Br. J. Pharmac. (1980), **70**, 5–7

METERGOLINE AND CYPROHEPTADINE SUPPRESS PROLACTIN RELEASE BY A NON-5-HYDROXYTRYPTAMINERGIC, NON-DOPAMINERGIC MECHANISM

G.M. BESSER, G. DELITALA, A. GROSSMAN & T. YEO

Department of Endocrinology, St. Bartholomew's Hospital, West Smithfield, London, EC1A 7BE

A dispersed rat anterior pituitary cell system has been used to investigate the effects of cyproheptadine and metergoline on prolactin secretion. Both drugs were potent inhibitors of prolactin secretion. However, the inhibition was not antagonized by either 5-hydroxytryptamine or a variety of dopamine antagonists. We conclude that both drugs act through mechanisms that are neither 5-hydroxytryptaminergic nor dopaminergic.

Introduction Cyproheptadine, a phenothiazine derivative, and metergoline, an ergot alkaloid, have potent anti-5-hydroxytryptamine activity and have been used by many workers to investigate the role of 5-hydroxytryptamine (5-HT) in the control of anterior pituitary hormones. However, the suppression of prolactin and growth hormone secretion in man by metergoline (Delitala, Masala, Alagna, Devilla & Lotti, 1976) and the inhibition of dihydroergocryptine binding in rat pituitaries by cyproheptadine (Caron, Beaulieu, Raymond, Gagne, Drouin, Lefkowitz & Labrie, 1978) have been taken to indicate that both agents may also be dopamine agonists. We present data showing that cyproheptadine and methergoline readily and reversibly suppress the release of prolactin from dispersed rat anterior pituitary cells and that this effect is not antagonized by either 5-HT or a variety of dopamine antagonists. These drugs appear to have additional activities independent of 5-HT antagonism or dopamine agonism.

Methods Columns of rat anterior pituitary cells were prepared and run as previously described by Besser, Delitala, Grossman, Stubbs & Yeo (1980). Anterior pituitaries from five female Wistar rats (200 to 230 g) were dispersed in a solution of trypsin (2 g/l) in Earle's balanced salt solution containing dopamine (5 μmol/l). Cells from four harvests were recovered by centrifugation, mixed with preswollen BioGel P2 and packed into a column constructed from a 2 ml disposable syringe (Gillette Surgical). The column was perfused at a flow rate of 0.5 mlzmin with Earle's balanced salts solution containing bovine serum albumin (2.5 g/l), penicillin (25 μ/l) and streptomycin (25 mg/l) and equilibrated with 95% O_2/5% CO_2. The drugs to be tested, in saline, were mixed with perfusing medium in a ratio of 1:9 (v/v) just before reaching the column. Fractions of 7.5 min (approximately 4 ml) were collected and the rat prolactin concentrations were determined by radioimmunoassay using reagents supplied by Dr A. F. Parlow and NIAMMD.

Metergoline (Montedison, Italy) was dissolved in hot tartaric acid solution (1 mmol/l) to give a 1 mmol/l solution. Cyproheptadine (MSD, Hoddesdon, U.K.) was dissolved in water. Subsequent dilutions were made with saline (0.9% w/v NaCl solution).

Results The ability of metergoline and cyproheptadine to suppress the secretion of prolactin from dispersed pituitary cells is shown in Figure 1. We have found that both drugs suppress prolactin secretion in a dose-related fashion at concentrations between 10 nmol/l and 10 μmol/l, the suppression occurring within 30 min for both drugs. When the drug was removed from the perfusion medium, prolactin secretion was restored within 60 min.

We have attempted to demonstrate antagonism of the prolactin-suppressing actions of cyproheptadine and metergoline by 5-HT and three dopamine antagonists, haloperidol, metoclopramide and perphenazine. 5-HT (up to 10 μmol/l) did not antagonize either drug at concentrations between 10 nmol/l and 100 nmol/l. Metoclopramide (10 nmol/l to 1 μmol/l) did not antagonize the action of metergoline (10 nmol/l) to 5 μmol/l) or cyproheptadine (100 nmol/l to 1 μmol/l). Neither haloperidol (100 pmol/l to 10 μmol/l) nor perphenazine (10 nmol/l to 100 nmol/l) antagonized the action of metergoline. Examples of our experiments are shown in Figure 1 (a, b, c). In contrast, the prolactin-suppressing effects of dopamine (5 μmol/l) were rapidly reversed by these dopamine antagonists at the concentrations discussed above (e.g. haloperidol, 100 nmol/l, Figure 1d).

Discussion The results presented here demonstrate that cyproheptadine and metergoline suppress prolactin secretion from dispersed pituitary cells. This effect was produced by concentrations as low as 10 nmol/l, was of rapid onset and the secretion of prolactin returned when the drugs were removed. The

0007-1188/80/090005-03 $01.00

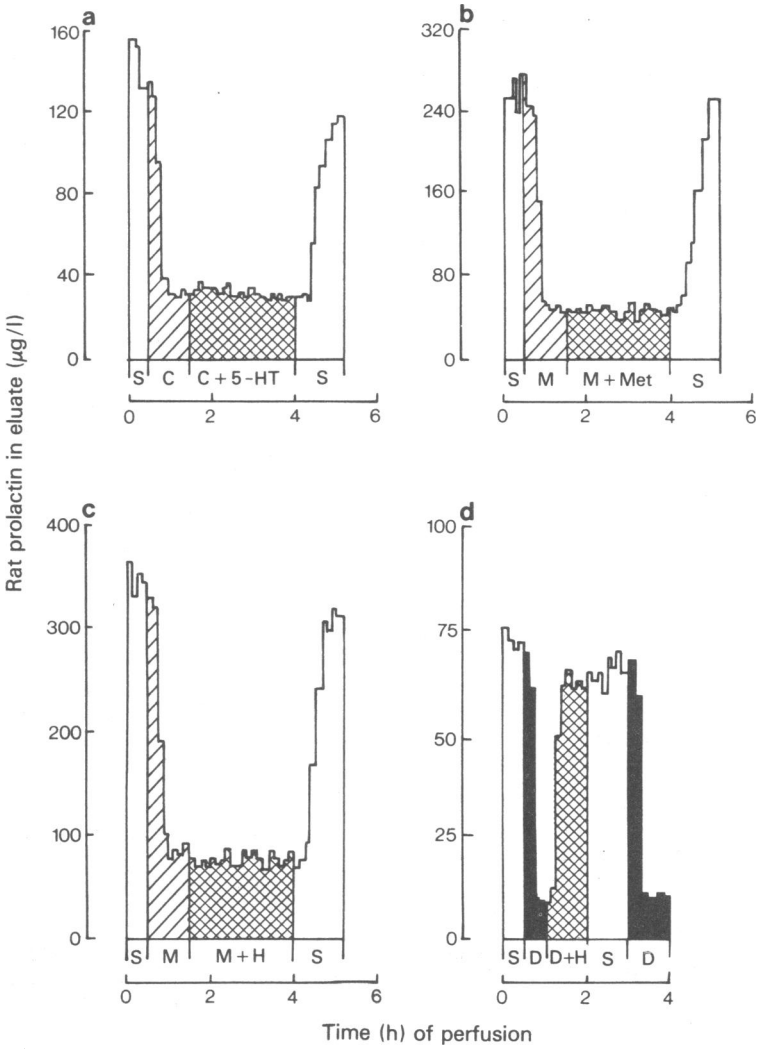

Figure 1 The effects of 5-hydroxytryptamine (5-HT), metoclopramide and haloperidol on the inhibition of prolactin release from dispersed pituitary cell columns produced by cyproheptadine, metergoline and dopamine: (a) shows the inability of 5-HT (10 μmol/l) to antagonize the prolactin lowering action of cyproheptadine (C, 100 nmol/l); (b) and (c) show a similar lack of antagonism of metoclopramide (Met, 10 nmol/l) and haloperidol (H, 10 nmol/l) respectively on the action of metergoline (M, 1 μmol/l); (d) shows that haloperidol (100 nmol/l) antagonized the action of dopamine (D, solid column, 5 μmol/l). Open columns (S) represent the action of 0.9% saline alone to the perfusing medium; hatched columns represent C or M; cross hatched columns represent the addition of two compounds.

action of both drugs was not blocked by 5-HT, suggesting that these agents were not acting in this instance through an anti-5-hydroxytryptaminergic mechanism. 5-HT itself has previously been shown to have no direct effect on pituitary prolactin secretion (Lamberts & MacLeod, 1978).

We were unable to demonstrate antagonism of the inhibition of prolactin release by cyproheptadine with metoclopramide, a dopamine antagonist believed to be specific for the dopamine$_2$ (DA_2) receptor (Jenner & Marsden, 1979). This agrees with the results of Lamberts & MacLeod (1978), who were unable to block the effect of cyproheptadine with haloperidol, which is believed to block both DA_1 and DA_2 receptors (Kebabian & Calne, 1979). Since histamine has been shown to have no direct effect on pituitary prolactin secretion (Rivier & Vale, 1977), the action of cyproheptadine is unlikely to be mediated by histaminergic mechanisms.

The blockade of prolactin release by metergoline

was not altered by the addition of metoclopramide, haloperidol or perphenazine. These agents readily antagonize the action of dopamine in this system at all concentrations used in these experiments. We have shown that other putative dopamine agonists such as bromocriptine, can also be antagonized by these agents in our system although rather high concentrations may be required (Delitala, Yeo, Grossman, Hathway & Besser, 1980). At low concentrations, in the absence of other drugs, metoclopramide, haloperidol and perphenazine have little effect on prolactin secretion. Paradoxically at higher concentrations (>100 nmol/l) also in the absence of other drugs, they suppress prolactin secretion (Besser *et al.*, 1980). Since addition of these dopamine antagonists did not overcome the suppression of prolactin release produced by metergoline, it appears unlikely that metergoline acts through a dopaminergic mechanism. This is in contrast to a report by MacLeod & Lamberts (1979) that the action of metergoline (1 μmol/l) could be antagonized by 10 nmol/l haloperidol. Furthermore, in another study the ability of metergoline to suppress prolactin secretion *in vivo* was apparently blocked by the dopamine antagonist pimozide (Cocchi, Locatelli, Carminati & Muller, 1978). However, the variation in baseline prolactin in the latter study made the changes difficult to interpret, and it has been argued that pimozide has a direct action on the entrance of calcium into the cell which is not shown by other dopamine antagonists. Our data suggest that the effectiveness of metergoline *in vivo* in lowering prolactin levels (Delitala *et al.*, 1976) may not be of a true dopaminergic mechanism and is independent of 5-HT receptors at least at the pituitary level. Since cyproheptadine and metergoline suppress prolactin by a mechanism that appears to be neither 5-hydroxytryptaminergic nor dopaminergic, this must be taken into account in any investigation using these agents as pharmacological tools.

References

BESSER, G.M., DELITALA, G., GROSSMAN, A., STUBBS, W.A. & YEO, T. (1980). Chlorpromazine, haloperidol, metoclopramide and domperidone release prolactin through dopamine antagonism at low concentrations but paradoxically inhibit prolactin release at high concentrations. *Br. J. Pharmac.* (in press).

CARON, M.B., BEAULIEU, M., RAYMOND, V., GAGNE, B., DROUIN, J., LEFKOWITZ, R.J. & LABRIE, F. (1978). Dopaminergic receptors in the anterior pituitary gland. *J. biol. Chem.*, **253**, 2244–2253.

COCCHI, D., LOCATELLI, V., CARMINATI, R. & MULLER, E. E. (1978). Mechanisms underlying the prolactin-lowering effect of metergoline in the rat. *Life Sci.*, **23**, 927–936.

DELITALA, G., MASALA, A., ALAGNA, S., DEVILLA, L. & LOTTI, G. (1976). Growth hormone and prolactin release in acromegalic patients following metergoline administration, *J. clin. Endocrinol. Metab.*, **43**, 1382–1386.

DELITALA, G., YEO, T., GROSSMAN, A., HATHWAY, N.R. & BESSER, G.M. (1980). A comparison of the effects of four ergot derivatives on prolactin secretion by dispersed rat pituitary cells. *J. Endocrinol.* (in press).

JENNER, P., & MARSDEN, C.D. (1979). A novel class of dopamine antagonists. *Life Sci.*, **25**, 479–486.

KEBABIAN, J.W. & CALNE, D.B. (1979). Multiple receptors for dopamine. *Nature*, **277**, 93–96.

LAMBERTS, S.W.J. & MACLEOD, R.M. (1978). Effects of cyproheptadine on prolactin synthesis and release by normal and suppressed pituitary glands and by dispersed pituitary tumour cells. *Endocrinology*, **103**, 1710–1717.

MACLEOD, R.M. & LAMBERTS, S.W.J. (1979). Clinical and fundamental correlates in dopaminergic control of prolactin secretion. In *Neuroendocrine Correlates in Neurology and Psychiatry*. ed. Muller, E.E. & Agnoli, A. Amsterdam: Elsevier/North-Holland Biomedical Press.

RIVIER, C. & VALE, W. (1977). Effects of γ-aminobutyric acid and histamine on prolactin secretion in the rat. *Endocrinology*, **101**, 506–511.

(*Received June 1, 1980.*)