A COMPARISON OF THE EFFECTS OF FOUR ERGOT DERIVATIVES ON PROLACTIN SECRETION BY DISPERSED RAT PITUITARY CELLS

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SUMMARY

The inhibitory effects of dopamine and various ergot alkaloids on prolactin secretion were studied using continuously perfused columns of dispersed rat anterior pituitary cells. Bromocriptine (5 nmol/l) and lisuride hydrogen maleate (5 nmol/l) both inhibited prolactin secretion, the effects persisting for more than 3 h after the end of the administration of the drugs. A similar although less long-lasting effect was observed with lergotrile (50 nmol/l) and the new ergoline derivative, pergolide (5 nmol/l). These effects contrasted with the rapid disappearance of the action of dopamine. The potency estimates of the ergots relative to that of dopamine were: lergotrile, 2-3; bromocriptine, 13; lisuride, 15; pergolide, 23.

The dopamine-receptor blocking drugs, metoclopramide and haloperidol, antagonized the prolactin release-inhibiting activity of the compounds; bromocriptine and lisuride showed the highest resistance to this dopaminergic blockade.

The results suggested that the direct effect of the ergot derivatives on dispersed pituitary cells was mediated through dopamine receptors and emphasized the long-lasting action of bromocriptine and lisuride in vitro.

INTRODUCTION

A variety of ergot derivatives inhibit lactation by suppressing prolactin secretion. They appear to act as dopamine agonists through mechanisms located at the pituitary level (Lu, Koch & Meites, 1971; Pastuels, Danguy, Frerotte & Ectors, 1971; MacLeod & Leh Meyer, 1974; Clemens, Smalstig & Shaar, 1975). Of these compounds, bromocriptine has been studied most widely and has had the widest clinical use, being effective in the treatment of physiological and pathological hyperprolactinaemia, acromegaly and Parkinson’s disease (Besser, Parke, Edwards, Forsyth & McNeilly, 1972; Liuzzi, Chiodini, Botalla, Cremascoli, Muller & Silvestrini, 1974; Thorner, Chait. Aitken, Benker, Bloom, Mortimer, Sanders, Stuart Mason & Besser, 1975; Calne, Kartzinel & Shoulson, 1976). The ergolines, related compounds lacking a peptide-like sequence, also suppress prolactin secretion both in vitro and in vivo and therefore hold therapeutic promise (Horowski, Wendt & Graf, 1978; Thorner, Ryan, Wass, Jones, Bouloux, Williams & Besser, 1978).

In this study we have investigated the effects of bromocriptine and the ergolines, n-D-6-methyl-8-isooergolenyl-N’-N-diethyl-carbamide hydrogen maleate (lisuride; Horowski & Wachtel, 1976), 2-chloro-6-methylergoline-8β-acetonitrile methanesulphonate (lergotril e; Clemens et al. 1975) and (8β)-8-[(methylthio)methyl]-6-propylergoline (pergolide; Fuller, Clemens, Kornfeld, Snoddy, Smalstig & Bach, 1979), on prolactin secretion by dispersed rat pituitary cells.
pituitary cells. The structures of these compounds are shown in Fig. 1. Their interaction with two dopamine-receptor blocking compounds, metoclopramide and haloperidol, was also assessed. Continuously perfused columns of dispersed rat anterior pituitary cells supported by BioGel beads were used. This system allowed study of the relative time-courses of prolactin inhibition induced by the drugs, as well as any interactions.

**MATERIALS AND METHODS**

Columns of dispersed anterior pituitary cells were prepared as previously described (Yeo, Thorner, Jones, Lowry & Besser, 1979). In brief, anterior pituitary glands from five female Wistar rats (body-weight 200-250 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 from four harvests were recovered by centrifugation and filtered through 100 μm nylon gauze. The cells, 2 × 10⁶ to 5 × 10⁶ cells per column, were mixed with 0·5 g preswollen BioGel P2 (200-400 mesh) and packed into a 2 ml plastic syringe acting as a column. Earle’s balanced salt solution, containing bovine serum albumin (2·5 g/l), penicillin (25 u/l) and streptomycin (25 mg/l), was used as the perfusion medium, instead of Medium 199 as previously described, since the simpler medium gave fewer problems with bacterial and fungal overgrowth. Viability of the cells was established by their ability to exclude Trypan blue stain and was greater than 95% just before columns were packed. A viable column was taken to be one that, when unsuppressed by dopamine, secreted similar amounts of prolactin throughout the experiment. The cell columns were perfused in a water bath at 37 °C at a flow rate of 0·5 ml/min; the test substances (suitably dissolved, see below, and diluted in 0·9% saline) were mixed with the perfusing medium in a ratio of 1 : 9 (v/v). Eluate fractions of 7·5 min (approx 3·7 ml) were collected.

*Dose-response relationships*

Dose–response relationships were developed using concentrations ranging from 1 nmol/l to 5 μmol/l of the four ergot derivatives and dopamine. Each concentration of each compound was perfused for at least 1 h through separate columns to allow suppression of prolactin release to become stable. The prolactin concentrations in the last six eluate fractions (45 min) collected during the administration of the drugs were compared with the mean of the six fractions collected just before the drugs were administered. The responses were expressed as percentage inhibition by the drug of the basal release of prolactin.

*Time-course*

The rate of onset of inhibition of prolactin secretion and recovery from this inhibition was studied using a submaximally effective dose of each drug. The cell column was perfused for 30 min with perfusion medium containing the drug and then replaced with perfusion medium without the drug.

*Effects of dopamine antagonists*

Metoclopramide (1 μmol/l; Beecham Research Laboratories, Brentford) or haloperidol (10 or 100 nmol/l; Searle, High Wycombe) were added to columns being perfused with each ergot derivative at the concentration which had been used to study the time-course. The cells were exposed to the ergot derivative for 30 min and then the antagonist and the derivative were perfused together for a further 2·5 h. The effect of the addition of the antagonist was compared with the effect of the ergot derivative alone in a control column.

Dopamine (Sigma, London) and metoclopramide were dissolved in 0·9% saline containing 100 mg ascorbic acid/l as antioxidant. Bromocriptine (Sandoz, Basle, Switzerland), lisuride hydrogen maleate (Schering, Berlin, Federal Republic of Germany)
and haloperidol were dissolved in 1 ml absolute ethanol and then diluted in tartaric acid (5 mmol/l) to give a solution of 1 mmol/l. Successive dilutions were made with 0.9% saline. Lergotriile and pergolide (Eli Lilly & Co., Indianapolis, U.S.A.) were dissolved in water. Prolactin was measured in the cell-column eluate by a double-antibody radioimmunooassay using reagents supplied by NIAMDD (Maryland, U.S.A.). Antibody S6 was used at a final dilution of 1 : 15 000 giving a sensitivity of 4 μg/l using the NIAMDD standard rat prolactin RP-1. The coefficient of variation at 100 μg/l was 9%. The inhibitory effects of the compounds in the secretion of prolactin were analysed using Student’s paired t-test. Best-fit lines for the long dose–response curves were fitted and potency ratios computed by the analysis of variance (Finney, 1978).

RESULTS

Dopamine and each of the ergot derivatives tested produced a significant reduction in prolactin secretion (P <= 0.05) which was dose-dependent and was seen with doses as low as 1 nmol/l (Fig. 1). Dopamine, bromocriptine, lisuride and pergolide (5 μmol/l) reduced prolactin secretion to less than 10% of basal levels. This concentration of lergotriile suppressed prolactin secretion to 15% of basal levels. Some points on the dose–response curve were repeated on two to six occasions. The results obtained were consistent with the data presented here. There was no significant deviation from parallelism of the linear portions of the log10 dose–response curves. Best estimates of the dose capable of reducing prolactin secretion by 50% for each compound are given in Table 1.

Table 1. Doses of dopamine and ergot derivatives required to inhibit prolactin secretion by dispersed rat pituitary cells to 50% of basal values (ID50), 95% confidence intervals and potency estimates relative to dopamine

<table>
<thead>
<tr>
<th>Compound</th>
<th>ID50 (molar)</th>
<th>95% Confidence intervals (molar)</th>
<th>Relative potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine</td>
<td>9 x 10^-8</td>
<td>2.9 x 10^-8 - 2.8 x 10^-7</td>
<td>1</td>
</tr>
<tr>
<td>Lergotriile</td>
<td>4 x 10^-8</td>
<td>1.2 x 10^-8 - 1.2 x 10^-7</td>
<td>2.3</td>
</tr>
<tr>
<td>Bromocriptine</td>
<td>7 x 10^-9</td>
<td>2.3 x 10^-9 - 2.2 x 10^-8</td>
<td>13</td>
</tr>
<tr>
<td>Lisuride</td>
<td>6 x 10^-9</td>
<td>1.9 x 10^-9 - 1.9 x 10^-8</td>
<td>15</td>
</tr>
<tr>
<td>Pergolide</td>
<td>4 x 10^-9</td>
<td>1.2 x 10^-9 - 1.3 x 10^-8</td>
<td>23</td>
</tr>
</tbody>
</table>

For definitions of ergot derivatives, see text.

The duration of action of the ergot derivatives after their removal from the perfusion medium was studied using doses that produced between 40 and 60% suppression of prolactin release (Fig. 2). Suppression was maintained longer after cessation of bromocriptine administration than after the other three ergot compounds. Prolactin release returned to basal levels within 90 min after the withdrawal of lergotriile and pergolide whereas secretion was still slightly suppressed 3 h after withdrawal of lisuride. There was only partial recovery of secretion of prolactin 3-5 h after withdrawal of bromocriptine. All ergot derivatives were longer acting than dopamine (Fig. 2). Bromocriptine and lisuride appeared to have a slower onset of action than the other two ergot derivatives.

Metoclopramide antagonized the inhibition of prolactin by each of the ergot derivatives. This antagonism was rapid and complete when metoclopramide (1 μmol/l) was added to the perfusion medium containing 5 nmol pergolide/l and 50 nmol lergotriile/l (Fig. 3). Prolactin secretion was active within 30 min of the addition of metoclopramide to the perfusate containing the derivative and remained unsuppressed as long as the antagonist was present. Metoclopramide (1 μmol/l) also antagonized bromocriptine and lisuride but only partially and the effect developed more slowly (Fig. 3). A lower concentration of
Fig. 1. Structures and log₁₀ dose–response curves for the inhibition of prolactin secretion by dopamine, lisuride, lergotriile, pergolide and bromocriptine. Each point represents the mean of six determinations ± S.E.M. Analysis of variance demonstrated that the log dose–response curves for the five compounds can be represented by lines of common slope but differing intercepts. See text for definitions of ergot derivatives.
Ergot alkaloids and prolactin secretion

Fig. 2. Concentrations of prolactin from rat anterior pituitary cell columns perfused with medium containing 5 µmol dopamine/1 (D, solid bars) and the four ergots (hatched bars), i.e. (a) bromocriptine (B, 5 nmol/1), (b) lisuride (Li, 5 nmol/1), (c) legrtrole (Le, 50 nmol/1) and (d) pergolide (P, 5 nmol/1). The open bars represent prolactin secretion when 0-9% saline (S) alone was added to the perfusion medium. See text for definitions of ergot derivatives.

metoclopramide (10 nmol/1), although effective in blocking the action of legrtrole and pergolide, showed only slight antagonism to bromocriptine and lisuride (data not shown).

Haloperidol also blocked the inhibitory effects of the four ergot derivatives; 10 nmol haloperidol/1 completely antagonized the effects of 50 nmol legrtrole/1 and 5 nmol pergolide/1 (Fig. 4). This dose of haloperidol had no effect on 5 nmol bromocriptine/1 or 5 nmol lisuride/1 (data not shown) but 100 nmol/1 was effective (Fig. 4).

DISCUSSION

Selected ergot derivatives have been shown to interact with dopaminergic neurones and to induce, in experimental animals, behavioural changes which are consistent with a dopamine mimetic effect (Corrodi, Fuxe, Hokfelt, Lidbrink & Ungerstedt, 1973). Most of these compounds also have antagonistic actions on adrenoreceptors and serotonin receptors (Votava & Lamplova, 1961; Corrodi et al. 1973). Since dopamine itself can inhibit prolactin secretion by a direct action on the pituitary gland, but serotonin cannot (Lamberts & MacLeod, 1978; Delitala, Yeo, Stubbs, Jones & Besser, 1980), it seems likely that these ergot derivatives also inhibit prolactin secretion, at least in part, by activating dopaminergic mechanisms. The results of the present study are consistent with this view.
We have shown that bromocriptine, lisuride, ergotriile and pergolide inhibit prolactin secretion by a direct action on rat anterior pituitary cells. All the ergot derivatives studied here had an action that persisted longer after the removal of the drug than in the case of dopamine. Bromocriptine had the longest action of all in vitro. We have consistently found that bromocriptine and lisuride have a slower onset of action than either pergolide or ergotriile. In a previous publication (Yeo et al. 1979), we have shown that the onset of action of bromocriptine (100 nmol/l) is much slower than that of dopamine (5 μmol/l). These concentrations gave nearly complete suppression of prolactin secretion. The slower onset of action of bromocriptine in vitro has been noted by other workers (MacLeod & Lamberts, 1979).

Haloperidol and metoclopramide, compounds which raise plasma prolactin levels in man and animals, are known to be competitive antagonists of dopamine (MacLeod & Lehmeyer, 1972, 1974; Dougan, Mearwick & Wade, 1974). In vitro, these agents block the dopamine-mediated inhibition of prolactin by acting directly on the dopamine receptors of pituitary lactotrophs (MacLeod & Lehmeyer, 1974; Yeo et al. 1979). The present data showed that the inhibition of prolactin release by the ergot derivatives can also be blocked by these dopamine...
Fig. 4. Effect of haloperidol (H, 10 or 100 nmol/l) in antagonizing the inhibition of prolactin secretion by dispersed rat pituitary cells by pergolide (P, 5 nmol/l) or bromocriptine (B, 5 nmol/l) (both hatched bars). Dopamine (D, 5 µmol/l, solid bars); P + H (10 nmol/l) or B + H (100 nmol/l) (both cross-hatched bars); 0.9% saline (S, open bars). See text for definitions of ergot derivatives.

antagonists, thus further supporting the concept of their common action on pituitary dopamine-receptor mechanisms. Lisuride appeared to act like a dopamine agonist in suppressing prolactin secretion although it has marked additional serotonin-antagonist actions (Kehr, 1977). The latter are unlikely to be involved in any effects of lisuride directly on the pituitary gland, since serotonin neither influences prolactin secretion from dispersed pituitary cells, nor is it possible to demonstrate that serotonin interferes with the action of lisuride in inhibiting the release of prolactin (Delitala et al. 1980).

Lisuride and lergotrile are effective in vivo in man in lowering levels of prolactin (Liuzzi, Chiodini, Oppizzi, Botalla, Verde, De Stefano, Colussi, Graf & Horowski, 1978; Thorner et al. 1978; Delitala, Wass, Stubbs, Jones, Williams & Besser, 1979) but are shorter acting than bromocriptine. It would seem that their lengths of action in vivo can be correlated with the in-vitro results and that their long-lasting activities, compared with dopamine, could be accounted for by persistent action at the pituitary level. In contrast with these findings, a recent report (Lemberger & Crabtree, 1979) has suggested that pergolide, at low doses, is exceptionally long-acting in vivo compared with the other ergot derivatives studied here. However, our data suggested that pergolide is relatively short-acting in vitro. This discrepancy might indicate that the pharmacokinetics of pergolide differ from those of the other ergot derivatives. In particular, persisting action at the pituitary level does not appear to be the explanation for its long action in vivo. Alternative explanations which remain to be explored include delayed clearance of the compound or production of longer-acting metabolites.
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REFERENCES


