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CHLORPROMAZINE, HALOPERIDOL, METOCLOPRAMIDE AND DOMPERIDONE RELEASE PROLACTIN THROUGH DOPAMINE ANTAGONISM AT LOW CONCENTRATIONS BUT PARADOXICALLY INHIBIT PROLACTIN RELEASE AT HIGH CONCENTRATIONS

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1 The effects of chlorpromazine, haloperidol, metoclopramide and domperidone on the release of prolactin from perfused columns of dispersed rat anterior pituitary cells were studied.

2 Chlorpromazine, haloperidol, metoclopramide and domperidone antagonized the dopamine-mediated inhibition of prolactin release at low concentrations.

3 Each dopamine antagonist displaced the dose-response curve for dopamine-induced suppression of prolactin release to the right in a parallel manner.

4 At higher concentrations, the four drugs became less effective as dopamine antagonists.

5 At high concentrations in the absence of dopamine, chlorpromazine, haloperidol, metoclopramide and domperidone paradoxically suppressed prolactin secretion by an unknown mechanism.

Introduction

Many types of neuroleptic agents, including the phenothiazines, butyrophenones and piperidine derivatives, increase prolactin secretion *in vivo* (Thorner & Besser, 1978). This property is also shown by some non-neuroleptic drugs, such as the substituted benzamides (McNeilly, Thorner, Volans & Besser, 1974). Although it has been suggested that these agents may affect prolactin secretion by actions at the hypothalamic level (Danon, Dikstein & Sulman, 1963; Kleinberg, Noel & Frantz, 1971; 1977), it is more probable that they increase prolactin by an action directly on the pituitary. Several of these drugs have been shown to block the inhibitory action of dopamine and dopamine agonists on prolactin secretion by pituitary cells *in vitro* (Macleod & Lehmeyer, 1974; Yeo, Thorner, Jones, Lowry & Besser, 1979), and binding studies have suggested that these drugs may act as antagonists at dopamine receptors on these cells (Creese, Burt & Snyder, 1975; 1976; Calabro & Macleod, 1978; Caron, Beaulieu, Raymond, Gagne, Drovin, Lefkowitz & Labrie, 1978). In this study, we have investigated the *in vitro* dose-response relationships of four drugs that are known prolactin secretagogues *in vivo*: chlorpromazine, a phenothiazine; haloperidol, a butyrophenone; metoclopramide, a substituted benzamide; and domperidone, a new anti-emetic structurally related to pimozide (Swann, Thompson & Qureshi, 1979). We have demonstrated that all four appear to act as competitive antagonists to dopamine at low concentrations, but paradoxically reduce prolactin secretion in the absence of dopamine when administered at high concentration.

Methods

Dispersed rat anterior pituitary cell columns

The preparation of columns of dispersed rat anterior pituitary cells supported on a matrix of BioGel P2 has been previously described in full (Yeo *et al.*, 1979). In brief, anterior pituitaries from five or six female Wistar rats (200 to 230 g) were dispersed in a solution of trypsin (2.5 g/l) in Earle's balanced salt solution containing dopamine (5 μmol/l). Dispersed cells were recovered by centrifugation, resuspended and filtered through 100 μm nylon gauze. The cells were mixed with preswollen BioGel P2 (200 to 400 mesh) and packed in a column constructed from a 2 ml plastic syringe (Gillette Surgical) with a needle placed through the septum. The columns were perfused with Earle's balanced salt solution containing 2.5 g/l bovine serum albumin (Armour) and penicillin (25 u/l) and streptomycin (25 mg/l) at a flow rate of 0.5 ml/min. Dopamine and other test substances, in saline, were mixed with the medium in a ratio of 1:9

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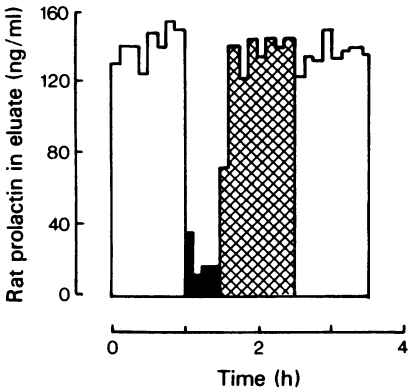

Figure 1 Prolactin concentrations in the eluate of a rat anterior pituitary cell column perfused with medium containing dopamine 5 μM (30 min, solid column) followed by dopamine 5 μM plus domperidone 100 nM (60 min, cross hatched column), open columns: perfusion with saline.

just before perfusing the column. Eluate fractions of approximately 4 ml were collected at 7.5 min intervals.

Dopamine (5-hydroxytyramine hydrochloride, Sigma), metoclopramide (Beecham Research Laboratories) and chlorpromazine (as the hydrochloride, May and Baker) were dissolved in 0.9% w/v NaCl solution (saline) containing ascorbic acid 50 μg/ml as antioxidant. Haloperidol (Searle) was dissolved in 1 ml absolute ethanol and then diluted in tartaric acid (5 mM) to give a 1 mM solution. Domperidone (Janssen) was obtained predissolved in dilute acetic acid in ampoules 5 mg/ml. Rat prolactin concentrations in the column eluate were determined by radioimmunoassay using reagents supplied by Dr A.F. Parlow and NIAMDD.

Dose-response relationships

Three experiments were performed for each drug:— *Antagonism of dopamine (5 μM).* A column was perfused with medium to which was added in sequence: saline (60 min); dopamine, 5 μM (30 min); dopamine 5 μM plus test concentration of drug (60 min); and saline (60 min). Figure 1 illustrates the experimental design. The dose range tested was as follows: chlorpromazine, 1 nM to 10 μM; haloperidol, 10 pM to 1 μM; metoclopramide, 1 nM to 100 μM; and domperidone 1 nM to 10 μM. The ability of the drug to antagonize the dopamine-induced suppression of prolactin se-

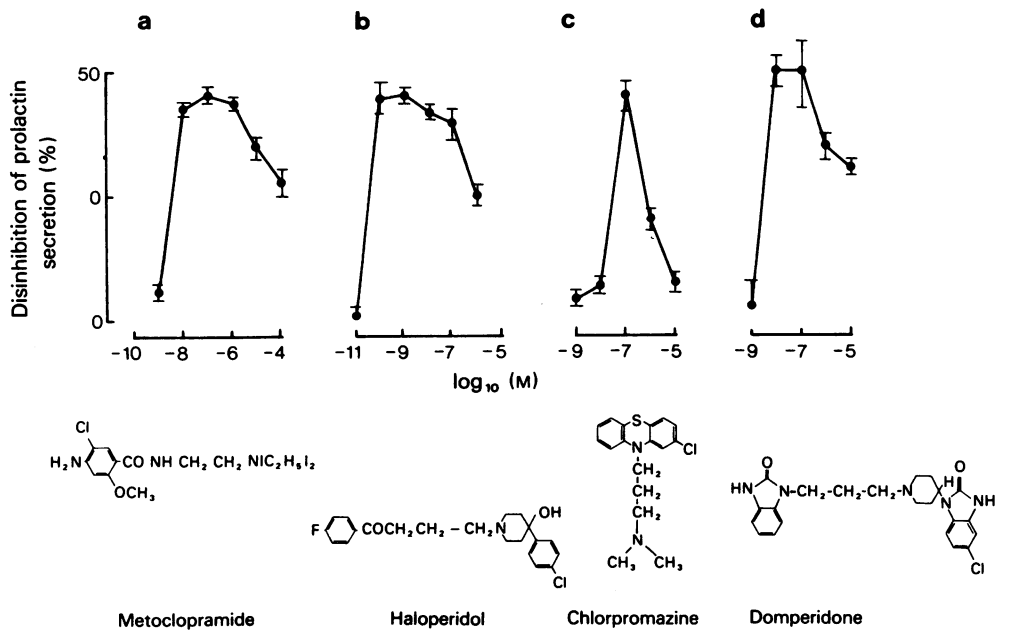

Figure 2 Log dose-response curves of the ability of metoclopramide (a), haloperidol (b), chlorpromazine (c) and domperidone (d) to reverse the suppression of prolactin secretion induced by 5 μmol/l dopamine. Each point represents a separate experiment, similar to that illustrated in Figure 1. The prolactin concentrations in each of the last 6 fractions during perfusion with dopamine plus antagonist are expressed as a percentage of the mean concentration when unsuppressed. The values given are the mean; vertical lines show s.d. A value of 100% would indicate that the dopamine-induced suppression of prolactin had been completely overcome.

cretion is given by the extent to which secretion returned to the unsuppressed basal level. For statistical analysis, prolactin levels in the last 6 fractions during perfusion with dopamine plus drug were compared with the mean level in the last 6 fractions during saline alone, and the result expressed as a percentage.

Effect on dose-response curve for dopamine. Dose-response curves for the suppression by dopamine of prolactin secretion were established in the presence and absence of each of the 4 antagonist compounds. The concentration of each compound used was the minimum dose found to produce potent dopamine antagonism (greater than 80%) as determined in the experiment described above.

Suppression of prolactin secretion by drug alone. The column was perfused with medium alone (60 min), medium plus chlorpromazine, haloperidol, metoclopramide or domperidone (60 min), and then medium alone (60 min). The last 6 fractions during drug administration were compared to the last 6 fractions during saline, and the secretion of prolactin expressed as a percentage of the mean during saline administration.

Results

The ability of the four compounds to antagonize the dopamine-induced inhibition of prolactin secretion is shown in Figure 2. Each compound gave good dopamine antagonism. Haloperidol was effective at 100 pM, metoclopramide and domperidone at 10 nM, and chlorpromazine at 100 nM. At high concentrations all compounds became less effective as dopamine antagonists. Figure 3 demonstrates that the dopamine dose-response curves were displaced in a parallel manner to the right in the presence of the lowest effective concentration of each compound as determined in the previous experiment.

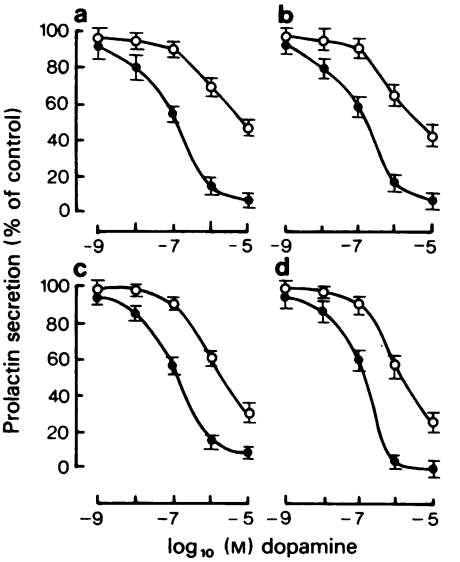

Figure 3 Effect of haloperidol (100 pM) (a), chlorpromazine (100 nM) (b), metoclopramide (10 nM) (c) and domperidone (10 nM) (d) on the log dose-response curve for dopamine suppression of prolactin secretion *in vitro*: (●) effect of dopamine alone; (○) effect of dopamine plus antagonist. Each point represents the mean of 3 separate experiments; vertical lines indicate s.d.

Dose-response curves for the inhibition of prolactin release by perfusion with the compound alone are given for each compound in Figure 4. It is evident that, whereas at low concentrations prolactin secretion is not affected, at high concentrations (at or

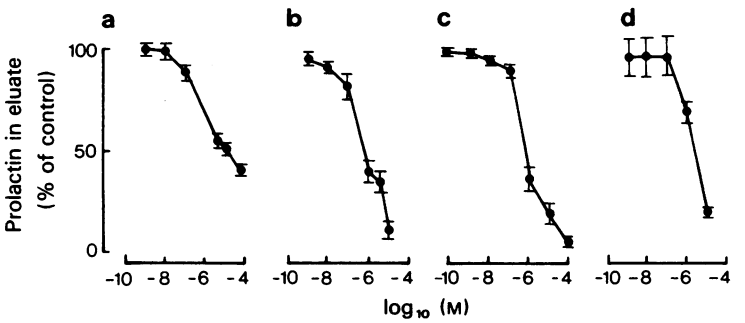

Figure 4 Log dose-response curves for the suppression of prolactin secretion from dispersed rat pituitary cell columns by metoclopramide (a), haloperidol (b), chlorpromazine (c) and domperidone (d) in the absence of dopamine. The prolactin concentrations in the last 6 fractions during drug administration are expressed as a percentage of the mean secretion during perfusion with medium to which saline alone was added. Each point represents the mean; vertical lines indicate s.d.

above 1 μM) each compound significantly decreased the release of prolactin.

Discussion

The administration of certain neuroleptics and related drugs *in vivo* produces hyperprolactinaemia (Thorner & Besser, 1978), and this has been attributed to their blockade of dopamine-receptors in the central nervous system. Dopamine has been demonstrated to be present at high concentrations in the hypothalamus (Fuxe & Hökfelt, 1966; Takahara, Arimura & Schally, 1974), and thus a hypothalamic site of action has been suggested for prolactin secretagogues with antidopaminergic activity (Danon *et al.*, 1963; Kleinberg *et al.*, 1971). However, it has been shown that perphenazine, haloperidol and pimozide could overcome the inhibitory action of dopamine directly on the pituitary (Macleod & Lehmeyer, 1974; Smalstig, Sawyer & Clemens, 1974; Caron *et al.*, 1978), and this was also shown for several neuroleptic agents and domperidone by Denef & Follebouckt (1978). Our data confirm that chlorpromazine, haloperidol, metoclopramide and domperidone all act directly on the pituitary at low concentrations to antagonize the dopamine inhibition of prolactin release. Removal of these agents allows dopamine rapidly to suppress prolactin secretion again. Furthermore, they demonstrate a parallel shift of the dopamine dose-response curves. The data suggest that these agents act as competitive antagonists at dopamine receptors. Binding studies are in keeping with these conclusions (Creese *et al.*, 1975; 1976; Calabro & Macleod, 1978; Caron *et al.*, 1978). Since dopamine has been demonstrated in the hypophyseal portal blood at a concentration that is effective in suppressing pituitary lactotrophs (Ben-Jonathan, Oliver, Weiner, Mical & Porter, 1977), it appears likely that dopamine acts at the pituitary level to inhibit prolactin secretion *in vivo*. It is therefore probable that dopamine antagonists also act at the pituitary level *in vivo* to produce hyperprolactinaemia. Their clinical value in differentiating between pituitary and hypothalamic causes of hyperprolactinaemia (Kleinberg *et al.*, 1971; 1977) is therefore dubious.

In addition, our data show that each of the four drugs investigated suppresses prolactin release in the absence of dopamine, when used at high concentrations. This was previously shown for pimozide and haloperidol by MacLeod & Lamberts (1978) and Caron *et al.* (1978) in static systems, but Denef & Follebouckt (1978) investigated 8 neuroleptics and related compounds and failed to demonstrate prolactin suppression by any of them except pimozide. They argued that the effect was independent of dopamine antagonist activity, and was therefore either related to a separate receptor system or was non-specific. The data presented here suggest that dopamine antagonists, other than haloperidol and pimozide and regardless of their specific chemical class, will suppress prolactin release if used at a sufficiently high concentration. There is evidence that a variety of compounds may suppress the release of prolactin, and that for some of these the effect may not be mediated by dopamine receptors: such compounds include cyproheptadine (Lamberts & MacLeod, 1978), γ-aminobutyric acid GABA (Lamberts & MacLeod, 1978; Besser, Delitala, Grossman & Yeo, 1979; Enjalbert, Ruberg, Arancibia, Fiore, Priam & Kordon, 1979; Grandison & Guidotti, 1979), and possibly metergoline (Besser, Delitala, Grossman & Yeo, 1980). Mechanisms other than these related to pituitary dopamine receptors could, therefore, be involved in the paradoxical inhibition of prolactin induced, *in vitro*, by neuroleptics and other dopamine antagonists. These could include a non-specific effect of the drug on the plasma membrane, or a drug-related action on the Ca^{2+}-dependent mechanism of the secretory process for prolactin. This mechanism has been recently suggested for pimozide (Denef, Van Neuten, Leysen & Janssen, 1979).

In conclusion, we have shown that chlorpromazine, haloperidol, metoclopramide and domperidone can act directly on the pituitary to raise prolactin, and that their mode of action is compatible with their acting as dopamine antagonists at low concentrations. In addition, all 4 drugs act at high concentrations to suppress directly pituitary prolactin secretion by an as yet undefined mechanism. Recent data indicate that haloperidol *in vivo* may initially increase prolactin secretion, but that there may then be a fall in the serum prolactin if the haloperidol administration continues (Langer, 1979). It is intriguing to speculate that this fall may, at least in part, be due to the intrinsic prolactin-suppressing action of high concentrations of haloperidol.

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