15 min (subgroups: A-II versus B-II, A-III versus B-III and A-IV versus B-IV: $p < 0.05$).

The plasma vasopressin level (*Fig. 1C*) in rats injected i.c.v. with 0.15 M NaCl decreased somewhat after 5 and 10 minutes of suckling but returned to the initial value at 15-th min of suckling. I.c.v. TRH did not affect the plasma vasopressin concentration.

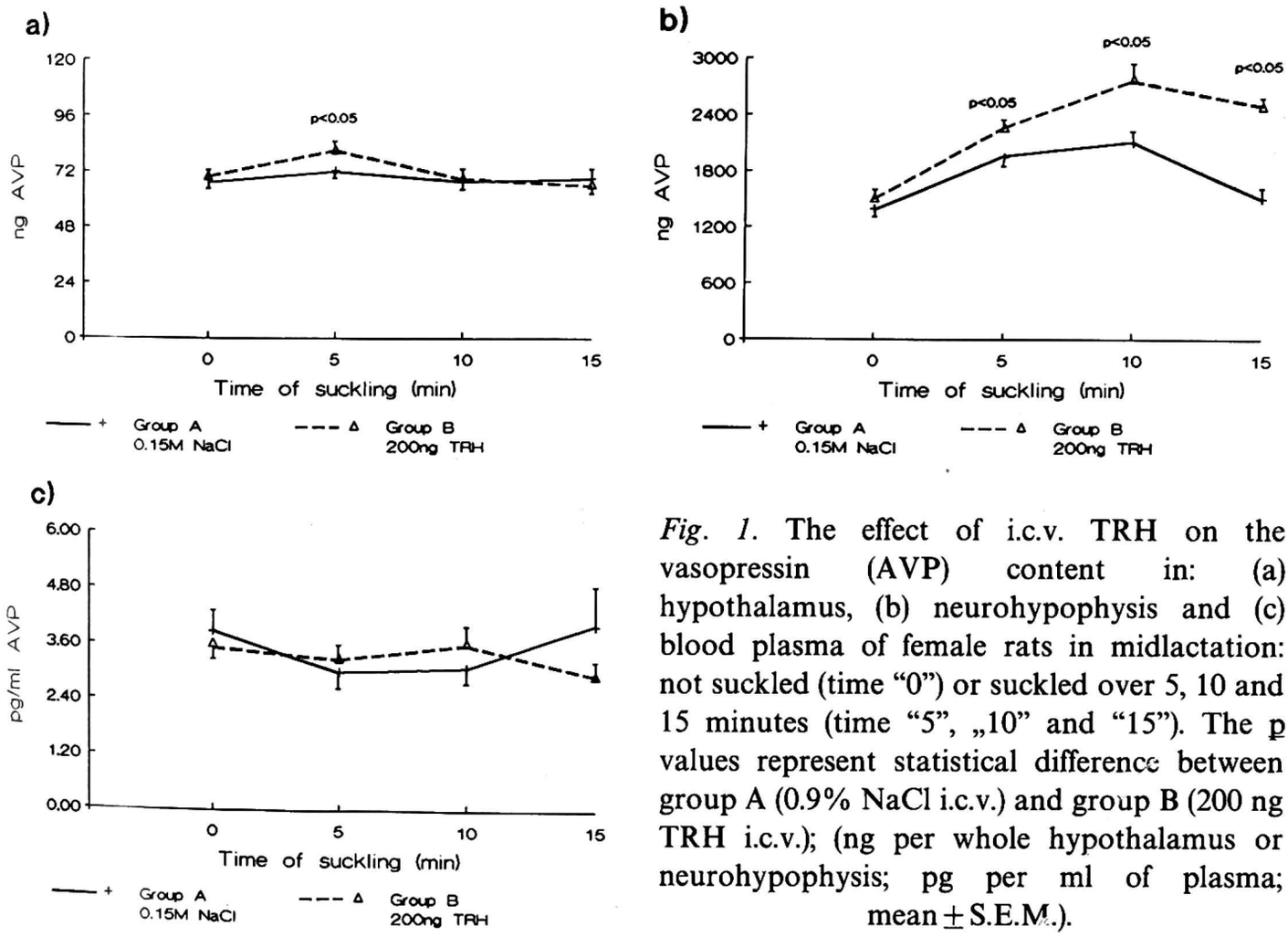

Fig. 1. The effect of i.c.v. TRH on the vasopressin (AVP) content in: (a) hypothalamus, (b) neurohypophysis and (c) blood plasma of female rats in midlactation: not suckled (time "0") or suckled over 5, 10 and 15 minutes (time "5", „10" and "15"). The p values represent statistical difference between group A (0.9% NaCl i.c.v.) and group B (200 ng TRH i.c.v.); (ng per whole hypothalamus or neurohypophysis; pg per ml of plasma; mean ± S.E.M.).

The effect of TRH on the oxytocin content in the hypothalamus and neurohypophysis as well as OT level in blood plasma (Fig. 2).

In females treated i.c.v. with 0.15 M sodium chloride, the oxytocin hypothalamic content (*Fig. 2A*) increased following 10 minutes ofsuckling. I.c.v. injections of TRH was followed by an increase of hypothalamic oxytocin content in females not suckled (time "0") (subgroup A-I versus B-I: $p < 0.05$) as well as at 5-th and 15-th minute of suckling (time "5" and "15") (subgroups: A-II versus B-II and A-IV versus B-IV: $p < 0.05$).

The neurohypophysial oxytocin content (*Fig. 2B*) in animals treated with vehicle decreased over the first 5 minutes of suckling; this effect was noted also

at 10-th and 15-th minute of suckling. The decrease of the neurohypophysial OT content as brought about by suckling was markedly attenuated by TRH (subgroups: A-III versus B-III and A-IV versus B-IV: $p < 0.05$).

The plasma oxytocin concentration (*Fig. 2C*) in animals injected with normal saline increased following 5 minutes of suckling (which is consistent with the simultaneous OT decrease in neurohypophysis) and returned to the initial values after 10 and 15 minutes. Both in not suckled (subgroup A-I versus B-I: $p < 0.05$) or suckled females TRH strongly inhibited the oxytocin release into the blood plasma as noted following 5, 10 and 15 minutes of suckling (subgroups: A-II versus B-II, A-III versus B-III and A-IV versus B-IV: $p < 0.05$).

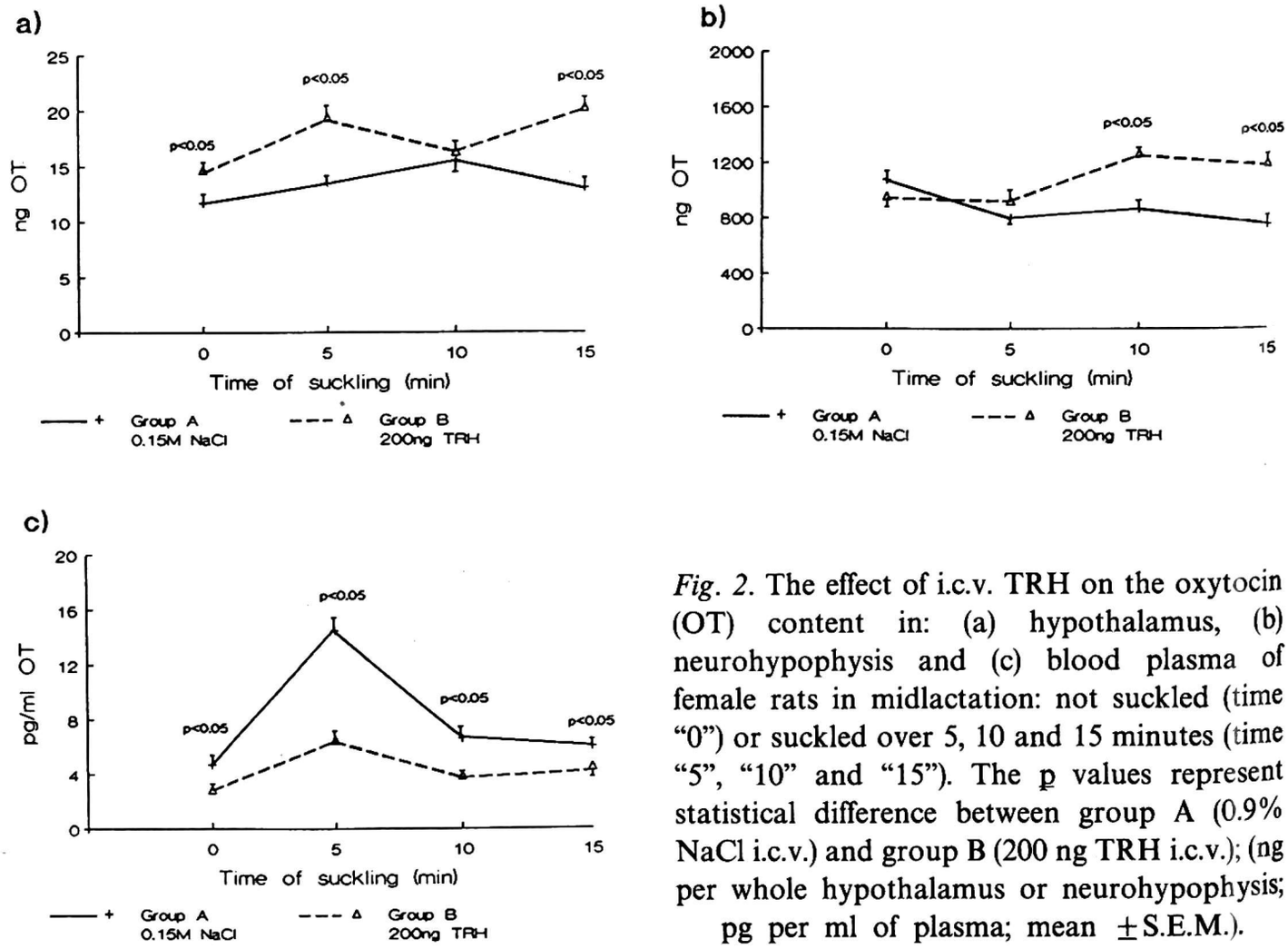

Fig. 2. The effect of i.c.v. TRH on the oxytocin (OT) content in: (a) hypothalamus, (b) neurohypophysis and (c) blood plasma of female rats in midlactation: not suckled (time "0") or suckled over 5, 10 and 15 minutes (time "5", "10" and "15"). The p values represent statistical difference between group A (0.9% NaCl i.c.v.) and group B (200 ng TRH i.c.v.); (ng per whole hypothalamus or neurohypophysis; pg per ml of plasma; mean ±S.E.M.).

The TRH influence on the prolactin blood plasma concentration (Fig 3).

The prolactin blood plasma concentration of animals from group A (i.e., treated i.c.v. with 0.15 M NaCl) raised up to a maximum at 10-th minute of suckling; at 15-th min the level of prolactin in blood plasma somewhat decreased. This increase was considerably attenuated in femals treated i.c.v. with TRH (subgroups: A-II versus B-II, A-III versus B-III and A-IV versus B-IV: $p < 0.05$).

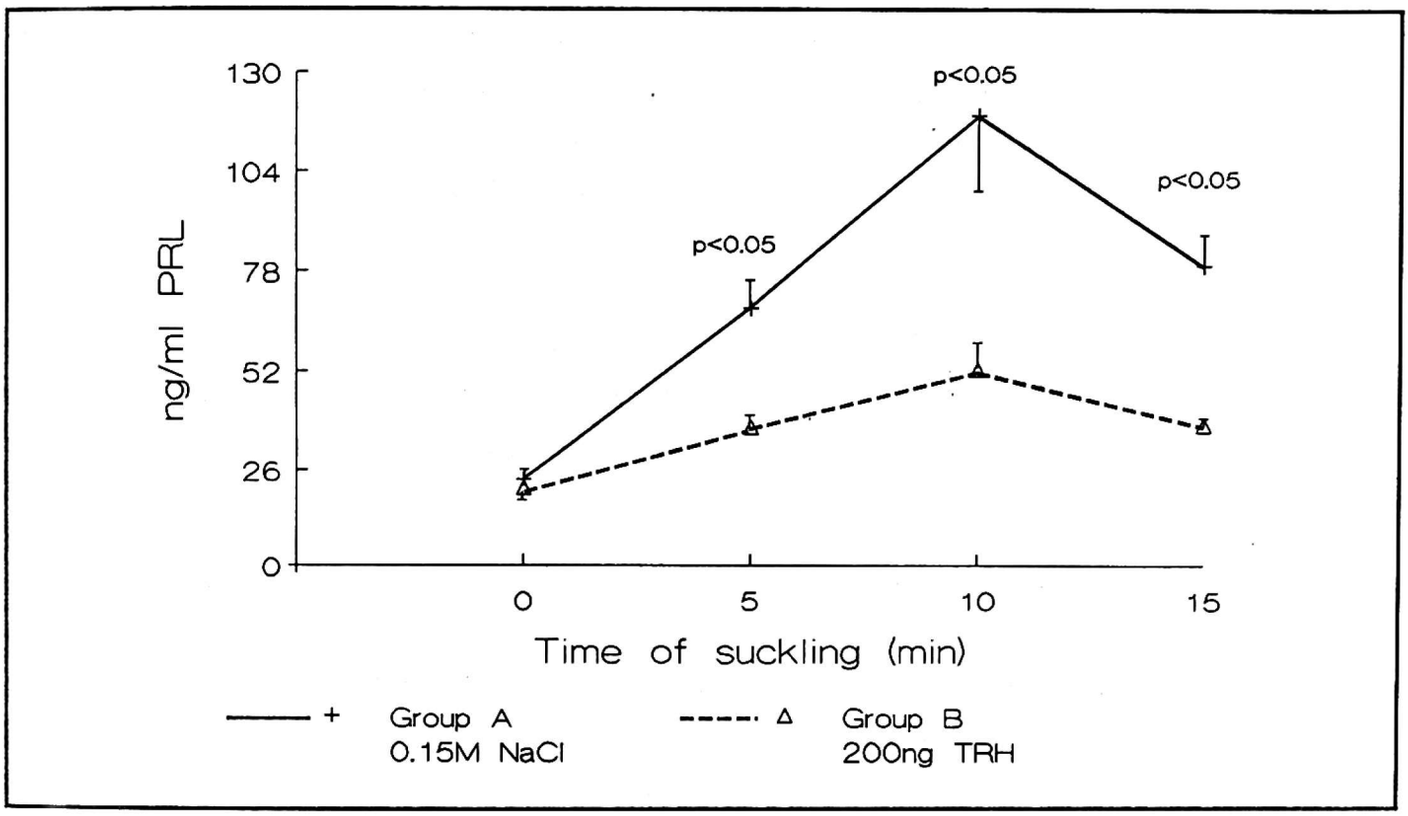

Fig. 3. The effect of i.c.v. TRH on the prolactin (PRL) concentration in blood plasma of female rats in midlactation: not suckled (time "0") or suckled over 5, 10 and 15 minutes (time "5", "10" and "15"). The p values represent statistical difference between group A (0.9% NaCl i.c.v.) and group B (200 ng TRH i.c.v.); (ng per ml of plasma; mean ±S.E.M.).

Experimental series II

The TRH influence on the plasma vasopressin concentration (Fig. 4A) and (Fig. 4B).

In females treated i.c.v. with normal saline and not suckled the vasopressin plasma concentration (*Fig. 4A*) increased somewhat over first

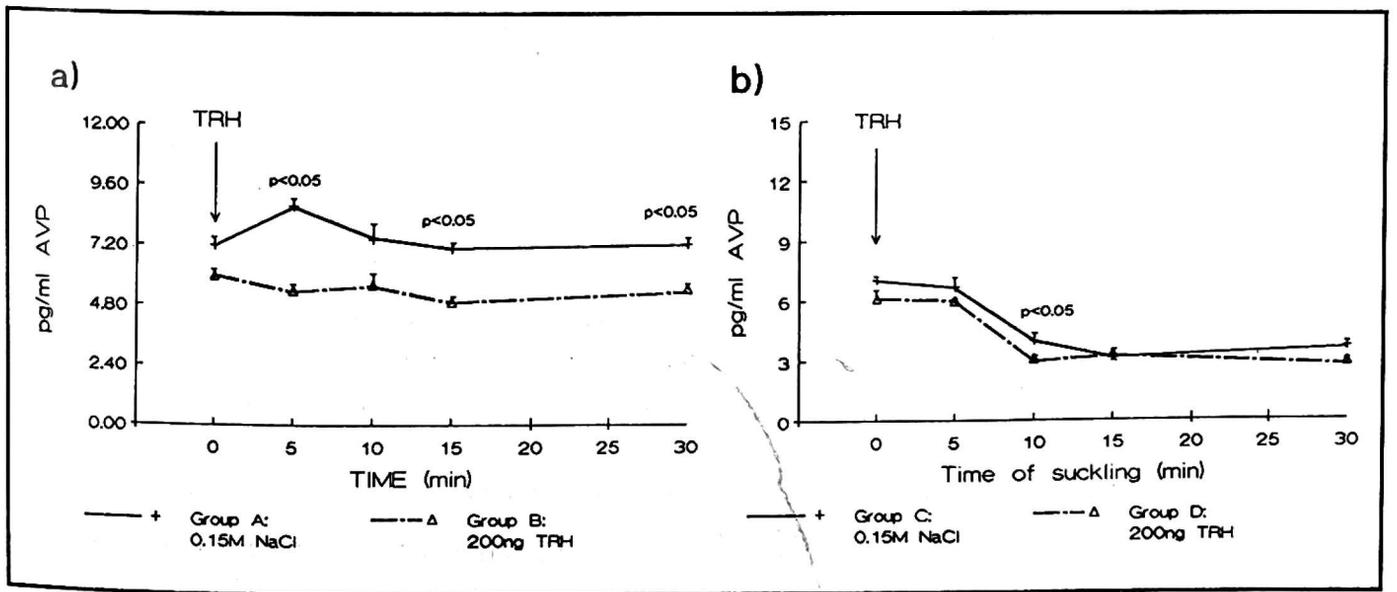

Fig. 4. Plasma arginine vasopressin (AVP) concentration as influenced by an i.c.v. injection (time "0") of TRH in female rats not suckled (a) or suckled (b). The p values represent statistical difference between group A (0.9% NaCl i.c.v.) and group B (200 ng TRH i.c.v)., (pg per ml of plasma; mean ±S.E.M.).

5 minutes (which was probably the reaction on the first blood sample collection) but then returned to the initial value. In animals treated i.c.v. with TRH, the vasopressin plasma level was distinctly lower in comparison with respective control values (subgroups: A-II versus B-II, A-IV versus B-IV and A-V versus B-V: $p < 0.05$).

In suckled females treated i.c.v. with 0.15 M NaCl the vasopressin plasma level was somewhat decreased from the 10-th up to the 30-th minute. In animals treated with TRH and suckled up to 30 minutes, some diminution of vasopressin plasma level only at time "10" was noted (subgroup A-III versus B-III: $p < 0.05$) (*Fig. 4B*).

The TRH influence on the plasma oxytocin concentration (Fig. 5A) and *(Fig. 5B).*

In females not suckled and injected i.c.v. with vehicle (*Fig. 5A*) the plasma oxytocin concentration somewhat decreased at 5-th and 15-th minute of the experiment. I.c.v. TRH administration was followed by the increase of oxytocin plasma level at 5-th, 10-th and 15-th minute of the experiment (subgroups: A-II versus B-II, A-III versus B-III; A-IV versus B-IV: $p < 0.05$).

In suckled females injected i.c.v. with 0,15 M NaCl (*Fig. 5B*) the plasma oxytocin concentration increased significantly and returned to the initial values at 30-th min. The single i.c.v. dose of TRH administered to suckled females inhibited the oxytocin release into blood plasma in response to suckling (subgroups: A-II versus B-II, A-III versus B-III, A-IV versus B-IV: $p < 0.05$).

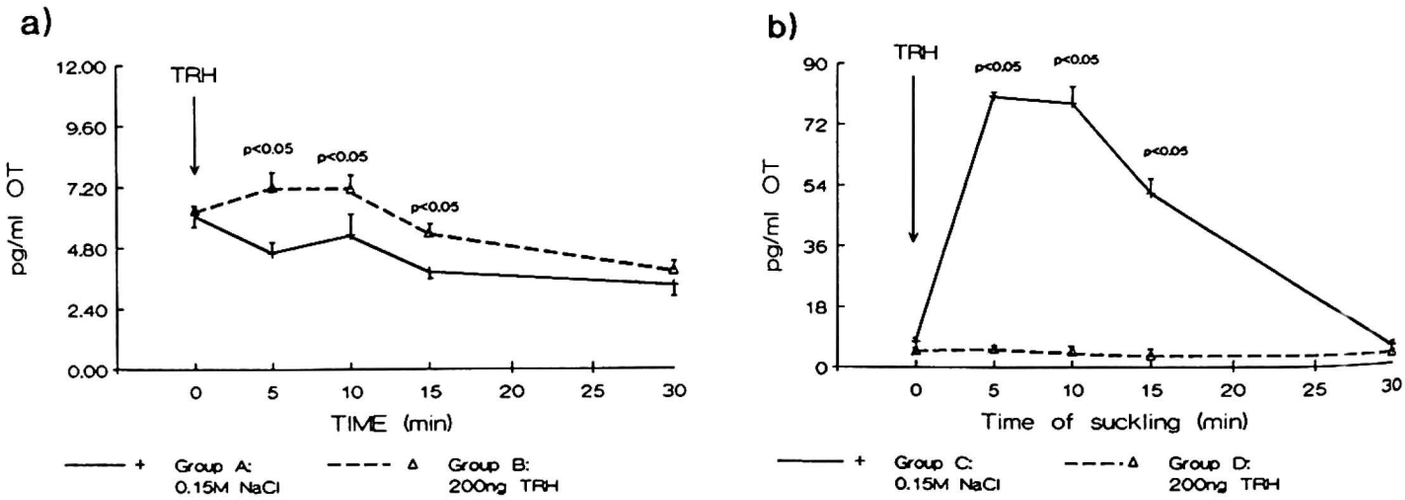

Fig. 5. Plasma oxytocin (OT) concentration as influenced by an i.c.v. injection (time "0") of TRH in female rats not suckled (a) or suckled (b). The p values represent statistical difference between group A (0.9% NaCl i.c.v.) and group B (200 ng TRH i.c.v.); (pg per ml of plasma; mean ± S.E.M.).

The TRH influence on the plasma prolactin concentration (Fig. 6A) and *(Fig. 6B).*

In females not suckled (*Fig. 6A*), injected i.c.v. with normal saline, the plasma prolactin concentration did not change from the start to the 30-th minute of experiment. TRH, injected i.c.v., was followed by an increase of prolactin plasma level 5-th, 10-th, 15-th and 30-th minute of experiment (subgroups: A-II versus B-II, A-III versus B-III, A-IV versus B-IV and A-V versus B-V: $p < 0.05$).

In suckled fmales, injected i.c.v. with 0.15 M sodium chloride solution, the prolactin plasma concentration raised progressively to the maximal value at 15-th min of suckling; thereafter, it somewhat decreased (at 30-th minute). TRH, administered i.c.v., inhibited the prolactin release at 5-th, 10-th, 15-th and 30-th minute of suckling (*Fig. 6B*; subgroups: A-II versus B-II, A-III versus B-III, A-IV versus B-IV and A-V versus B-V: $p < 0.05$).

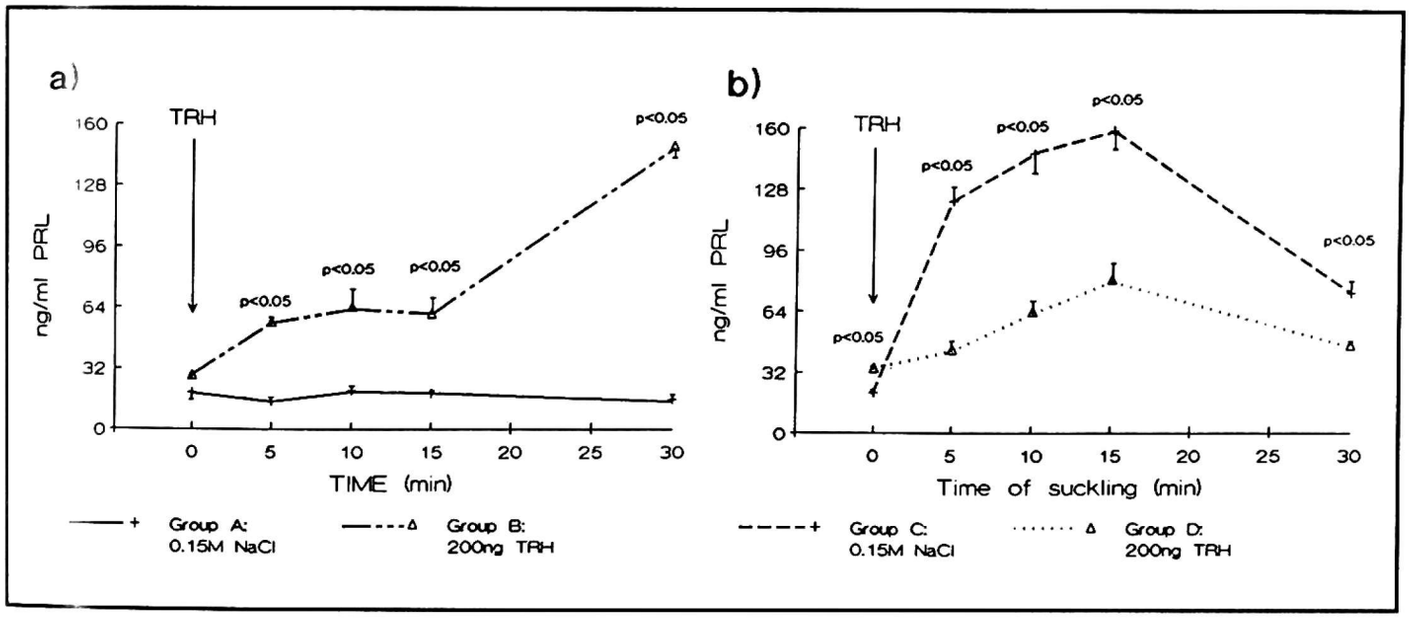

Fig. 6. Plasma prolactin (PRL) concentration as influenced by an i.c.v. injection (time "0") of TRH in female rats not suckled (a) or suckled (b). The p values represent statistical difference between group A (0.9% NaCl i.c.v.) and group B (200 ng TRH i.c.v.); (ng per ml of plasma; mean ± S.E.M.).

DISCUSSION

Suckling and the secretion of neurohypophysial hormones and prolactin in lactating rats.

Several studies confirmed that the release of oxytocin and prolactin is enhanced in response to suckling. An increased bursting activity (discharge of periodic high frequency action potentials) in the oxytocin-secreting neurons is evoked by suckling, parallelly to the milk-ejection reflex (22). The periodic bursts are related to a pulsatile oxytocin release from the neurohypophysis into the blood; the highest oxytocin secretion occurs usually from 5 to 30 minutes after the pups are allowed to suck (23, 24). The release of oxytocin within the hypothalamic supraoptic and paraventricular nuclei in response to suckling has also been shown by push-pull perfusion and microdialysis techniques (22, 25). Prolactin plasma concentration reaches highest level after 15—30 minutes of suckling (23, 26). Recent studies revealed that oxytocin is involved in the mechanism of suckling-induced prolactin release (13). It has therefore been proposed that oxytocin (whose release is known to be clearly related to the circadian rhythms) may be a neurohormonal factor which stimulates in a rhythmic manner the lactotrophs and induces rhytmic prolactin release (15). It

is supposed that oxytocin is involved in the co-ordination of the characteristic bursting electrical activity of oxytocin neurons, as shown during suckling (27). In female rats during suckling as well as parturition oxytocin may be released within the neurons of the paraventricular and supraoptic nuclei in the process of the exocytosis from their dendrites or axons (25, 28). Accordingly, oxytocin released in the SON and PVN acts in the positive feedback mechanism on its own secretion (29). This phenomenon has been called „intranuclear release" (25). The answer to the question whether oxytocin release during the suckling or parturition is accompanied by increased vasopressin liberation (22) is still not clear. Most reports do not support this assumption (25, 30). E.g., Neumann *et al.* (25) could not show that both parturition or suckling (classical stimuli for the oxytocin release) did result in an increased vasopressin release.

Our results in series I as well as in series II are quite consistent with the above data. Suckling induced distinct release of oxytocin after 5, 10 and 15 minutes from the onset of the suck in the experiments of series I and II. Accordingly, the neurohypophysial oxytocin content decreased just after 5 minutes of suckling and did not return to the initial level up to the end of experiment. On the other hand, in series II a diminution of blood plasma oxytocin level was noted in not suckled females injected i.c.v. with 0.15 NaCl solution. The mechanism of this decrease is unclear; it may be interjected, however, that after i.c.v. injection of normal saline some authors (e.g., 31) have noted functional fluctuations within the endocrine system.

The plasma vasopressin concentration decreased somewhat after 10 and 15 minutes of suckling but was not changed in not just suckled females; these results appear to be consistent with those of Neumann *et al.* (25). It seems therefore that during suckling, when the oxytocinergic neurons are stimulated, the vasopressinergic ones are rather suppressed.

The effect of TRH on the release of oxytocin, vasopressin and prolactin in lactating rats.

Some data suggest a modulating role for TRH in the release of neurohypophysial hormones; the respective experiments, however, were performed on males (1—5). What is more, the results obtained so far are not consistent. TRH was shown to increase the blood vasopressin and oxytocin level in the rabbit (1, 2). In the rat, however, Kasting (4) as well as Siren *et al.* (5) could not show any increase of the vasopressin and oxytocin release following intravenous injection of TRH. Similarly, no change in oxytocin and vasopressin release has been noted following intravenous injections of TRH in humans (32). Skowsky and Swan (33) and also Ciosek and Guzek (6) reported that TRH increased the vasopressin release from the neurointermediate lobes *in vitro* but inhibited that of oxytocin. On the other hand, TRH inhibited

the vasopressin and oxytocin release from the rat hypothalamo-neurohypophysial explants *in vitro* (34). I.c.v. administration of TRH increased the hypothalamo-neurohypophysial vasopressin content and decreased that of oxytocin in the dehydrated, salt-loaded or haemorrhaged male rats (6, 7, 8, 9, 10).

The present findings show that TRH, when administered i.c.v., inhibits the release of vasopressin from the hypothalamo-neurohypophysial system both in not suckled and just suckled females. Therefore, TRH may possibly act in the rat central nervous system as an inhibitory neuromodulating factor for the vasopressin release. May be, administration of relatively large doses of TRH (200 ng as used in this experiment) suppresses the activity of TRH-ergic neurons and also depresses TRH receptors localized in the PVN and SON. Moreover, it is probable that the mutual connections between TRH-synthesizing neurons (which are present in the medial and periventricular parvocellular subdivisions of the PVN (35, 36), may be modified by TRH applied i.c.v.

A number of studies demonstrated that TRH is a potent stimulator of prolactin secretion (37, 38). Most probably, TRH receptors exist on the lactotroph plasma membrane in the anterior pituitaries (39). Some authors, however, noted only a small, transient increase in plasma prolactin following TRH administration to lactating rats (37). On the other hand, i.c.v. injections of TRH were noted to inhibit PRL secretion from the anterior pituitary of male rats, possibly by increasing the release of endogenous dopamine from the hypothalamus (40). In the case of hypothyroidism (when hypothalamic TRH content increased) the number of lactotrophs in the anterior pituitary and PRL release significantly decreased (41). Indeed, Riskind *et al.* (42) concluded that TRH may be not a major prolactin-releasing factor during suckling in the rat: thought it increased the plasma TSH level nevertheless it did not affect the plasma concentrations of prolactin under such conditions.

During lactation, changes in the nervous and hormonal systems modify the hypothalamic TRH metabolism in the rat. Also, there is an increased TRH release from the median eminence into the hypophysial portal blood in response to suckling (43). Electrical stimulation of an isolated mammary nerve was reported to increase the TRH release into hypophysial portal blood in lactating rats (43). The suckling-induced TRH release was shown to be accompanied by enhanced TRH synthesis: a suckling stimulus after removing the pups for 8 hours induced a rise in paraventricular TRH mRNA (44). On the other hand, nevertheless, just the removal of the pups might affect the TRH biosynthesis. TRH mRNA level in the PVN as well as the mediobasal hypothalamic TRH content are known to remain unchanged 8 h after removal of the pups (43); as long as 56 h of suckling interruption increased paraventricular TRH mRNA without changes in mediobasal hypothalamic TRH (45). In other studies, however, no changes in TRH output were observed in the conscious, lactating rats during suckling (46).

Present study shows that TRH modifies in different manner the oxytocin and prolactin release in not suckled or suckled lactating female rats. TRH intensified the oxytocin as well as prolactin release in not suckled females but, on the contrary, TRH strongly inhibited the oxytocin and prolactin release into the blood of femals just suckled. Similar observation (i.e., diminished PRL secretion after TRH administration in females just suckled but increased PRL release in females not just suckled) has been reported earlier by Collu and Taché (47). Hence, it may be assumed that the effect of TRH on the oxytocin and prolactin release depends on the current activity of TRH neurons just during suckling or between the suckling episodes. Moreover, TRH could modify the activity of other hypothalamic factors involved in the modulation of prolactin secretion. Indeed, there are earlier observations that TRH may influence the release of some anterior pituitary hormones. E.g., TRH was noted to intensify the adrenocorticotropic hormone release in patients with Cushing syndrome (48) or to enhance the secretion of growth hormone in those with acromegaly (49). So, in accordance with the above data and also in conformity with this experiment it may be suggested that during suckling exogenous TRH, through possible functional modification of TRH-ergic neurons at the hypothalamic level, may result in suppression of the afferent suckling signals reaching the oxytocinergic neurons.

It may be hypothesized, furthermore, that during suckling a positive feedback mechanism between oxytocin and prolactin secretion possibly exists: oxytocin is supposed to penetrate the anterior pituitary and stimulate the prolactin release (50); on the other hand, prolactin has been shown to stimulate oxytocin release from the neurohypophysis both *in vivo* and *in vitro* (24).

The site of TRH action on reflex mechanisms related to vasopressin and oxytocin release remains unclear. Following i.c.v. injection, possible TRH influence on a number of brain structures containing susceptible neural junctions may be expected. It cannot be excluded, however, that exogenous TRH may act directly on TRH paraventricular neurons through axodendritic and/or axosomatic synapses between TRH-IR (TRH-immunoreactive) fibres and TRH-IR neurons (51). Moreover, exogenous TRH could act on TRH paraventricular neurons indirectly, its influence on other mediator(s) or modulator(s) being an intermediary event. Indeed, inhibitory dopamine terminals exist in the median eminence; these terminals, localized on TRH-IR fibres, are thought to inhibit the TRH release (52). What is more, some pharmacological evidence indicates existence of a facilitatory noradrenergic mechanism in the PVN (53); it seems therefore possible that noradrenaline — being an intermediary link within the chain of events triggered by exogenous TRH — may act *via* inhibitory interneurons innervating the TRH-IR nerve cell bodies and dendrites (52).

Concentrations of noradrenaline and adrenaline have been shown to increase in the blood plasma after i.c.v. TRH administration (54). Noradrenaline, in turn, is known to inhibit the vasopressin and oxytocin release (55). On the other hand, systemic TRH administration is followed by reduction of the noradrenaline turnover in the PVN (56). Hence, TRH may modify its own secretion through modulation of the noradrenergic mechanisms in the PVN.

In conclusion, it may be supposed that TRH is of some importance for regulation of vasopressin, oxytocin as well as prolactin release in lactating female rats. As for vasopressin release, TRH acts as an inhibitory neuromodulating factor. TRH seems to be the inhibiting factor for oxytocin and prolactin release activated by suckling.

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