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A Sensitive Assay for Studying Dopaminergic Activity in Cultures of Rat Pituitary Cells

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Abstract

The dopaminergic and antidopaminergic activity of drugs is frequently assayed in pituitary cell cultures. Here we describe a modified version of the assay based on the use of pituitary cells from prepubertal female rats.

Under our experimental conditions (50 000 cells well^{-1}, 2-day culture and 2-h drug-exposure) the assay yielded high selectivity and sensitivity for drug dopaminergic activity. D_2 agonistic activity of bromocriptine could be observed at a concentration as low as 10^{-15} M, the antagonistic activity of haloperidol at 10^{-16} M. The assay also proved reproducible and simple enough for routine screening of dopaminergic activity.

The assay enabled dopaminergic agonist and antagonist activity to be revealed at very low drug concentrations. The high sensitivity of the assay could be of advantage in studying dopaminergic activity in samples containing active substances present at low concentrations or for disclosing the activity of substances with low dopaminergic potency.

Despite the numerous classes of drugs and natural compounds with potent dopaminergic activity, suitable pharmacological methods for their study remain scarce. Because dopamine inhibits prolactin secretion through D_2 receptors, dopaminergic activity is frequently determined by investigating effects on prolactin secretion in-vitro (Willner 1983; Becù-Villalobos et al 1992). Although in-vitro assays based on monolayer-cell cultures are simple enough to be used for routine testing of dopaminergic activity, the sensitivity might be inadequate, particularly when testing the dopaminergic activity of natural compounds, which might be present at very low concentrations in biological fluids or tissue extracts. Improvement of the sensitivity of the monolayer-cell culture assay might, therefore, enable the evaluation of dopaminergic activity in biological samples.

The sensitivity of the in-vitro assay depends primarily upon the sex and the hormonal status of rats (Hoefer et al 1984). Cells obtained from male donors respond to dopamine inhibition less than do cells from females (Hoefer et al 1984). Although assays using female rats give better sensitivity, their reproducibility suffers because the number of dopamine receptors in the anterior pituitary gland of adult female rats varies as a result of hormonal changes occurring during the oestrus cycle (Heiman & Ben-Jonathan 1982). This might explain why cells from adult female rats undergo rapid changes in their responsiveness to dopamine (Heiman & Ben-Jonathan 1982). Achieving a reproducible assay in adult female donors entails constant checking of their endocrine status, which can be a drawback in an assay intended for routine screening of dopaminergic activity (see, for example, the experimental procedures described by Heiman & Ben-Jonathan (1982)). To address these problems we have designed an assay that uses 25-day-old prepubertal female rats. In-vivo sensitivity to dopamine agonists is much higher at this age than in younger rats and approaches the sensitivity in adults (Ojeda & McCann 1974; Bero & Kuhn 1987). Hoefer et al (1984) reported that cell density and culturing time could also influence assay sensitivity in cultures from adult rats; for this reason we also examined whether these culture conditions influenced the sensitivity in our assay based on the use of prepubertal rats.

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Materials and Methods

Animals

Female Wistar rats, 21 days old, were obtained from Charles River (Italy). The rats were housed in an animal room illuminated from 0700 to 1900 h and kept at a constant temperature of 22°C; food and water were freely available.

Chemicals

Bromocriptine methanesulphonate, cyproheptadine hydrochloride, dopamine hydrochloride and haloperidol, were obtained from Sigma (St Louis, MO); spiperone hydrochloride and SKF83566 hydrochloride were obtained from Research Biochemical (Natick, MA).

Cell dispersion

Rats, 25 days old, were killed with CO_2 and the pituitary glands were removed. The glands were cut into small pieces and dispersed by trypsin into single cells according to procedures previously described (Denef et al 1978). After dispersion, the cells were counted and the trypan blue dye-exclusion test showed viability higher than 95%. The cells were suspended in an appropriate volume of medium before being transferred to 24-multiwell plates. After 2 days' culture the medium was changed and the cultured cells were exposed to the drugs. Unless otherwise specified, 50 000 cells well^{-1} were plated and then incubated with drugs for 2 h. To examine the influence of cell density and the length of drug exposure on the sensitivity of the assay 50 000, 100 000 or 150 000 cells well^{-1} were incubated as a monolayer in 0·5 mL culture medium. After the medium had been changed, cells were exposed for 2, 4 or 6 h to medium alone or to medium containing dopamine. Throughout the experiment the plates were kept in a humidi-

fied CO_2–air incubator (1·5% CO_2–98·5% air) at 37°C. The culture medium was essentially the same as that previously described (Baes & Denef 1987) except that phenol red was omitted because its oestrogenic activity is known to interfere with prolactin secretion (Berthois et al 1986). Dexamethasone (4 nM) and 3,3′,5-triiodo-L-thyronine (50 pM) were added to improve cell attachment (Koppenaal et al 1992). Drugs were dissolved either in absolute ethanol (haloperidol) or in 50% (v/v) aqueous ethanol and then diluted with medium containing 1·1 mM ascorbic acid to prevent oxidation. All substances were tested in triplicate. The media harvested after exposure of cells to the drugs were used for determination of prolactin concentration.

Prolactin determination

The concentration of prolactin in harvested media was determined by radioimmunoassay (RIA), using reagents provided by NIDDK (Bethesda, MD). Data were expressed in terms of NIDDK rat prolactin reference preparation (NIDDK + r-PRL-RP-3). The [^{125}I] prolactin tracer was supplied by Dupont de Nemours Italiana (Milan, Italy). All pituitary cell cultures were assayed in triplicate. The coefficient of variation within assays was 4·5%; that between assays was 14·4%.

Statistical analysis

The significance of differences between means was calculated by one-way analysis of variance, followed by either Dunnett's test or by the Student-Newman-Keuls test.

Results

Baseline prolactin secretion and the inhibitory effect of dopamine were evaluated in pituitary cell cultures from 25-day-old female prepubertal rats; the cell densities were 50 000, 100 000 and 150 000 cells well^{-1} after incubation for 2, 4 and 6 h. The data from these experiments (not shown) indicated that basal prolactin levels increased with increasing cell density or incubation time. Because a cell density higher than 50 000 cells well^{-1} or incubation longer than 2 h failed to increase sensitivity to dopamine further, as Hoefer et al (1984) have reported, in subsequent experiments we used 50 000 cells well^{-1} and incubated plates for 2 h.

Dopamine concentrations ranging from $2·5 \times 10^{-9}$ to 10^{-7} M inhibited prolactin secretion (Fig. 1); 5×10^{-8} M dopamine caused maximum inhibition (80 ± 4·5%). Concentration–response curves (10^{-9}–10^{-7} M) yielded an IC50 of $5·4 \pm 1·2 \times 10^{-9}$ M. At 10^{-10} M, dopamine slightly increased prolactin secretion in 45% of experiments.

To confirm that the assay had maintained its specificity under our conditions we assayed pituitary cells with various antagonists. Cyproheptadine 10^{-8} M and SKF83566 10^{-8} M (a D_1 antagonist) left the inhibitory effect of dopamine unchanged. In contrast, 10^{-8} M haloperidol and 10^{-8} M spiperone (a D_2 antagonist) both antagonized 10^{-8} M dopamine-induced inhibition of prolactin secretion, showing that this effect is mediated by D_2 receptors (Table 1).

Bromocriptine inhibited prolactin secretion at a concentration as low as 10^{-15} M; a concentration of 10^{-9} M caused maximum inhibition (80 ± 2·3%). The IC50 was $1·7 \pm 0·9 \times 10^{-14}$ M. This effect was antagonized by 10^{-8} M spiperone but not by 10^{-8} M SKF83566 (Fig. 2).

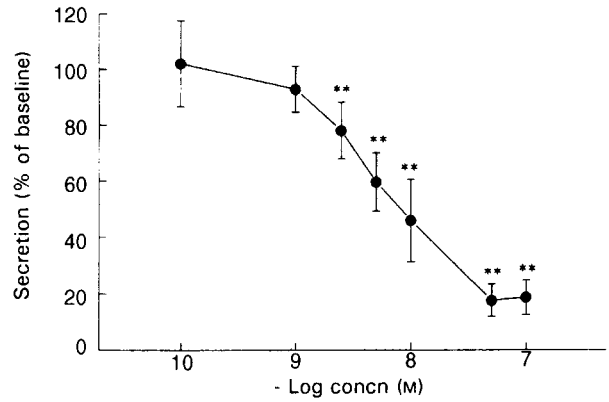

FIG. 1. Concentration–response curve for the effect of dopamine (10^{-9}–10^{-7} M) on prolactin secretion in cells from 25-day-old female rats. Results are means ± s.d. of six experiments. $^{**}P < 0·01$ compared with baseline value.

Table 1. Effect of 10^{-8} M cyproheptadine, SKF83566, spiperone and haloperidol on 10^{-8} M dopamine-mediated inhibition of prolactin secretion.

Treatment	Prolactin secretion (% of baseline)
Baseline	100 ± 2·4
Dopamine	45 ± 5·7*
Dopamine + cyproheptadine	53 ± 7·1*
Dopamine + SKF83566	42 ± 3·1*
Dopamine + spiperone	102 ± 12·3†,‡,§
Dopamine + haloperidol	106 ± 11·7†,‡,§

Results are means ± s.d. from six experiments. $^{*}P < 0·01$ compared with baseline; $^{\dagger}P < 0·01$ compared with 10^{-8} M dopamine; $^{\ddagger}P < 0·01$ compared with cyproheptadine; $^{\S}P < 0·001$ compared with SKF83566.

Haloperidol at concentrations from as low as 10^{-16} M antagonized dopamine (5×10^{-9} M) inhibition of prolactin secretion (Table 2).

Discussion

Our in-vitro assay using pituitary cells from 25-day-old female prepubertal rats under the experimental conditions described above revealed the dopamine antagonist activity of haloperidol at concentrations much lower than those previously reported. Under experimental conditions similar to ours, Caron et al (1978) found a K_d value for haloperidol of about 10^{-10} M, whereas we found a K_d value (calculated as described by Caron et al) of $1·9 \times 10^{-16}$ M.

In our experiments the maximum inhibition achieved by bromocriptine (80%) is in the range (70–85%) previously reported (Delitala et al 1980; Login & Trugman 1989; Kacsòh et al 1993). Although our findings also accord with the observation by Kacsòh et al (1993) that the inhibitory effect of bromocriptine, like that of dopamine, is mediated solely by D_2 receptors, we found an IC50 value (10^{-14} M) much lower than those previously reported by several authors. Login & Trugman (1989), using adult females but experimental conditions otherwise similar to ours obtained a IC50 value of 5×10^{-10}; similar IC50 values (between 7×10^{-9} and 10^{-10} M) were also obtained by Delitala et al (1980), who used continuously perfused columns of dispersed anterior pituitary cells, and by Kacsòh et al (1993), who prepared cultures of whole pituitary glands from 2-day-old rats.

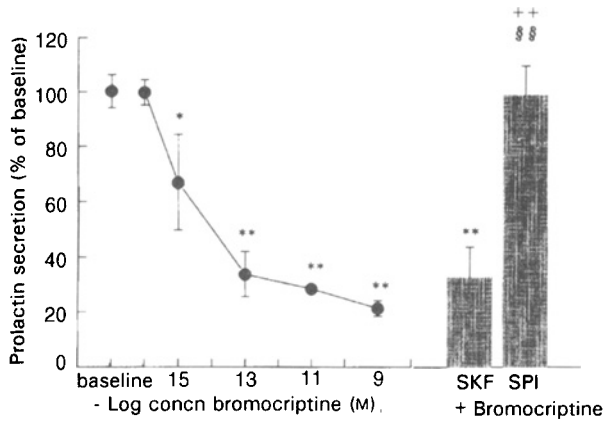

FIG. 2. Inhibitory effect of bromocriptine (10^{-16}–10^{-9} M) on prolactin secretion. The effect of 10^{-11} M bromocriptine was antagonized by 10^{-8} M spiperone (SPI) but not by 10^{-8} M SKF83566 (SKF). Results are means ± s.d. of three experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with baseline value; $^{++}P < 0.01$ compared with 10^{-11} M bromocriptine; $^{§§}P < 0.01$ compared with bromocriptine + SKF.

Table 2. Effect of haloperidol (10^{-16}–10^{-8} M) on 5×10^{-9} M dopamine-mediated inhibition of prolactin secretion.

Treatment	Prolactin secretion (% of baseline)
Baseline	100 ± 10·2
Dopamine	57 ± 16·3*
Dopamine + 10^{-16} M haloperidol	77 ± 10·2†
Dopamine + 10^{-14} M haloperidol	86 ± 7·4†
Dopamine + 10^{-12} M haloperidol	84 ± 5·9†
Dopamine + 10^{-10} M haloperidol	102 ± 19·1‡
Dopamine + 10^{-8} M haloperidol	102 ± 14·1‡

Results are means ± s.d. of six experiments. $^{*}P < 0.01$ compared with baseline; $^{†}P < 0.05$, $^{‡}P < 0.01$ compared with 5×10^{-9} M dopamine.

Many groups have found remarkably similar IC50 values for dopamine (between 3×10^{-8} and 9×10^{-8} M) even though their experimental conditions varied substantially (Caron et al 1978; Delitala et al 1980; Login & Trugman 1989; Lamberts et al 1990). We found an IC50 value of 5×10^{-9} M, which is approximately a tenth of those reported above. The sensitivity to dopamine was, therefore, also enhanced, even though the increased sensitivity was less than that observed for bromocriptine and haloperidol. Our results are also in agreement with literature data relating to the maximum inhibition achieved (80%) and the sensitivity to the tested antagonists.

Thus our in-vitro assay achieves higher sensitivity than previously reported assays yet maintains its specificity.

The higher sensitivity might depend on dopaminergic regulation of prolactin secretion, which under our experimental conditions differed from that previously described. For example, very low concentrations (lower than the minimum concentration having an inhibitory effect on prolactin secretion) of dopaminergic agonists essentially lacked the stimulatory effect on prolactin secretion reported by others (Kramer & Hopkins 1982; Tagawa et al 1992).

In summary, the use of pituitary cells from 25-day-old female prepubertal rats enables dopaminergic agonist and antagonist activities to be revealed at very low drug concentrations. The high sensitivity of this assay could be an advantage in studying dopaminergic activity in samples containing active substances at low concentrations. It might also be helpful in disclosing the activity of substances that have low dopaminergic potency.

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References

Baes, M., Denef, C. (1987) Evidence that stimulation of growth hormone release by epinephrine and vasoactive intestinal peptide is based on cell-to-cell communication in the pituitary. Endocrinology 120: 280–290

Becù-Villalobos, D., Thyssen, S. M., Rey, E. B., Lux-Lantos, V., Libertun, C. (1992) Octopamine and phenylethylamine inhibit prolactin secretion both in vivo and in vitro. Proc. Soc. Exp. Biol. Med. 199: 230–235

Bero, L. A., Kuhn, C. M. (1987) Role of serotonin in opiate-induced prolactin secretion and antinociception in the developing rat. J. Pharmacol. Exp. Ther. 240: 831–836

Berthois, Y., Katzenellenbogen, J. A., Katzenellenbogen, B. S. (1986) Phenol red in tissue culture media is a weak estrogen: implications concerning the study of estrogen-responsive cells in culture. Proc. Natl. Acad. Sci. USA 83: 2496–2500

Caron, M. G., Beaulieu, M., Raymond, V., Gagné, B., Drouin, J., Lefkowitz, R. J., Labrie, F. (1978) Dopaminergic receptors in the anterior pituitary gland. J. Biol. Chem. 253: 2244–2253

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. 87: 95–103

Denef, C., Hautekeete, E., De Wolf, A., Vanderschueren, B. (1978) Pituitary basophils from immature male and female rats: distribution of gonadotrophs and thyrotrophs as studied by unit gravity sedimentation. Endocrinology 103: 724–734

Heiman, M. L., Ben-Jonathan, N. (1982) Dopaminergic receptors in the rat anterior pituitary change during the estrous cycle. Endocrinology 111: 37–41

Hoefer, M. T., Heiman, M. L., Ben-Jonathan, N. (1984) Prolactin secretion by cultured anterior pituitary cells: influence of culture conditions and endocrine status of the pituitary donor. Mol. Cell. Endocrinol. 35: 229–235

Kacsòh, B., Tòth, B. E., Avery, L. M., Grosvenor C. E. (1993) In vitro control of prolactin (PRL) and growth hormone secretion of neonatal rat pituitary glands: effects of ovine PRL, salmon calcitonin, endothelin-3, angiotensin II, bromocriptine and somatostatin. Life Sci. 52: 259–269

Koppenaal, D. W., Tijssen, A. M. I., de Koning, J. (1992) The effect of gonadotrophin surge-inhibiting factor on the self-priming action of gonadotrophin-releasing hormone in female rats in vitro. J. Endocrinol. 134: 427–436

Kramer, I. M., Hopkins, C. R. (1982) Studies on the kinetics of dopamine-regulated prolactin secretion. Mol. Cell. Endocrinol. 28: 191–198

Lamberts, S. W. J., van Koetsveld, P. M., Hofland, L. J. (1990) The effect of clozapine on prolactin secretion at the level of the lactotroph. Life Sci. 46: 1013–1019

Login, I. S., Trugman, J. M. (1989) The dopamine agonist PHNO ((+)-4-propyl-9-hydroxynaphthoxazine) inhibits cyclic adenosine 3′,5′-monophosphate formation and prolactin release from anterior pituitary cells. Neuropharmacology 28: 647–650

Ojeda, S. R., McCann, S. M. (1974) Development of dopaminergic and estrogenic control of prolactin release in the female rat. Endocrinology 95: 1499–1505

Tagawa, R., Takahara, J., Sato, M., Niimi, M., Murao, K., Ishida, T. (1992) Stimulatory effects of quinpirole hydrochloride, D_2-dopamine receptor agonist, at low concentrations on prolactin release in female rats in vitro. Life Sci. 51: 727–732

Willner, P. (1983) Dopamine and depression: a review of recent evidence. III. The effects of antidepressant treatments. Brain Res. Rev. 6: 237–246