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Reduced tuberoinfundibular dopaminergic neuronal function in rats after long-term withdrawal of estrogen treatment[1]

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Summary. Hypothalamic fragments from female rats treated repeatedly with estradiol valerate (EV) and bearing prolactin (PRL)-secreting tumors contained, seven months after the last EV injection, lower concentrations of dopamine (DA) than age-matched controls. Depolarizing concentrations of K^+ (35 mM) and amphetamine (50 μM) evoked in PRL-secreting tumor bearing rats an endogenous DA release significantly lower than in controls.
Key words. Tuberoinfundibular dopaminergic system; prolactin; estrogen-treated rats; prolactin secreting adenoma.

Considerable evidence suggests that hypothalamic infundibular dopaminergic (TIDA) neurons are involved in the control of prolactin (PRL) secretion from the anterior pituitary gland. Dopamine released from the medial palisade zone reaches the anterior pituitary, via portal vessels, activates dopaminergic receptors on mammotrophs and inhibits PRL secretion[1]. An increased PRL secretion, in turn, exerts a positive feedback on DA terminals in the median eminence by increasing dopamine synthesis[2-4] and release[5,6] from TIDA nerve endings. Although dopamine is not the sole prolactin-inhibiting factor, it is probably the most physiologically important[7]. Among the factors that influence the activity of TIDA neurons, estrogens exert a prominent role. Short-term (3–5 days) estrogen treatment increases the activity of TIDA neurons, as revealed by increases in the rate of synthesis[8] and turnover[9,10] of dopamine in the median eminence and in the concentration of dopamine in hypophyseal portal blood[11]. Long-term treatment with estradiol (many weeks), on the other hand, results in a decrease in the concentration and synthesis of dopamine in the median eminence[12,13]. We have shown in previous work[14] that multiple injection of 2 mg estradiol valerate at 3-week intervals into mature cycling rats is accompanied by a striking rise in plasma PRL concentration, development of pituitary-secreting tumors (prolactinoma), a progressive decrease in median eminence dopamine concentration and unresponsiveness of PRL secretion to nomifensine, a drug which promotes release of dopamine and inhibits its reuptake from nerve terminals[15]. The neurochemical mechanisms by which long term estrogen treatment cause these alterations are unclear. The changes may represent an irreversible decrease in the number of TIDA neurons, in view of the cytopathological changes present in the arcuate nucleus of long-term estradiol treated rats[14,16-18] or, alternatively, may be related to a transient decrease in the activity and function of TIDA neurons.

In the present study, we investigated the activity of TIDA neurons of long-term estradiol valerate treated rats bearing pituitary prolactinomas, seven months after discontinuation of estrogen treatment. As a direct index of TIDA function, we evaluated in vitro the release of endogenous dopamine from arcuate-periventricular nucleus-median eminence complexes.

Experimental procedure. Female Sprague-Dawley rats (Nossan Corezzana Inc., Milano, Italy) weighing 160–200 g were housed (5 per cage) in an air conditioned (24 °C) and light controlled (light on 06.00–20.00 h) room with free access to chow and water. Normal vaginal cyclicity (4 days) was established for at least two weeks before initiating the experiments. Each rat was then injected with 2 mg s.c. of 17-β-estradiol valerate (Progynon Depot, Schering, Berlin, West Germany), 5 times at 3-week intervals. Seven months after the last injection, estradiol valerate-treated rats and age-matched vehicle-injected controls were decapitated and trunk blood collected for serum PRL determination by a double antibody RIA[19] with reagents provided by the National Pituitary Agency (NIAMDD). Plasma samples were assayed in duplicate: the volume used was 25 μl undiluted or diluted 1:10. PRL values were expressed in terms of NIADDK rat PRL-RP-3 with a biological potency of 30 IU/mg. The intra-and interassay coefficients of variation were 8.7% and 9.8%, respectively. The sensitivity of the assay was 0.75 ng/ml. Arcuate-periventricular nucleus-median eminence fragments were obtained by dissecting with fine scissors under a stereomicroscope a triangular shaped area (1 mm × 1 mm, about 2 mg in weight) according to Cuello et al.[20]. After a 30 min preincubation, pools of three fragments were incubated in flasks containing 1 ml Krebs-Ringer Bicarbonate (KRB) buffer (118 nM NaCl, 5mM KCl, 1.15 mM $MgSO_4$, 1.15 mM NaH_2PO_4, 2.5 mM $Ca Cl_2$, 25 mM $NaHCO_3$, 11 mM glucose, 10 μM tyrosine and 1

Endogenous dopamine release from TIDA neurons expressed as percent of dopamine total content (dopamine release during the whole experiment plus dopamine content measured in the fragments at the end of the experiment) in control and prolactinoma-bearing rats

	Control rats	Prolactinoma-bearing rats
5 mM K^+ KRB medium	1.07 ± 0.2	0.99 ± 0.2
35 mM K^+ KRB medium	7.96 ± 1.0	6.48 ± 0.6
5 mM K^+ KRB medium + d-amphetamine	3.90 ± 0.6	3. 3 ± 0.6

For experimental details see legend of the figure.

mM ascorbic acid) adjusted to pH 7.4. When a higher K^+ concentration was used the Na^+ concentration was accordingly reduced. The flasks were incubated at 37 °C in a Dubnoff metabolic shaker (66 shakes/min) and aerated with a 95% O_2 mixture for the whole duration of the experiments. When tissue was subjected to drugs or different KCl concentrations, the previous KRB buffer was replaced by the new medium under study. After a 10- or 20-min incubation, 100-µl samples were collected, and the tissue was blotted dry, weighed and homogenized in 0.1 M perchloric acid on ice. Incubation samples and tissue homogenates were centrifuged at 10,000×g for 10 min at 4 °C. The supernatant was removed and stored at −50 °C. Both tissue and medium dopamine were assayed by the sensitive radioenzymatic procedure of Peuler and Johnson[21], slightly modified[22]. The amount of dopamine measured in 100 µl samples was adjusted for the volume of incubation medium and divided by three to obtain the dopamine release as pg/ml medium/fragment.

For the morphological study, after opening the skulls, some representative pituitaries were quickly removed, immersed in 2% glutaraldehyde-1.5% paraformaldehyde solution, phosphate buffered at pH 7.4, and left in the fixative solution for at least 48 h. Paramedial sagittal sections, about 1 mm thick were cut free-hand, postfixed in 2% OsO_4 solution (pH 7.4) for 2 h. Afterwards, semithin sections for light microscopic study were cut and stained with toluidine blue, and thin sections cut for electron microscopy were stained with uranyl acetate and lead citrate[23].

Statistical analysis. All results were expressed as means±SEM, Differences between the means were determined by Student's t-test for unpaired data. The 0.05 level of probability was used as the minimum criterion of significance for all data.

Results. PRL levels. Seven months after the last estradiol valerate administration plasma PRL concentrations of prolactinoma-bearing rats (n=21) were significantly higher as compared to age-matched controls (n=23) (486.3±115 vs 27.3±10 ng/ml, $p < 0.01$).

Effect of depolarizing K^+ concentration on the release of endogenous dopamine from TIDA neurons. The hypothalamic fragments were incubated for 10 min in a KRB medium containing 5 mM K^+ to determine the spontaneous efflux of endogenous dopamine. Basal dopamine release from the hypothalamic fragments of prolactinoma-bearing rats did not differ from that of age-matched controls (fig.).

When the arcuate-periventricular nucleus-median eminence fragments were exposed to a depolarizing K^+ concentration (35 mM), dopamine release of prolactinoma bearing rats was significantly lower than that of controls (fig.). The endogenous total dopamine (dopamine released plus residual dopamine measured in the fragment at the end of the experiment) of hypothalamic preparations from prolactinoma bearing rats, was also significantly lower than that of controls (6464±790 vs 9642±1319 pg/ml medium/fragment). However, when dopamine released at each time interval was expressed as percentage of total endogenous dopamine, no sgnificant difference between prolactinoma bearing rats and controls was detected (table).

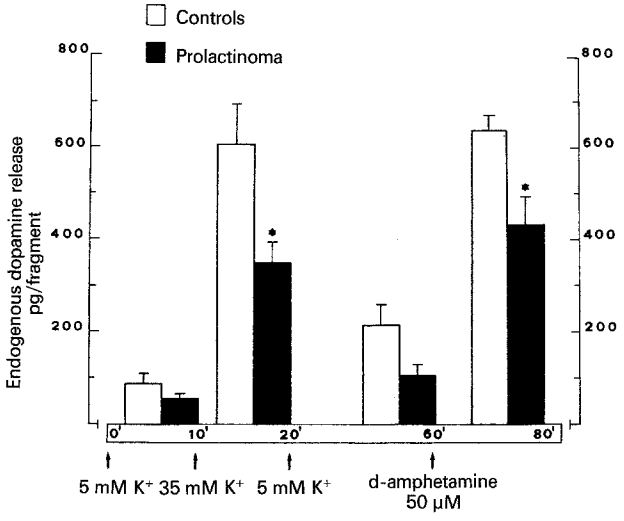
Effect of the pharmacological dopamine releaser d-amphetamine on TIDA neurons. D-amphetamine (50 µM) added to the KRB medium enhanced dopamine release (pg/ml medium/fragment) from control preparations more than release from preparations from prolactinoma bearing rats (fig.). When d-amphetamine-induced dopamine release was expressed as percent of total endogenous dopamine, however, no significant difference between prolactinoma bearing rats and controls was detected (table).

Pituitary weight and morphology. Great differences in the pituitary size were encountered among estradiol valerate-treated rats. In some of these animals pituitary weight was barely larger than that of controls; in others it was up to 10 times as large. Mean anterior pituitary weights in estradiol valerate-treated rats and controls were: 37.4±3.1 mg vs 10.8±0.6 mg, respectively ($p < 0.001$). Light microscopical observation showed that estradiol valerate-treated rats harbored pituitary tumors. The smaller glands contained microadenomas, sometimes only a few hundred µm in diameter; the larger glands had macroadenomas with diameters up to 3–4 mm (data not shown). Neoplastic cells, independently from the size of the adenoma, had a comparable appearance in all tumors, either by light or electron microscopy.

Discussion. Chronic administration of huge doses of estradiol valerate at 3-week intervals causes the development of micro- and macroadenomas in the pituitary gland of female rats[14,18]. As shown by the present study these tumoral changes are permanent, still being present seven months after estrogen withdrawal. Dopamine content in the arcuate-periventricular nucleus-median eminence complex of prolactinoma bearing rats after seven months of estrogen withdrawal was lower than in controls, as already reported for prolactinoma-bearing rats under estrogen treatment[14,18]. Moreover, application of a depolarizing concentration of K^+ or a dopamine releaser e.g. amphetamine was less effective in releasing dopamine from TIDA neurons of prolactinoma-bearing rats than from TIDA neurons of controls. Similarly to these findings, it has been recently shown by Sarkar et al.[24] that the electrically-induced release of dopamine from the median eminence in vitro was significantly decreased in rats under estrogen treatment as compared to controls.

In all, our data demonstrate that biochemical changes in rat TIDA neurons related to repetitive administration of huge doses of estradiol valerate are still detectable long-term after withdrawal of the steroid and point to the irreversibility of this lesion. It has been suggested[14,18] that the estradiol-induced decrease in median eminence dopamine content is associated with TIDA neuronal loss, an interpretation consistent with the results of our study. However, since percentual DA release after ionic or pharmacologic challenges was comparable in prolactinoma-bearing and control rats, it would appear that in the TIDA neurons undamaged by estrogens the mechanisms involved in neurotransmitter release are unimpaired.

The way of thinking can be affected by the fact that the values of DA contained in the fragments are only an approximation; the DA broken down within the tissue piece during the incubation



Effects of K^+ (35 mM) and d-amphetamine (50 µM) on endogenous dopamine release from arcuate-periventricular nuclei-median eminence fragments of prolactinoma-bearing rats (solid columns) and age-matched controls (open columns). The hypothalamic fragments were preincubated in the presence of 5 mM K^+ (10 min) and then exposed to 35 mM K^+ (10 min). After a rest period (5 mM K^+ for 40 min), the tissue was incubated (20 min) in the presence of 5 mM K^+ + 50 µM d-amphetamine.
Each column represents the mean ±SE of seven determinations (21 hypothalamic fragments). Asterisks denote significant difference ($p < 0.05$) from corresponding control values (unpaired Student's t-test).

time is not measured. In principle, tissue breakdown might differ in different states (i.e. in prolactinoma-bearing rats vs controls). The participation of PRL in the disrupting effect of estradiol on TIDA neurons is difficult to evaluate, for estrogens increase PRL release, and long-lasting hyperprolactinemia can itself prove toxic to TIDA neurons[18]. In the experiments of Demarest et al.[12] long-term treatment with estradiol reduced the activity of TIDA neurons whereas continuously elevated PRL induced by chronic haloperidol administration increased dopamine turnover in the same neurons[24]. On the other hand the recent demonstration that chronic elevation of PRL produces a decline in TIDA function[25] in the absence of any rise in plasma estrogens, suggests that PRL per se may have a disrupting effect.

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A daily rhythm in hCG binding to ovarian follicles of the cyclic hamster

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Summary. On each day of the estrous cycle hCG binding to follicle increased from 09.00 to 21.00 h; then hCG binding was static until 09.00 h of the next day. FSH binding did not exhibit rhythmicity. This pattern of hCG binding may be related to the pulsing of LH on each cycle day.
Key words. hCG receptor; FSH receptor; rhythm; follicles; steroids.

There is evidence of circadian rhythms in secretion patterns of most central nervous system controlled pituitary hormones, e.g. thyrotropin, prolactin, growth hormone and luteinizing hormone[2,3], whose adaptive role is poorly understood. Everett and Sawyer[4] reported that barbiturates prevent ovulation, if administered at 14.00 h on proestrus. Subsequently several studies[5-7] suggested a critical period during proestrus when administration of blocking agents prevents ovulation. Everett[8] defined the critical period in proestrous rats between 14.00 h to 16.00 h. Other investigators have reported critical periods for LH release on other cycle days[9,10]. Dominguez and Smith[9] noted that pentobarbital, a blocker of LH secretion, was effective in delaying ovulation for one day if the injection was given at 12.45 h on any day of the cycle while injections at 09.00 und 17.00 h were ineffective.

Bolton[11] has shown that serum LH levels in female hamsters were significantly elevated at 13.00 h on day 1, 19.00 h on day 2 and 16.00 h on day 4; a high level of LH in serum was also noted at 19.00 h on day 3 although it was not statistically significant from lower values on that day thus, a circadian pattern in LH release appeared evident on each day of the cycle. Since LH alters the number of LH receptors in the ovary[12] and since serum LH levels in hamsters exhibit a circadian rhythm, we were interested in determining whether follicular LH receptors exhibited circadian alteration throughout development of the 4-day estrous cycle. An additional aim was to determine whether follicular steroid levels coincided with any change in gonadotropin receptors.

Materials and methods. Cyclic hamsters, weighing 80–100 g, were maintained on a 14-h light: 10 h dark schedule with lights on from 05.00 to 19.00 h in a room of 21–23 °C. Day 1 (the day of ovulation, estrus) was determined by the characteristic vaginal discharge. Three consecutive 4-day cycles were monitored before using the animals.

Classification of follicles. Follicles were classified according to a previous description[13]. The stages are: stage III: preantral follicles with 6–7 layers of granulosa cells; stage IV: preantral follicles with eight or more layers of granulosa cells; stage V: follicles