In-vivo and in-vitro effects of domperidone on the release of prolactin and LH in male and female rats

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Summary. Domperidone at a dose of 4 mg/kg significantly raised circulating prolactin levels in male and female rats although this increase was approximately 4-fold higher in females compared with males. No effect on LH secretion was observed. The LH and prolactin responses to Gn-RH and TRH respectively were investigated with isolated perfused pituitary glands obtained from domperidone-pretreated animals. For females, the basal release of prolactin was raised by domperidone pretreatment and the responsiveness of the pituitary to Gn-RH was markedly attenuated. When domperidone was added to the perfusing media, high concentrations (100 µg/ml) reduced both the basal and TRH-stimulated prolactin secretion from pituitaries of untreated male and female rats although lower doses (1 µg/ml and 10 ng/ml) were ineffective in altering prolactin release. The pituitary LH responses to Gn-RH were similarly reduced by the presence of 100 µg domperidone/ml in the medium.

Introduction

It is now generally accepted that dopamine released from hypothalamic neurosecretory cells reaches the anterior pituitary gland via the hypophyseal portal vessels and exerts a tonic inhibition on prolactin secretion (Macleod, 1976). Dopamine-receptor blocking drugs will therefore increase the release of prolactin by effectively removing this inhibition (Thorner & Besser, 1978). In-vitro studies on isolated pituitary tissue have confirmed that dopamine antagonists can block the effects of dopamine on prolactin secretion although at high concentrations these drugs can inhibit the release of prolactin in the absence of dopamine (MacLeod & Lamberts, 1978; Caron *et al.*, 1978). The mechanism of this paradoxical effect is unknown.

The discovery that the anti-emetic drug, domperidone, is a potent dopamine antagonist which does not readily cross the blood–brain barrier (Laudron & Leysen, 1979) stimulated interest in its effects on prolactin secretion (Cocchi *et al.*, 1980; Kato *et al.*, 1980). We have used domperidone to induce hyperprolactinaemia in female rats and attempted to characterize *in vitro* and *in vivo* the actions of this drug. Initial experiments were carried out to determine whether the presence of different concentrations of domperidone in the perfusing media of isolated pituitary glands could alter the basal and TRH (thyrotrophin-releasing hormone)-stimulated release of prolactin. Subsequent observations were made on the secretion of prolactin and LH from perfused pituitaries obtained from rats pretreated with domperidone. They include the effects of the drug on the pituitary responsiveness to both TRH and gonadotrophin-releasing hormone (Gn-RH).

Materials and Methods

Adult male (180–200 g) and female (200–250 g) Porton–Wistar rats were maintained under controlled lighting (lights on 06:00–18:00 h) and temperature (20°C) and had free access to food and water. Daily vaginal smears were taken and only those females which exhibited at least 2 complete 4-day oestrous cycles were used. Animals pretreated with domperidone (Janssen, Marlow, Bucks.), 4 mg/kg, were injected subcutaneously at 11:00 h on the day preceding the experiment and again at 09:00 h on the day of the experiment. Female rats received their first injection of domperidone on dioestrus day 2 so that pituitary sensitivity to hypothalamic releasing hormones was always tested on the day of pro-oestrus. Control animals were injected with the same volume (0.2 ml) of saline (9 g NaCl/l) alone. All rats were decapitated between 11:00 and 11:30 h, and trunk blood was collected for subsequent hormone assay. The pituitary glands were excised from the sella turcica and, after removal of the neurohypophysis, the anterior lobe was bisected and both hemipituitaries were placed in a single perfusion chamber.

The chambers, with a total volume of 200 µl, were perfused with Krebs–Ringer–bicarbonate (0.236 M-NaCl, 0.5 M-NaHCO₃, 0.475 M-KCl, 0.118 M-MgSO₄·7H₂O, 0.118 M-KH₂PO₄, 0.252 M-CaCl₂·H₂O) solution containing glucose (2 g/l) and bovine serum albumin (2.5 g/l) (KRBG). The perfusate, which was gassed with 95% O₂ and 5% CO₂ throughout the experiment, was delivered to the tissue at the rate of 0.2 or 0.4 ml/min by a peristaltic pump (Desaga, Uniscience Ltd, Cambridge). The perfusion chambers were suspended in a water-filled container which was maintained at 37°C and constantly gassed with 95% O₂ and 5% CO₂. The KRBG was delivered to the tissue in silicone tubing which passed through the container before entering the chambers. This ensured that the perfusate was fully saturated with O₂ and CO₂ and was at the same temperature as the pituitary glands. In all experiments the hemipituitaries were perfused for an initial period of 2 h and subsequently 5- or 10-min perfusate samples were collected on a fraction collector (Ultrorac 2070, LKB, Croydon, Surrey) for a further 2-h period.

The direct effects of domperidone on the release of prolactin and LH *in vitro* was tested by perfusing anterior pituitary glands for untreated rats with KRBG containing the drug at a concentration of 10 ng/ml, 1 µg/ml or 100 µg/ml. Anterior pituitary glands of male rats were challenged with two 5-min perfusate pulses of TRH (Calbiochem, Bishop's Stortford, Herts) at a concentration of 100 ng/ml in KRBG while experiments on female pituitaries involved 2 similar pulses but with 10 ng synthetic Gn-RH [(Pyro)Glu-His-Tri-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂] (Hoechst AG, 6230 Frankfurt)/ml. For all observations an experimental gland was paired with a control.

Radioimmunoassay of prolactin and LH. Prolactin and LH were measured by a double-antibody radioimmunoassay using material and protocols supplied by the NIAMDD Rat Pituitary Hormone Distribution Program. The results are expressed in terms of NIAMDD-rat-PRL-RP1 and rat/LH-RP1 and the sensitivities of the assays were 1 ng prolactin/ml and 10 ng LH/ml. The inter- and intra-assay variations were calculated to be 10.1 and 8.9% for prolactin and 10.0 and 9.0% for LH.

Analyses of results. The pituitary sensitivity to TRH and Gn-RH was taken to be the difference in the mean concentration of prolactin and LH respectively between the peak value directly following the pulse and the value preceding the peak. Significance between the experimental and control groups was determined by an unpaired Student's *t* test. The same statistical test was employed to determine significant differences in the mean basal release of prolactin between groups of observations.

Results

In-vitro effects of domperidone on prolactin and LH secretion

When pituitary glands from untreated male rats were perfused with 100 µg domperidone/ml throughout the experiment there was a significant reduction in the basal secretion of prolactin (*P*

Text-fig. 1. Release of prolactin from perfused male rat pituitary glands and the responses to 5-min 'pulses' of 10 ng TRH/ml when domperidone is included in the perfusing medium at concentrations of (a) 100 µg/ml, (b) 1 µg/ml and (c) 10 ng/ml. Values are mean $\pm$ s.e.m. for 6 observations. The asterisks (*) indicate a significant reduction ($P < 0.05$) in the increment of TRH-stimulated prolactin secretion.

< 0.05) and the responses to TRH were markedly attenuated ($P < 0.05$). Domperidone doses of 1 $\mu\text{g}/\text{ml}$ and 10 ng/ml in the media had no significant effect on basal prolactin release or on the responses to the first pulse of TRH although in both cases the basal levels of prolactin were lower in the presence of domperidone. These doses of the drug appeared to prevent the reduction of TRH-stimulated prolactin release usually observed with the second pulse of the releasing hormone. There was a significantly greater response ($P < 0.05$) to the second TRH challenge by

Text-fig. 2. Comparison of the effects of 4 mg domperidone/kg pretreatment with 100 μg domperidone/ml in the incubating medium on (a) prolactin and (b) LH release from isolated pituitary glands of female rats. Values are mean $\pm$ s.e.m. for 6 observations. In (a) the summated mean levels of release in both experimental groups ($\bullet$, $\blacksquare$) are significantly different ($P < 0.005$) from the control values ($\circ$, $\square$). In (b) the summated mean basal release of LH is significantly higher ($P < 0.005$) in the domperidone-incubated group ($\bullet$) compared with the paired controls ($\circ$). The magnitude of the response to Gn-RH (difference between peak concentration and the preceding basal level) are indicated by letters and significant differences were found between responses a and b ($P < 0.005$) and c and d ($P < 0.01$).

pituitaries perfused with 1 µg domperidone/ml compared to the control values which showed the typical reduced response. These results are summarized in Text-fig. 1.

Similar experiments were not carried out with anterior pituitaries of female rats because the TRH responses in this sex were inconsistent and frequently absent. However, the effect of 100 µg domperidone/ml is shown in Text-fig. 2(a). There was again a significant reduction in the basal secretion of prolactin compared with that of the paired controls ($P < 0.005$). Conversely the same concentration of domperidone markedly increased the release of LH ($P < 0.005$) although the pituitary response to Gn-RH was virtually abolished (Text-fig. 2b).

In-vivo treatment of male and female rats with domperidone

Two injections of domperidone (4 mg/kg) administered 24 and 2 h before blood sampling significantly raised circulating levels of prolactin in both sexes ($P < 0.005$), although female rats showed a far greater response. In males the mean $\pm$ s.e.m. prolactin concentration was 203 ± 14 ng/ml compared with 56 ± 11 ng/ml in the saline-injected controls whereas the corresponding figures in females were 982 ± 99 compared with 58 ± 12 ng/ml. Plasma LH levels were not altered by the domperidone treatment.

In-vitro release of prolactin and LH from pituitaries of domperidone-pretreated male and female rats

Pretreatment of male rats with domperidone had no effect on either the basal or TRH-stimulated release of prolactin from perfused pituitary glands, despite the fact the circulating prolactin levels were raised before death (Text-fig. 3). However, in the female rats made hyperprolactinaemic by domperidone treatment, there were marked differences in the

Text-fig. 3. Release of prolactin from perfused pituitary glands of male rats. Treatment with domperidone had no significant effect on the mean basal or TRH-stimulated release of prolactin. Values are mean $\pm$ s.e.m. for 6 observations.

release of prolactin from their isolated pituitary glands—the mean basal release of prolactin was significantly higher ($P < 0.005$) compared with that of the controls (Text-fig. 2a).

Text-figure 2(b) shows the effects of domperidone pretreatment on the responses of pituitaries from female rats to pulses of Gn-RH. Comparable to the *in-vitro* effects of the drug, pituitary responsiveness was attenuated ($P < 0.01$) although there was no significant difference on the basal release of LH.

Discussion

The observation that high concentrations of domperidone *in vitro* can inhibit the release of prolactin by a direct action on the pituitary gland is in agreement with a recent study by Besser, Delitala, Grossman, Stubbs & Yeo (1980) on dispersed rat anterior pituitary cell columns. Such an effect may be due to agonistic properties of the drug which only become apparent at high doses. However, the experiments also showed that the TRH-stimulated release was also suppressed which may indicate some non-specific action of domperidone. Deneff, Van Neuten, Leysen & Janssen (1979) have suggested that high concentrations of pimozide, a neuroleptic structurally related to domperidone, may interfere with a Ca^{2+} -dependent mechanism of prolactin secretion. Although this would be a plausible explanation for the paradoxical inhibition of prolactin release by dopamine antagonists, our results, which demonstrate that domperidone can inhibit prolactin secretion but stimulate LH release in the same preparation, cast some doubt on this proposal since LH secretion is also Ca^{2+} -dependent.

The degree of hyperprolactinaemia induced by domperidone treatment was far greater in females than in males, the difference being in the order of 800 ng/ml. Similarly, the release of prolactin from their perfused pituitary glands was significantly higher in the pretreated females compared with the control groups whereas no differences were observed between hyperprolactinaemic and control male rats. Oestrogen increases prolactin secretion (MacLeod, 1976) and probably stimulates prolactin synthesis primarily and its release only secondarily (Nagy, Valdenegro & MacLeod, 1980). Thus the higher levels of prolactin secretion from female pituitaries may be due to a larger releasable pool of prolactin induced by the presence of oestrogen.

Domperidone pretreatment of female rats or its presence in the perfusing media inhibited the pituitary LH response to Gn-RH. Similar *in-vivo* observations have been made for female rats treated with perphenazine (Vasquez, Nozian & Mahesh, 1980) and pimozide and haloperidol have the same effects as those reported here for domperidone (D. A. Carter & S. A. Whitehead, unpublished observations). The reduced pituitary responsiveness to Gn-RH following domperidone treatment may be the result of an indirect effect of the elevated prolactin levels on the hypothalamic–pituitary axis (Gudelsky & Porter, 1980) or a direct action of the drug on the dopaminergic control of Gn-RH secretion (Sarkar & Fink, 1981) which could alter the pituitary sensitivity. The latter possibility is unlikely since domperidone treatment did not alter circulating levels of LH. On the other hand, McNeilly (1980) has suggested that high prolactin levels may increase the sensitivity of the hypothalamic–pituitary axis to the negative feedback effects of ovarian steroids and this could account for the diminished pituitary responses.

Since the presence of domperidone in the perfusate also attenuated the LH responses to Gn-RH, a direct effect of the drug cannot be discounted although it is unlikely that such high concentrations of the drug were reaching the pituitary *in vivo*.

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