Identification of a D₁ dopamine receptor, not linked to adenylate cyclase, on lactotroph cells

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1 We studied the lactotroph cells of the rat by both *in vivo* and *in vitro* pharmacological techniques for the presence of D₁-receptors. Both approaches revealed the presence of a D₂-receptor, stimulated by quinpirole (resulting in an inhibition of prolactin secretion) and blocked by domperidone.

2 Administration of fenoldopam, the most selective D₁-receptor agonist currently available, resulted in a dose-dependent decrease of prolactin secretion *in vivo* (after pretreatment with α -methyl-*p*-tyrosine) and *in vitro* (cultured pituitary cells). This increase was dose-dependently blocked by the selective D₁-receptor antagonist, SCH 23390, and although the effect of fenoldopam was less than that obtained by D₂-receptor stimulation, these data suggest that a D₁-receptor also controls prolactin secretion.

3 In order to detect the location of these dopamine receptors, autoradiographic studies were performed by use of [³H]-SCH 23390 and [³H]-spiperone as markers for D₁- and D₂-receptors, respectively. Specific binding sites for [³H]-SCH 23390 were demonstrated. Fenoldopam dose-dependently reduced [³H]-SCH 23390 binding, but had no effect on [³H]-spiperone binding. Immunocytochemical labelling of prolactin cells after incubation with [³H]-SCH 23390 revealed that the granulae and hence, D₁ binding sites were present on the lactotroph cells.

4 Radioligand binding studies performed on membranes from anterior pituitary cells revealed the presence of the D₂-receptor (54 fmol mg⁻¹ protein) with a K_d of 0.58 nM for [³H]-spiperone, but failed to detect D₁-receptors.

5 Finally, we studied the effect of dopamine and of fenoldopam on the adenosine 3':5'-cyclic monophosphate (cyclic AMP) content of anterior pituitary cells. Although cyclic AMP increased upon prostacyclin administration, indicating an intact adenylate cyclase system, fenoldopam failed to increase the cyclic AMP production.

6 It is tempting to speculate that fenoldopam reduces prolactin secretion through interaction with a non-cyclase-linked D₁-receptor on the lactotroph cells.

Keywords: Dopamine; D₁-receptors; D₂-receptors; prolactin secretion

Introduction

Some ten years ago Keibian & Calne (1979) proposed the division of central dopamine receptors into two subtypes, according to biochemical and pharmacological criteria: the D₁-receptors, which are positively coupled to adenylate cyclase, and the D₂-receptors, which are negatively coupled to this enzyme. The main pharmacological characteristics of D₂-receptors are their high affinity for neuroleptics and their lower affinity for dopamine receptor agonists (Seeman & Grigoriadis, 1987). Independently, two subtypes of dopamine receptors were identified in the periphery, which were designated as DA₁- and DA₂-receptors. Their characteristics are very similar, but not identical, to those of the D₁- and D₂-receptors in the central nervous system (Stoof & Keibian, 1984).

Dopamine has been shown to be an important regulator of prolactin secretion from the anterior pituitary gland (Denef, 1986). Stimulation of this pituitary dopamine receptor results in a small decrease in prolactin secretion. Therefore the hypothesis of a tonic inhibition of prolactin secretion by dopamine has been put forward (Gunnert & Moore, 1988). Blockade of the dopamine receptor by drugs such as domperidone, markedly increases the prolactin release. In rat anterior pituitary homogenates, dopamine inhibits basal adenylate cyclase by approximately 50% (Enjalbert *et al.*, 1986), and both radioligand binding and autoradiographic studies have

demonstrated the presence of D₂-binding sites on the prolactin secreting cells (Enjalbert & Bockaert, 1983; Foord *et al.*, 1983; Nikitovich-Winer *et al.*, 1987).

These findings have led to the suggestion that the pituitary dopamine receptor, involved in prolactin secretion, is a D₂-receptor. Moreover, prolactin secretion by lactotroph cells has been currently used as a model to evaluate D₂-receptor activity of dopamine-like drugs.

In contrast to the well established presence of a D₂-receptor, initial studies concerning the presence of a D₁-receptor yielded contradictory results. The selective D₁-receptor antagonist, SCH 23390 (Hyttel, 1983), has been shown to stimulate prolactin secretion in the rat (Apud *et al.*, 1985); however, when [³H]-SCH 23390 was used as an autoradiographic ligand, no specific binding sites in broken cells from the anterior pituitary gland could be demonstrated (Rovescalli *et al.*, 1987). SK&F 38393-A, a relatively selective D₁-receptor agonist, has been reported to either stimulate (Saller & Salama, 1986) or inhibit prolactin secretion (Cocchi *et al.*, 1987). This prompted us to investigate the effects of fenoldopam, the most selective D₁-receptor agonist currently available (Hahn *et al.*, 1982), on prolactin secretion in the conscious rat and from cultured pituitary cells, and to compare these results with those obtained with quinpirole, a selective D₂-receptor agonist (Lefevre-Borg *et al.*, 1987). In this study, it is shown that fenoldopam and quinpirole both lower prolactin secretion, suggesting the presence of both, D₂- and D₁-receptors. Whereas initial attempts to detect D₁-receptors by binding of [³H]-SCH 23390 to broken cell preparations

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were unsuccessful, their presence on intact cultured pituitary cells can be demonstrated by autoradiography.

Methods

In vivo experiments

The *in vivo* studies were done in 19 groups of either 6 or 12 normotensive male Wistar Kyoto rats (KUL Proefdierencentrum, Leuven, Belgium), which were equipped with an indwelling silastic catheter, implanted in the right external jugular vein under sodium pentobarbitone anaesthesia (40 mg kg⁻¹, i.p.). The catheters were exteriorized and kept open by daily flushing with a heparinized saline solution.

Rats were housed individually in wire-bottom cages in a light controlled room with a 10:14 h light:dark cycle (lights on at 07 h 00 min), having food and water freely available. All experiments were conducted 4 to 6 days after surgical procedure.

In a preliminary series of experiments saline ($n = 6$), fenoldopam (100 µg kg⁻¹; $n = 6$) or quinpirole (100 µg kg⁻¹; $n = 6$) was administered after an initial blood sampling (0.7 ml). Two additional blood samples were drawn 15 and 40 min after injection of the different drugs.

As the synthesis and release of prolactin are under a predominantly inhibitory influence of hypothalamic dopamine, we also assessed the effects of the different dopamine receptor agonists on α -methyl-*p*-tyrosine (AMPT) treated rats. AMPT, a tyrosine hydroxylase inhibitor, decreases temporarily the endogenous dopamine production at the medio-basal-hypothalamic dopaminergic neurones. This results in a diminished dopaminergic tone at the pituitary gland and a subsequent increase of plasma prolactin (Buydens *et al.*, 1988).

Therefore, in the second series of experiments AMPT (250 mg kg⁻¹, i.p.) was administered, after initial blood sampling. A second blood sample was taken 1 h later, after which either saline ($n = 48$) or the selective dopamine D₁ receptor antagonist, SCH 23390 (100 µg kg⁻¹; $n = 48$) was administered intravenously. A third blood sample, drawn 10 min later, was immediately followed by injection of either saline ($n = 12$), fenoldopam (10, 30, 100 or 300 µg kg⁻¹, $n = 12$ for each dose) or the selective dopamine D₂-receptor agonist, quinpirole (10, 30 or 100 µg kg⁻¹, $n = 12$ for each dose). The doses of fenoldopam and quinpirole used are those producing maximal reductions in renal and mesenteric vascular resistance (Van der Niepen *et al.*, 1988; Dupont *et al.*, 1987). Two additional blood samples were taken 15 and 40 min after injection of these drugs. Blood samples were always immediately centrifuged and plasma was stored at -20°C until assayed for prolactin. Red blood cells were resuspended in heparinized saline (0.25 ml) and reinfused after the following blood sampling to minimize the effect of blood loss.

In vitro experiments

For each experiment, total pituitary cell populations were prepared from anterior pituitaries of 20 female adult nulliparous Wistar rats as described by Velkeniers *et al.* (1988). The cells were subsequently grown in 24-well dishes (Falcon) at concentrations of 5×10^4 cells in 1 ml of culture medium, and maintained at 37°C in 5% CO₂ humidified atmosphere. Culture medium consisted of Dulbecco's modified Eagle's medium (DMEM) supplemented with NaHCO₃ 1 g l⁻¹ HEPES (4-[2-hydroxyethyl] piperazin-N-1-ethanesulphonic acid) 15 mM, Tes (2-[tris-(hydroxymethyl)-methyl]-amino} ethanesulphonic acid) 15 mM (Calbiochem, San Diego, CA, U.S.A.), and 10% newborn calf serum (Gibco), penicillin 35 µg ml⁻¹ and streptomycin 50 µg ml⁻¹ (Sigma) adjusted to pH 7.5. Release experiments were carried out 3 days after cell plating. Cells were washed twice in DMEM and finally 1 ml DMEM (control) or 1 ml DMEM with fenoldopam (10⁻⁶, 10⁻⁷, 10⁻⁸, 10⁻⁹ M) or quinpirole (10⁻⁶, 10⁻⁷, 10⁻⁸, 10⁻⁹ M), were added

for 2 h, each in the presence of saline, domperidone (10⁻⁶, 10⁻⁷, 10⁻⁸, 10⁻⁹ M) or SCH 23390 (10⁻⁶, 10⁻⁷, 10⁻⁸, 10⁻⁹ M).

As SCH 23390 has been reported to have some affinity for 5-HT₂ receptors (Peroutka, 1988), we also investigated the possible involvement of 5-hydroxytryptamine receptors in prolactin secretion. Therefore, the 5-HT receptor antagonists, mianserin (10⁻⁶ M), ketanserin (10⁻⁶ M) or RU 24969 (10⁻⁶ M) were added for 2 h, together with fenoldopam (10⁻⁶, 10⁻⁷, 10⁻⁸, 10⁻⁹ M) in 1 ml DMEM.

At the end of incubation period, medium was removed and centrifuged at 2000 *g* in order to exclude cellular material. The supernatants were kept frozen at -20°C until prolactin hormone assay.

Membrane preparation and radioligand binding

Pituitary glands from 100 rats were dissected and kept on ice. All subsequent steps were performed at 4°C. Glands were homogenized in Tris-HCl 10 mM (pH 7.4), NaCl 145 mM, MgCl₂ 2 mM and sucrose 50 mM (Buffer A) with an Ultraturax homogenizer (15 s at maximum speed) and a Potter Elvehjem homogenizer (5 up and down strokes at maximum speed). The homogenates were then centrifuged in a Sorvall centrifuge at 900 *g* for 5 min. The pellet was discarded and the supernatant was centrifuged subsequently 29,000 *g* for 20 min. The pellet was resuspended in Tris-HCl 10 mM (pH 7.4), NaCl 145 mM, MgCl₂ 2 mM (Buffer B) and centrifuged at 29,000 *g* for 20 min. This washing step was repeated twice. The final pellet was resuspended in buffer B and immediately used for binding assays. Protein concentrations were determined by the method of Lowry *et al.* (1951), with bovine serum albumin (BSA) as a standard. Binding assays were done in duplicate in 0.5 ml of buffer B, containing 0.5 to 6 nM [³H]-SCH 23390 or 0.03 to 2 nM [³H]-spiperone and membrane protein (1.4 and 0.9 mg ml⁻¹, respectively). Mianserin (300 nM) was included to prevent binding of [³H]-spiperone to 5-HT₂ receptors. After incubation of the membranes for 20 min at 30°C, 4 ml of ice cold buffer was added to each tube and samples were rapidly filtered through Whatman GF/B glass fibre filters (2.5 cm diameter). The filters were washed rapidly 3 times with 4 ml of ice cold buffer, placed in 20 ml polyethylene scintillation vials with 1 ml of 0.1 N NaOH and 10 ml of scintillation fluid (Pico Fluor 15 from Packard), and counted in a Packard liquid scintillation spectrometer.

Non-specific binding was determined in the presence of 10 µM (+)-butaclamol (for [³H]-SCH 23390) or of 1 µM (+)-butaclamol (for [³H]-spiperone). Specific binding was calculated by subtracting non-specific binding from total binding.

Autoradiographic studies

Radioligand bindings Dispersed rat pituitary cells were cultured for 3 days on 12 coverslips (15 mm Thermanox, Miles Laboratories Inc., Naperville, U.S.A.) as described previously (Velkeniers *et al.*, 1988). Each experiment was repeated twice. After washing in sodium phosphate 10 mM (pH 7.4)/NaCl 120 mM (Buffer C), 6 coverslips were slightly fixed with 0.5% glutaraldehyde and 0.04% paraformaldehyde in Buffer C for 15 min at 4°C. All 12 coverslips were then pre-incubated with sodium phosphate 20 mM (pH 7.4), NaCl 120 mM, MgCl₂ 2 mM (Buffer D) for 5 min at 4°C. Binding, as described by De Keyser *et al.* (1988a), was performed during 30 min at 30°C for fixed cells and during 60 min for 4°C for unfixed (i.e. living) cells in Buffer D, supplemented with 0.1% BSA (fraction V, Sigma, St. Louis, U.S.A.) and 100 KIU ml⁻¹ aprotinin (Boehringer, Mannheim, West Germany). Radioligand concentrations were 0.5 nM for [³H]-spiperone (83 Ci mmol⁻¹; Amersham), to detect D₂-receptors, and 2 nM for [³H]-SCH 23390 (85 Ci mmol⁻¹; New England Nuclear) to detect D₁-receptors. Mianserin was included to mask binding to 5-HT₂ receptors and α_1 -adrenoceptors: 300 nM for [³H]-spiperone and 20 nM for [³H]-SCH 23390. Non-specific binding was determined with unlabelled (+)-butaclamol (1 µM for

[³H]-spiperone and 10 μ M for [³H]-SCH 23390), in addition to the respective radioligand. Specific binding was qualitatively evaluated with increasing fenoldopam concentrations, from 10⁻⁹ M to 10⁻⁶ M. After incubation, cells were washed twice with Buffer D for 5 min at 4°C to remove unbound ligand. Then ligand was fixed with 0.5% glutaraldehyde in Buffer C for 10 min at 4°C, as suggested by Junger (1978). After a brief rinse in cold distilled water, the coverslips were quickly air-dried and processed for immunocytochemistry.

Immunocytochemistry The avidin-biotin complex method (Hsu *et al.*, 1981) was used, employing a commercially available staining kit (Boehringer, Mannheim, West Germany). Buffer C (pH 7.2) was used as a diluent for all reagents and for the washes. The incubations with normal goat serum (10:100; 15 min) and with biotinylated goat anti-rabbit-IgG (1:100; 30 min) were performed at room temperature, except for rabbit-anti-prolactin (NIH; Bethesda, U.S.A.) (1:5000; 12 h overnight; 4°C). After incubation with the avidin-biotin complex, peroxidase activity was visualised with 3.3 diaminobenzidinetetrahydrochloride (0.15% w/v) and hydrogen peroxide (1% v/v) in Buffer C. Each incubation period, except the normal serum step, was followed by three washes of 5 min each.

Autoradiography For localization of [³H]-SCH 23390 and [³H]-spiperone, coverslips were dipped in liquid emulsion (nuclear K2 emulsion, Ilford) diluted with distilled water (1:1). After an exposure time of 4 weeks at -4°C, the autoradiograms were developed in Kodak D19 at 20°C for 8 min, rinsed in distilled water, fixed in Ilford Rapid Fixer for 10 min and thoroughly washed in distilled water. Cells were then counterstained with haematoxylin to visualise cell nuclei. After mounting, coverslips were scanned with Axiophot (Zeiss, West Germany).

Adenylate cyclase assay

Harvested cells were centrifuged (1000 *g*) for 10 min at 4°C in Krebs-Ringer-HEPES buffer (composition mM: NaCl 110, KCl 5, MgCl₂ 1, CaCl₂ 1.8, glucose 25, HEPES 10 and BSA 1 mg ml⁻¹, pH 7.4). The pelleted cells were resuspended in the same buffer (10⁶ cells ml⁻¹, final concentration). When indicated, cells were treated with drugs under culture conditions, centrifuged and resuspended in buffer as above. For cyclic AMP determinations, cells were incubated with 0.5 mM theophylline and the indicated compounds for 15 min at 37°C in a final volume of 200 μ l. The incubation was stopped by addition of 100 μ l of 4 mM EDTA (pH 7.4) and boiling for 6 min. Denaturated proteins were precipitated by centrifugation at 13,000 *g* for 2 min, and the cyclic AMP contents of the supernatants determined by the method of Gilman (1970), with protein kinase A used as binding protein.

Hormone assay and data analysis

Prolactin was measured by double antibody radioimmunoassay procedures, with use of anti-rat PRL antibodies and anti-rabbit-IgG, kindly provided by Dr Raiti (National Institute of Arthritis, Diabetes, Digestive Disease and Kidney). Rat prolactin was used as a standard.

The data were analysed by two-way analysis of variance with repeated measures. When significant *F* values occurred due to the treatment, the data were reanalysed by one-way analysis of variance, followed by the Student-Newman-Keuls procedure. A *P* value of less than 0.05 was considered significant. Data are expressed as means $\pm$ s.e.mean. Dose-response curves and 50% effective doses (ED₅₀) were calculated by use of weighted iterative nonlinear least squares regression.

Materials

The sources of the drugs used were as follows: (+)-butaclamol hydrochloride (Research Biochemicals Inc., Wayland, U.S.A.),

mianserin (Organon, Oss, The Netherlands), fenoldopam (Smith Kline and French Laboratories, Philadelphia, U.S.A.), quinpirole hydrochloride (Lilly Research Laboratories, Indianapolis, U.S.A.), SCH 23390 [(R)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine] (Schering Corporation, Bloomfield, U.S.A.) and RU 24969 (5-methoxy 3(1,2,3,6-tetrahydro-4-pyridinyl)1H indole succinate; Roussel-UCLAF, Paris, France). Spiroperidol, domperidone and ketanserin were kindly donated by Janssen Pharmaceutica (Beerse, Belgium). [³H]-SCH 23390 (80 Ci mmol⁻¹) was supplied by Amersham (U.K.) and [³H]-spiperone (24 Ci mmol⁻¹) by New England Nuclear. All other chemicals were of the highest grade commercially available.

Results

In vivo experiments

Saline, fenoldopam or quinpirole did not significantly change basal serum prolactin levels in conscious rats (4.21 $\pm$ 2.63 ng ml⁻¹ before and 4.39 $\pm$ 2.11 ng ml⁻¹ 40 min after injection of saline, *n* = 6; 4.85 $\pm$ 2.35 before and 4.17 $\pm$ 1.99 ng ml⁻¹ after fenoldopam, *n* = 6; and 3.84 $\pm$ 1.72 ng ml⁻¹ before and 3.02 $\pm$ 2.12 ng ml⁻¹ after quinpirole, *n* = 6).

AMPT increased serum prolactin from 4.34 $\pm$ 2.88 to 48.6 $\pm$ 25.6 ng ml⁻¹ (*n* = 96) after 1 h and it remained elevated for at least 6 h. In AMPT-treated rats, saline or SCH 23390 did not significantly change serum prolactin. Quinpirole significantly and dose-dependently decreased prolactin secretion (ED₅₀ = 4 μ g kg⁻¹) (Figure 1). Fenoldopam also significantly reduced prolactin secretion in a dose-dependent manner (ED₅₀ = 24 μ g kg⁻¹) (Figure 1). The maximal inhibitory effect of fenoldopam was significantly less pronounced than that of quinpirole (59.8 $\pm$ 3.5% vs. 93.0 $\pm$ 2.3%, *P* $\leq$ 0.01). Pretreatment with SCH 23390 (100 μ g kg⁻¹) reduced the effect of fenoldopam (prolactin levels were 40.7 $\pm$ 15.2 before and 35.6 $\pm$ 19.6 ng ml⁻¹ after administration of fenoldopam with SCH 23390, *n* = 6), but did not affect basal nor quinpirole-inhibited prolactin secretion (Figure 1).

In vitro experiments

Both, fenoldopam and quinpirole dose-dependently reduced prolactin secretion from cultured pituitary cells (Figure 2a,b). SCH 23390 provoked a dose-dependently rightward shift of the dose-response curve of fenoldopam (Figure 2a), but did

Figure 1 Concentration-response relationships for the effects of quinpirole (□), fenoldopam (Δ), quinpirole plus SCH 23390 (■) and fenoldopam plus SCH 23390 (▲) on prolactin secretion *in vivo* after pretreatment with α -methyl-*p*-tyrosine. Responses are calculated as a % decrease from the baseline level. Values are expressed as means from 6 to 12 experiments. Standard errors of the mean are not shown for reasons of clarity, but were less than 4.1%.

Figure 2 Prolactin secretion from cultured pituitary cells: effect of increasing concentrations of fenoldopam (a) and quinpirole (b). In the experiments with fenoldopam, cells were pretreated with saline (□), domperidone (10⁻⁶ M, ○), SCH 23390 (10⁻⁶ M, ●; 10⁻⁷ M, △ or 10⁻⁸ M, ▲). In the experiments with quinpirole, cells were pretreated with saline (□), SCH 23390 (10⁻⁶ M, ●), domperidone (10⁻⁶ M, ○; 10⁻⁷ M, △ or 10⁻⁸ M, ▲). Responses are calculated as a % decrease from the baseline value. Values are expressed as mean; $n = 12$. Standard errors of the mean are not shown for reasons of clarity, but were less than 2.8%.

not affect the dose-response curve of quinpirole (Figure 2b). Domperidone did not antagonize the prolactin lowering effect of fenoldopam (Figure 2a), but dose-dependently inhibited the effect of quinpirole (Figure 2b). Schild plot analysis revealed a pA_2 value of 8.83 for SCH 23390 (slope of the Schild regression = 0.96 and linearity, represented by $r = 0.98$) and a pA_2 value of 9.39 for domperidone (slope = 0.98 and $r = 0.97$).

Agents acting on 5-HT receptors have been found to affect prolactin secretion *in vivo* (Seeman & Grigoriadis, 1987). Therefore, we examined the ability of three 5-HT antagonists, mianserin, ketanserin and RU 24969 (each in a concentration of 10⁻⁶ M), to inhibit the prolactin lowering effect of fenoldo-

Figure 3 Specific binding of [3H]-SCH 23390 and [3H]-spiperone to pituitary membranes. Crude membrane preparations were incubated with increasing concentrations of radioligand as described in Methods. Data shown (means $\pm$ s.d. of 2 determinations) refer to specific binding of [3H]-SCH 23390 (○) and [3H]-spiperone (●). Inset: Scatchard transformation of [3H]-spiperone binding to pituitary membranes. Transformation of binding yielded a linear plot: K_D 0.58 nM; B_{max} 54 fmol mg⁻¹ protein.

Figure 4 Autoradiographic localization of [3H]-SCH 23390 and [3H]-spiperone. After 4 weeks of exposure, binding sites are visualized by black dots. Cells were counterstained with haematoxylin to visualise cell nuclei. Specific binding revealed D₂-receptors (a) and D₁-receptors (b). Non-specific binding was negative in both cases: (c) for D₂-receptors, and (d) for D₁-receptors. Scale bar represents 20 μ m.

pam. None of the antagonists had a significant effect on basal prolactin secretion or fenoldopam-induced inhibition of prolactin secretion.

Radioligand binding

No specific binding of [3H]-SCH 23390 (in a dose from 0.5 to 6 nM) was detected in crude membrane preparations of pituitary glands (Figure 3). Under the same experimental conditions, high affinity specific binding of [3H]-spiperone (from

Figure 5 Immunocytochemical labelling for prolactin of dispersed rat pituitary cells. Prolactin cells stained brownish (one-arrow, a), whereas immunocytochemical negative cells (two arrows, a) were identifiable only by their blue nucleus. Autoradiographic labelling (with [^3H]-SCH 23390) was only present on immunocytochemical positive cells (one arrow, b), whereas immunocytochemical negative cells were unlabelled with silver grains (two arrows, b). Scale bar represents 20 μm .

0.05 to 2.0 nM) to these membranes was observed (Figure 3). Scatchard analysis of [^3H]-spiperone binding showed an equilibrium dissociation constant of 0.58 nM and a B_{max} value of 54 fmol mg^{-1} protein.

Autoradiographic studies

No differences in labelling were found between fixed and unfixed cells. Non-specific binding was negative in both conditions. Specific binding sites for both [^3H]-SCH 23390 and [^3H]-spiperone were demonstrated on cultured cells (Figure

4). More cells were labelled for D_2 -receptors than for D_1 -receptors. Fenoldopam, in doses of 1 nM up to 1 μM , dose-dependently reduced the number of positive cells for [^3H]-SCH 23390, but had no effect on [^3H]-spiperone binding. Immunocytochemical staining of prolactin cells, after incubation with [^3H]-SCH 23390 or [^3H]-spiperone, is shown in Figure 5. Pituitary cells, staining for prolactin, also contained the granulae, representing [^3H]-SCH 23390 binding.

Effect on basal adenylate cyclase

Basal cyclic AMP production of the dispersed rat pituitary cells was 2.1 pmol/ 10^6 cells, and increased to 6.2 pmol/ 10^6 cells after administration of prostacyclin (PGE_1 , 20 μM) for 15 min. Under these experimental conditions, dopamine (0.1 μM , 1 μM , 10 μM , 100 μM and 1 mM) dose-dependently inhibited cyclic AMP production. Fenoldopam (0.1 μM , 1 μM , 10 μM , 100 μM and 1 mM) did not affect cyclic AMP formation (Table 1).

Discussion

Our results confirm and extend previous work showing the presence of specific D_2 -receptors on lactotroph cells, involved in prolactin secretion (Foord *et al.*, 1983; Enjalbert *et al.*, 1986). We and others (Dupont *et al.*, 1986; Boesgaard *et al.*, 1989) were unable to demonstrate any effect of fenoldopam, the most selective D_1 -receptor agonist currently available, on prolactin secretion in *in vivo* base-line conditions. This is in agreement with the currently accepted theory that the anterior pituitary dopamine receptors are of the D_2 -type (Caron *et al.*, 1978; Sibley *et al.*, 1982; Dumbille-Ross & Seeman, 1987) and that prolactin secretion is tonically inhibited by dopamine and that D_2 -receptor agonists, such as bromocriptine (Delpozo *et al.*, 1972) and quinpirole (Lefevre-Borg *et al.*, 1987) can lower prolactin. Blockade of the hypothalamic dopaminergic influence, by AMPT, resulted in an immediate increase in plasma prolactin. When the *in vivo* experiments were repeated after pretreatment with AMPT, fenoldopam, as well as quinpirole, provoked a significant and dose-dependent reduction of prolactin secretion. SCH 23390, a selective D_1 -receptor antagonist (Hyttel, 1983), abolished the effect of fenoldopam, suggesting that D_1 -receptors are also able to modulate the secretion of prolactin. The prolactin lowering effect of fenoldopam was less pronounced than that of quinpirole. This difference might be related to several factors, such as the number of D_1 - and D_2 -receptors, the intrinsic activity of the agonists used and the differences in the efficiency of the receptor-effector coupling mechanisms. In subsequently performed *in vitro* experiments we were able to demonstrate that both fenoldopam and quinpirole dose-dependently reduced prolactin secretion from cultured pituitary cells. These data indicate that this D_1 -receptor is localized on a pituitary cell rather than an extra-pituitary site (i.e. hypothalamic or at the portal vascular bed), as suggested by Cocchi *et al.* (1987). The pA_2 values for SCH 23390 and for domperidone, obtained by Schild plot analysis, are in agreement with the affinity of these drugs for D_1 - and D_2 -receptors respectively (Seeman & Grigoriadis, 1987). The 5-HT receptor antagonists used in this study did not significantly affect the fenoldopam-induced inhibition of prolactin secretion. Thus, the 5-HT $_{1A}$, 5-HT $_{1B}$ and 5-HT $_2$ receptor subtypes (Peroutka, 1988) are not involved. This is in agreement with the D_1 -selectivity of fenoldopam (Hahn *et al.*, 1982) and the fact that no 5-HT $_2$ receptors have been detected in the pituitary so far.

The dopamine receptors in anterior pituitary membranes have been studied in the past with different radiolabelled ligands, such as dihydroergocryptine, spiperone and dopamine itself (Creese *et al.*, 1983). In all these studies this binding site fulfilled the criteria of a D_2 -receptor. In this study, we also detected high affinity binding sites for [^3H]-spiperone in a crude membrane preparation from pituitary glands. The

Table 1 Adenosine 3':5'-cyclic monophosphate (cyclic AMP) contents of pituitary cells: effect of prostaglandin E_1 (PGE_1), dopamine and fenoldopam

Addition	Cyclic AMP (pmol/ 10^6 cells)
None	2.1 $\pm$ 0.3
PGE_1	6.2 $\pm$ 0.5
Dopamine (0.1 μM)	4.8 $\pm$ 0.4
Dopamine (1 μM)	4.4 $\pm$ 0.3
Dopamine (10 μM)	4.4 $\pm$ 0.2
Dopamine (100 μM)	4.1 $\pm$ 0.2
Dopamine (1 mM)	4.0 $\pm$ 0.2
Fenoldopam (0.1 μM)	2.1 $\pm$ 0.2
Fenoldopam (1 μM)	2.3 $\pm$ 0.3
Fenoldopam (10 μM)	2.0 $\pm$ 0.2
Fenoldopam (100 μM)	2.4 $\pm$ 0.2
Fenoldopam (1 mM)	2.3 $\pm$ 0.2

Cells were treated for 15 min at 37°C with the indicated compounds after which the cyclic AMP content was measured as described in Methods. Values are means $\pm$ s.e. mean of 4 determinations.

binding parameters (K_d and B_{max}) are similar to those previously reported for pituitary D_2 -receptors (Seeman & Grigoriadis, 1987). In agreement with the study of Rovescalli *et al.* (1987) no specific binding for [3H]-SCH 23390 could be demonstrated, indicating that no binding sites for [3H]-SCH 23390 are present or that the binding site is lost during preparation. This latter explanation is favoured by the autoradiographic studies on cultured cells, which revealed specific binding sites for both [3H]-spiperone and [3H]-SCH 23390. As fenoldopam dose-dependently reduces [3H]-SCH 23390 binding and has no effect on [3H]-spiperone binding, the binding sites for [3H]-SCH 23390 appear to correspond to D_1 -receptors, rather than to D_2 -receptors. [3H]-SCH 23390, as well as [3H]-spiperone, granulae were present on lactotroph cells, as demonstrated by immunohistochemical labelling, suggesting that D_1 - and D_2 -binding sites are located on the prolactin secreting cells.

In view of the initial assumption that D_1 - and D_2 -receptors exert an opposite control on the adenylate cyclase activity (Swennen & Denef, 1982; Stoof & Kebabian, 1981), it seems contradictory that both receptors are capable of inhibiting prolactin release. However, D_1 - and D_2 -receptors have been described as controlling other membrane signalling pathways as well (Andersen *et al.*, 1990); indeed, the chain of molecular events, linking receptor stimulation to inhibition of prolactin release, still remains to be elucidated. D_1 -receptors have for example been shown to stimulate the phosphatidylinositol turnover pathway (Berridge, 1987); the proximal tubular D_1 -receptor e.g. has been shown to stimulate phospholipase-C

activity independent of adenylate cyclase (Felder *et al.*, 1989). Moreover, a D_1 -receptor, not linked to adenylate cyclase, has recently also been identified in the amygdala (Mailman *et al.*, 1986), the stellate ganglia (Sabouni *et al.*, 1987) and the human frontal cortex (De Keyser *et al.*, 1988b). In this context, the failure of fenoldopam to increase the cyclic AMP levels in pituitary cells might reflect linking of pituitary D_1 -receptors to an alternative membrane signalling pathway. Dopamine itself did not inhibit cyclic AMP formation of dispersed rat pituitary cells, which can be explained by the relatively low basal level of cyclic AMP. Malfunctioning of the adenylate cyclase system was ruled out on basis of the ability of PGE₁ to produce a 3 fold increase in the cellular cyclic AMP levels.

We conclude from our pharmacological, radioligand binding, autoradiographic and adenylate cyclase studies that, in contrast to the report of Kebabian & Calne (1979), the anterior pituitary gland contains more than one type of dopamine receptor. It is tempting to speculate that fenoldopam reduces prolactin secretion through interaction with a non-cyclase-linked D_1 -receptor on the lactotroph cells.

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