Persistence of a defective tuberoinfundibular dopaminergic function in rats after long-term removal of oestrogen treatment. An in vivo study

D. Cocchi1, A. Peñalva2, R. Torpia, G. L. Rossi3
and E. E. Muller

Department of Pharmacology, Chemotherapy and Toxicology, University of Milan, Milan, Italy and
Institute of Animal Pathology3, University of Berne, Berne, Switzerland

Abstract. The function of the tuberoinfundibular dopaminergic (TIDA) neurons of 49 rats bearing oestradiol-valerate (EV)-induced prolactin (Prl) secreting tumours (prolactinomas) was evaluated in vivo, 7 months after discontinuation of EV-treatment, with neuroactive drugs acting via stimulation or inhibition of DA neurotransmission. Based on the size and morphologic appearance of the pituitary and on determination of plasma Prl levels, rats previously treated with EV could be divided into those bearing macro- (31/49) and those bearing microprolactinomas (18/49). Administration of the indirect DA agonist drug nomifensine (10 mg/kg iv) lowered plasma Prl levels in control rats, but failed to do so in rats bearing either macro- or microprolactinomas. Administration of the DA receptor antagonist domperidone (50 µg/kg ip) or the synthetic enkephalin analogue FK 33-824 (1 mg/kg ip) failed to induce a rise in plasma Prl in rats with macro-, but induced a clear-cut rise in plasma Prl in those with microprolactinomas.

Prl unresponsiveness to all three neuroactive drugs indicates that long time after EV withdrawal TIDA neuronal function is still highly impaired in rats bearing EV-induced macroprolactinomas. The impairment of TIDA neuronal function would be of lesser extent in rats bearing microprolactinomas as revealed by a defective response to only one of the three applied neuroendocrine probes.

It is now clearly established that hypothalamic infundibular dopaminergic (TIDA) neurons play a crucial role in the control of tonic prolactin (Prl) secretion from the anterior pituitary gland. These neurons secrete DA into the hypophyseal portal vasculature, activate DA receptors on lactotrophs and inhibit Prl secretion (Mac Leod 1976). An increased Prl secretion, in turn, exerts a positive feedback on DA terminals in the median eminence by increasing DA synthesis (Anunziato 1980) and release (Perkins & Westfall 1978; Foreman & Porter 1981).

It is known that both short- and long-term oestrogen administration in rats influences the activity of TIDA neurons. Short-term (3–5 days) oestrogen treatment enhances the activity of TIDA neurons, as revealed by increased rate of synthesis (Demarest & Moore 1979) and turnover (Cuello et al. 1973; Eikenburg et al. 1977) of DA in the median eminence and concentration of DA in hypophyseal portal blood (Gudelsky et al. 1981). Long-term treatment with oestradiol, on the other hand, results in a decrease in the concentration and synthesis of DA in the median eminence (Du Pont et al. 1981). In this context, we have shown in a previous work that repeated injections of 2 mg oestradiol valerate (EV) at 3-week intervals into mature cycling rats is accompanied by strikingly raised plasma Prl concentrations, development of Prl-secreting tumours (prolactinoma), a progressively decreased median eminence DA concentration and unresponsiveness of Prl secretion to drugs acting via inhibition or stimulation of DA neurotransmission (Casanueva et al. 1982).
The nature of the biochemical mechanisms by which long-term oestrogen treatment causes these alterations is still unclear. The observed changes may be due to irreversibly decreased number of TIDA neurons, in view of the cytopathologic changes in the arcuate nucleus of the hypothalamus of long-term oestradiol treated rats (Brawer et al. 1978, 1983; Casanueva et al. 1982) or, alternatively, to reversibly decreased activity and function of TIDA neurons.

The objective of our investigation was to probe the function of TIDA neurons of long-term EV-treated rats bearing pituitary prolactinomas (PP-rats), 7 months after discontinuation of oestrogen treatment. As an index of TIDA function we evaluated in vivo the competence of some neuroactive drugs, acting via stimulation or inhibition of DA neurotransmission, to induce the expected changes in plasma Prl secretion.

Materials and Methods
Female Sprague-Dawley rats (Nossan Corezzana Inc., Milan, Italy) weighing 160–200 g, were housed (5 or 6 per cage) for at least 1 week in standard environmental conditions with free access to chow and water. Each rat was then injected with 2 mg sc of oestradiol-17β valerate (EV: Progynon, Schering, Berlin, West Germany) 5 times at 3-week intervals. Seven months after the last injection, EV-injected and control rats underwent acute administration of 1) nomifensine (NOM, Hoechst, Frankfurt/Main, West Germany, 10 mg/kg iv), a drug which activates CNS-DA function by releasing DA and blocking DA re-uptake, but unable to stimulate directly DA receptors (Nicholson & Turner 1977); 2) domperidone (DOM, Janssen Italia S.p.A., Rome, Italy, 50 μg/kg ip), a potent blocker of DA receptors, unable to cross the blood-brain barrier (BBB; Reyntjens et al. 1978); 3) FK 33-824 (DAMO, Sandoz AG, Basle, Switzerland, 0.5 mg/kg ip), a potent opioid peptide analogue of met-enkephalin (Roamer et al. 1977).

All the experiments were started at 10 a.m. disregarding the oestrus cycle in controls and cycle disruption in EV-treated rats. NOM was dissolved in a small volume of HCl 1 N then buffered to pH 6; the other drugs were dissolved in saline. Control rats received isotonic amounts of the appropriate diluent. In the experiments with DOM and FK 33-824, blood samples were obtained by retro-orbital venous puncture at time 0 and again 15, 30 and 60 min after drug administration. To minimize the stress effect of blood sampling, rats were handled for at least 2 weeks before the experiments. In the case of NOM, to better detect its Prl-lowering effect in controls with low plasma Prl levels, blood was sampled through a permanent catheter inserted in the jugular vein 2 days earlier. In this way blood sampling could be performed in freely moving animals without any handling stress effect.

At the end of the experiments, heparinized blood was immediately centrifuged, plasma separated and kept frozen at −20°C until assayed for Prl by a double antibody radioimmunologic method, using reagents kindly provided by NIAMD (reference preparation used: NIADDK-RP-3 with a biologic potency of 30 IU/mg). The sensitivity of the assay was 0.5 ng/ml. The intra-assay coefficient of variation was 4.5% and the inter-assay was 11%.

Morphologic studies
At the completion of the experiments, after killing by decapitation, the skulls of representative rats were opened, the pituitaries were quickly removed, fixed by immersion in 2% glutaraldehyde 1.5% paraformaldehyde solution, phosphate buffered at pH 7.4 and left in this solution for at least 48 h. Paramedial sagittal sections, about 1 mm thick, were cut free-hand, post-fixed in 2% OsO₄ solution (pH 7.4) for 2 h as previously described (Rossi et al. 1979). After dehydration, infiltration and embedding in Spurr’s low viscosity medium, semithin sections were cut and stained with toluidine blue for light microscopy. From selected tissue areas, thin sections were then cut, stained for contrast with uranyl acetate and lead citrate, studied and photographed by means of a Philips EM 300 electron microscope.

Analysis of data
Since basal values between control and experimental animals were significantly different, Prl data were expressed as ratio between baseline (B) and suppressed (S) values (S/B) in the experiment with NOM and as absolute increments from baseline (A) in the experiment with DOM.

In the experiment with FK 33-824, results were expressed as integrated areas under the curves (AUC ng/ml/30 min). In all the experiments, significance of differences was calculated for each time by Dunnett’s t-test preceded by ANOVA.

Results
Prl levels
Seven months after the last EV administration animals could be divided into two groups. The first one (18 out of 49 rats) had pituitaries barely larger than controls (mean weight 23.7 ± 2.0 vs 15.8 ± 0.8 mg, P < 0.01) and mean Prl values of 63.4 ± 15.1 ng/ml (P < 0.05 vs mean control values of 10.6 ± 2.3 ng/ml); the second one, comprising the remaining 31 rats, had pituitaries up to 10 times as large as those of controls (mean weight 66.7 ± 10.7 vs 15.8 ± 0.8 mg, P < 0.05) and mean Prl values of
Representative electron microscopic appearance of neoplastic cells from a micro- (a) and a macroadenoma (b) of female rats 7 months after interruption of a chronic treatment with oestradiol valerate (2 mg sc, 5 times at 3-week intervals). The structure of the two tumours is comparable, cells are clear, contain a well developed endoplasmic reticulum, large mitochondria and few, small secretory granules. Magnification: 6900 X.

637 ± 107 ng/ml (P < 0.001 vs mean control values, P < 0.001 vs the first group of EV-treated rats).

Morphologic studies

By light microscopy all pituitaries of EV-treated rats resulted to be tumourous. The smaller glands contained microadenomas with a maximum diameter of 500 µm, the larger ones macroadenomas with diameters up to 3–4 mm. All neoplastic cells had comparable appearance, independently from the size of the adenoma. Cells were round or ovoid, with clear cytoplasm, large nuclei and few small secretory granules. Electron microscopy confirmed that tumour cells from micro- (Fig. 1a) and from macroadenomas (Fig. 1b) did not differ in structure. These cells were characterized by a clear cytoplasm, numerous and large mitochondria, a well developed endoplasmic reticulum and few small secretory granules (Fig. 1a,b).

No adenomatous cells could be evidenced in the pituitaries of the control rats examined.

Effect of neuroactive drugs on Prl secretion

Administration of NOM lowered significantly basal Prl levels in control rats (P < 0.05 vs placebo-treated rats at times 30, 60 and 120 min) but did not modify basal Prl levels either in rats bearing an EV-induced macroprolactinoma or in rats bearing a microprolactinoma (Fig. 2). Administration of placebo did not alter basal Prl levels in any of the three groups of rats. For the sake of clarity, the latter data have not been reported. Administration of DOM induced a striking Prl-releasing effect in control rats (P < 0.01 vs respective controls at 15, 30 and 60 min) and a similar stimulation of Prl release was present in EV-treated animals bearing microprolactinomas (P < 0.01 vs controls at 15, 30 and 60 min). In contrast with these findings, DOM
Effect of nomifensine (10 mg/kg iv) on plasma Prl levels in rats bearing micro- or macroprolactinomas and in controls. For the sake of clarity, data corresponding to placebo treatment were omitted. Data are expressed as ratio between post-treatment and baseline Prl levels (S/B). Means ± SEM are reported (number of animals in parenthesis). *P < 0.05 vs saline group (not reported).

Effect of domperidone (50 µg/kg ip) or saline on plasma Prl levels in rats bearing micro- (MI) and macroprolactinomas (MA) and in controls. Data are expressed as increments (Δ) over baseline values. Each point represents the mean ± SEM of 4–6 determinations. *P at least < 0.05 vs respective saline group.

Discussion

It is known that repeated administration of huge doses of EV at 3-week intervals is associated to the development of prolactinomas in the pituitary gland of female rats (Brawer et al. 1978; Casanueva et al. 1982). Our study shows that 7 months after EV withdrawal EV-induced tumoural changes are still present, yet size and morphologic appearance of the pituitary and plasma Prl levels allow to
distinguish between micro- and macroprolactinomas.

In all rats, irrespective of whether bearing micro- or macroprolactinomas, acute injection of NOM, an indirect-acting DA agonist which releases DA and blocks DA re-uptake into the neuron terminals (Nicholson & Turner 1977), proved to be ineffective in lowering Prl release. NOM was instead effective in control rats.

Although other interpretations may be possible (see below), the inability of NOM to lower plasma Prl in these rats very likely resides in a permanent defect in TIDA neuronal function following EV treatment. It appears unlikely that this failure might result from the inability of the lactotrophs to respond to the NOM-induced release of DA, since Prl responsiveness to the direct DA agonist bromocriptine was increased in EV-treated rats developing prolactinomas (Casanueva et al. 1982). Likewise, a compression of the hypothalmo-hypophyseal portal vasculature by the enlarged pituitary gland, resulting in reduced amount of DA reaching the lactotrophs, might account for the ineffectiveness of NOM in rats bearing macroprolactinomas, but not in those rats with microprolactinomas. However, the view that TIDA neuronal function has become deficient in all EV-treated rats and that this defect is irreversible is apparently contradicted by the different pattern of Prl secretion present after DOM and FK 33-824. In fact, failure to stimulate Prl release after administration of these compounds was present only in rats with macroprolactinomas; in those with microprolactinomas both compounds were effective in stimulating Prl release, not differently than in controls. DOM, a DA receptor antagonist, does not cross the BBB (Reyntjens et al. 1978) and increases Prl secretion by blocking pituitary DA receptors (Cocchi et al. 1980). Hence, stimulation of Prl release by DOM, as well as by other DA receptor antagonists, requires physiologic modulation of Prl-secreting cells by DA delivered through the hypophyseal portal circulation (Gudelsky & Porter 1980). The ability of opioid peptides to increase Prl secretion is believed to be mediated via inhibition of the TIDA system (Gudelsky & Porter 1979), though the involvement of serotonin (5-HT) pathways has also been advocated (Koenig et al. 1979; Spampinato et al. 1979).

The effectiveness of these compounds to raise plasma Prl in rats with microprolactinomas would then denote preserved or restored TIDA neuronal function in these animals.

Another possibility would be a partial defect in TIDA neuronal function coupled perhaps to hypersensitive pituitary DA receptors; such combination may explain the only slightly elevated baseline Prl levels. This would account for DOM's and FK 33-824's ability to elicit a plasma Prl rise in these rats, without denying for the opioid peptide an additional mechanism, mediated by a serotonergic pathway (Koenig et al. 1979; Spampinato et al. 1979) and, possibly, a Prl-releasing factor. Interestingly, an increased Prl-releasing activity has been found in the hypothalamus of rats bearing EV-induced prolactinomas 35 and 200 days after the last oestrogen injection (Nakagawa et al. 1980).

In rats with macroprolactinomas no TIDA neuronal function would be spared from destruction and hence no Prl response to DOM and FK 33-824 elicited. In these rats, the direct neurotoxic effect of oestrogens on DA producing neurons of the TIDA system (Sarkar et al. 1984a) would be enhanced by the disrupting effect of constantly elevated Prl levels. In this context, Sarkar et al. (1984b) have clearly demonstrated that chronic elevation of plasma Prl levels, in the absence of any rise in oestrogen levels, produces a decline in TIDA function. Viewed in this frame NOM appears to be a more subtle probe of TIDA function than DOM and FK 33-824, since it would unravel more discrete alterations in the TIDA system. Superiority of NOM over DOM and FK 33-824 as probe of TIDA function had already been reported (Peñalva et al. 1984). In experimental models of hyperprolactinaemia of rats both DOM and FK 33-824 were unable to functionally differentiate (e.g. by Prl response) between a state of TIDA overactivity and a state of TIDA hypoactivity, a task accomplished only by NOM (Peñalva et al. 1984).

In conclusion, the present results indicate that in rats bearing EV-induced macroprolactinomas TIDA neuronal function is still highly impaired 7 months after EV withdrawal, as judged by Prl unresponsiveness to all the neuropharmacologic probes which were used. In rats bearing microprolactinomas, the impairment of TIDA function would be of lesser extent, and was revealed only by one of the three neuroendocrine probes employed. Whether the last condition results from an initially less severe lesion following EV treatment or reflects instead a transient state of restoration towards normality cannot be elucidated by our studies.
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