0026-895X/91/040531-09$03.00/0

MOLECULAR PHARMACOLOGY, 39:531-539

Regulation of Dopamine D2 Receptors in a Novel Cell Line (SUP1)

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Received April 17, 1990; Accepted January 17, 1991

SUMMARY

A prolactin-secreting cell line, SUP1, has been established from rat pituitary tumor 7315a. In radioligand binding experiments, the D2 receptor antagonist (*S*)-(−)-3-[^{125}I]iodo-2-hydroxy-6-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide ([^{125}I]IBZM) labeled a single class of sites in homogenates of SUP1 cells (K_d = 0.6 nM; B_{max} = 45 fmol/mg of protein). The sites displayed a pharmacological profile consistent with that of D2 receptors. Inhibition of the binding of [^{125}I]IBZM by dopamine was sensitive to GTP, suggesting that D2 receptors in SUP1 cells are coupled to guanine nucleotide-binding protein(s). In the presence of isobutylmethylxanthine, dopamine decreased the level of cAMP accumulation in SUP1 cells. Dopamine also inhibited prolactin secretion from SUP1 cells. Both the inhibition of cAMP accumulation and the inhibition of prolactin secretion were blocked by D2 receptor antagonists, suggesting that these effects of dopamine were mediated by an interaction with D2 receptors. The regulation of D2 receptors in SUP1 cells by D2 receptor agonists was investigated. Exposure of SUP1 cells to dopamine or to the D2 receptor agonist *N*-propylnorapomorphine led to increased expression of D2 receptors, with no change in the affinity of the receptors for [^{125}I]IBZM. An increase in the density of D2 receptors in SUP1 cells was evident within 7 hr of exposure to dopamine. Spiroperidol, a D2 receptor antagonist, blocked the effect of dopamine on receptor density. These results suggest that exposure of D2 receptors in SUP1 cells to agonists leads to an up-regulation of D2 receptors. Dopamine retained the ability to inhibit cAMP accumulation in SUP1 cells exposed to dopamine for 24 hr, suggesting that D2 receptors in SUP1 cells are not desensitized by prolonged exposure to agonist. SUP1 cells should be a useful model system for future studies of the regulation of the expression and function of D2 receptors in cultured cells.

Dopamine receptors have been classified into D1 and D2 subtypes on the basis of pharmacological and biochemical criteria (for review, see Ref. 1). D1 and D2 receptors are widely distributed throughout the central nervous system. Although the density of D2 receptors is less than the density of D1 receptors in most regions of the brain (2), D2 receptors have been implicated in the pathophysiology of disorders including schizophrenia and tardive dyskinesia (3).

The regulation of the density and function of D2 receptors in the striatum has been investigated in experiments involving drug treatments and lesions of dopaminergic tracts. Denervation of dopaminergic tracts to the striatum with 6-hydroxydopamine and blockade of D2 receptors with neuroleptics leads to an increase in the density of receptors, accompanied by a behavioral supersensitivity to D2 receptor agonists (4). Stimulation of D2 receptors with agonists might be expected to lead to a decrease in receptor density and to desensitization of D2 receptor-mediated responses. However, the effect of exposure to agonists on D2 receptors is not clear. Treatment of animals with L-3,4-dihydroxyphenylalanine, an indirect D2 receptor agonist, does not consistently result in a decrease in the density of D2 receptors (for review, see Refs. 5 and 6). Paradoxically, treatment of animals with the indirect agonist amphetamine leads to a supersensitive behavioral response to D2 receptor agonists (7).

The pituitary has been used as a model tissue for the study of D2 receptors, because this tissue expresses only the D2 subtype of dopamine receptors. Dopamine interacts with D2 receptors in lactotrophs of the anterior pituitary and inhibits prolactin secretion (8). In primary cultures of cells from the anterior pituitary, activation of D2 receptors by dopamine results in a decrease in cAMP accumulation (9), a decrease in phosphoinositide formation and/or hydrolysis (10), and a decrease in levels of intracellular calcium (11) that may be secondary to an increase in potassium conductance (12). Any of these effects of dopamine might inhibit prolactin secretion,

This work was supported by United States Public Health Service Grants NS-18591 and NS-18479 and by a Grant-in-Aid from the American Heart Association. K.J.I. was supported by a predoctoral fellowship from the National Science Foundation.

ABBREVIATIONS: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DMEM, Dulbecco's modified Eagle's medium; B_{max}, maximal number of binding sites; IBZM, (*S*)-(−)-3-iodo-2-hydroxy-6-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide; BSA, bovine serum albumin; IBMX, isobutylmethylxanthine; NPA, *N*-propylnorapomorphine; 5-HT, serotonin; G protein, guaninenucleotide-binding protein.

because vasoactive intestinal peptide increases prolactin secretion through stimulation of adenylyl cyclase activity (13), thyrotropin-releasing hormone increases prolactin secretion through stimulation of phosphoinositide hydrolysis (14), and elevated intracellular calcium levels promote secretory events in many types of cells, including lactotrophs (9).

It has been difficult to study the coupling of D2 receptors to second messenger systems in lactotrophs, because cells in primary cultures of anterior pituitary are heterogeneous and paracrine interactions may occur. Methods of dispersion of pituitary tissue into single-cell suspensions, with subsequent enrichment of a given cell type by differential centrifugation, have been described (12, 15). These methods do not result in pure preparations of lactotrophs. An alternative approach has been the use of carcinogen- or estrogen-induced tumors of lactotroph origin. The 7315a transplantable pituitary tumor is one example of such a tumor (16). It was induced by administration of trimethylaniline to a female Buffalo rat and has been maintained in Buffalo rats for more than 20 years. 7315a tumors secrete prolactin (16) and express D2 receptors coupled to the inhibition of adenylyl cyclase activity (17, 18). Cells dispersed from 7315a tumors have been used as a model system to investigate some of the properties of lactotrophs, because relatively large numbers of lactotrophs, uncontaminated by other cells of pituitary origin, can be obtained. Results of studies with 7315a cells suggested that dopamine interacts with D2 receptors in these cells and inhibits prolactin secretion stimulated by increased intracellular calcium but does not affect basal prolactin secretion (19). The ability of dopamine to inhibit prolactin secretion was augmented by the presence of the antiestrogen tamoxifen, suggesting that estrogens may counteract the ability of dopamine to inhibit prolactin secretion from 7315a cells (20). Estrogens have also been shown to block the effect of dopamine on prolactin secretion in primary cultures of cells from the anterior pituitary (21). Because the ability of dopamine to inhibit prolactin secretion is dependent on the presence of functional D2 receptors in lactotrophs, it has been suggested that estrogens antagonize the effects of dopamine by causing a decrease in the density of pituitary D2 receptors (22, 23) or an uncoupling of D2 receptors from adenylyl cyclase (24, 25). Estrogens have also been shown to cause a decrease in the density of D2 receptors in tumors derived from the anterior pituitary (26).

The density and function of D2 receptors may be regulated by changes in hormonal status as well as by exposure to agonists or antagonists. Because it is possible to control the exposure of cultured cells to dopamine, other neurotransmitters, and steroids, the availability of lactotroph cell lines expressing D2 receptors would aid studies of the regulation of D2 receptors as well as studies of the coupling of these receptors to second messenger systems and to prolactin secretion. Previous attempts to use prolactin-secreting tumors to establish permanent cell lines that maintain the expression of D2 receptors have been largely unsuccessful. One important exception appears to be the MMQ cell line that was recently established from the 7315a tumor (27). MMQ cells respond to dopamine with a decrease in forskolin-stimulated cAMP accumulation and a decrease in forskolin-stimulated prolactin secretion. The ability of the D2 antagonist haloperidol to blunt these effects of dopamine suggests that they are mediated by D2 receptors. The human retinoblastoma cell lines WERI 27 and Y-79 have been reported to express D2 receptors, as measured in radioligand binding assays (28, 29). Serum deprivation and exposure to dibutyryl-cAMP cause Y-79 cells to differentiate and to express a high density of D2 receptors that are functionally coupled to the inhibition of adenylyl cyclase activity (29).

We now report the establishment of a cell line (SUP1) from the 7315a tumor that shares several characteristics of the MMQ cell line; in SUP1 cells, as in MMQ cells, dopamine inhibits cAMP accumulation and prolactin secretion through an interaction with D2 receptors. In addition, the results of radioligand binding assays demonstrate that SUP1 cells express a measurable density of D2 receptors. We have used SUP1 cells as a model system to investigate the regulation of receptor expression in cultured cells. We report here that the density of D2 receptors in SUP1 cells is increased after exposure of the cells to D2 receptor agonists.

Materials and Methods

Establishment of SUP1 cell line. Female Buffalo rats carrying the 7315a tumor (donated by R. M. MacLeod, University of Virginia, Charlottesville, VA) were sacrificed by decapitation. Tumors were dissected from adhering tissue and incubated at room temperature for 30 min in L15 medium buffered with 15 mM HEPES (pH 7.4), supplemented with 100 units/ml penicillin and 100 μg/ml streptomycin. Tissue was then minced with a razor blade and incubated for 15 min at 37° in L15 medium containing 15 mM HEPES (pH 7.4), 0.4% collagenase, 0.001% DNAse, 0.125% hyaluronidase, 1% BSA, and penicillin/streptomycin. After centrifugation for 5 min at 180 × *g*, the tissue was resuspended in medium A [DMEM without phenol red (GIBCO), supplemented with 10% fetal bovine serum (HyClone) that had been treated with charcoal to remove biogenic amines and steroids (30), 2 mM glutamine, 150 μg/ml oxaloacetate, 50 μg/ml pyruvate, 0.2 unit/ml insulin, and penicillin/streptomycin]. A single-cell suspension was obtained by filtration of the tissue through a Millipore Swinnex-25 filter. Cells were again collected by centrifugation, resuspended in medium A, seeded in T-25 tissue culture flasks, and grown in an atmosphere of 10% CO_2 in air. After several days, cells growing in suspension were cloned by limiting dilution. Individual clones were expanded and screened for the presence of binding sites for [^{125}I]IBZM. One of the cell lines that expressed binding sites for [^{125}I]IBZM, designated SUP1, was recloned and used in subsequent experiments.

Experiments reported here were carried out over an extended period of time, and several different lots of fetal bovine serum were used in the culture medium. When cultured in the presence of different lots of serum, SUP1 cells expressed different densities of D2 receptors (from 25 to 80 fmol/mg of protein; compare Figs. 1 and 7, for example). However, regardless of the initial density of receptors in the cells, the change in the density of receptors observed after experimental manipulations was reproducible.

Cell culture. SUP1 cells were grown in medium A and were maintained in logarithmic phase growth (less than 10^6 cells/ml) in T-75 or T-175 flasks (Falcon). Cells were triturated and fed or passaged every third day. Drug treatments involved addition of drugs or vehicle to flasks of cells at densities of 4–6 × 10^5 cells/ml. Dopamine and NPA were prepared as 50 mM solutions in 2.5 mM ascorbate containing 0.002% BSA. Spiroperidol was prepared as a 5 mM solution in dimethyl formamide.

Radioligand binding assays. Cells were collected by centrifugation for 10 min at 180 × *g*, washed once in phosphate-buffered saline (138 mM NaCl, 4 mM KCl, 5 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 11 mM glucose, pH 7.4), lysed in hypotonic buffer (5 mM HEPES, 5 mM EDTA, pH 7.4) for 10 min at 4°, homogenized with a Brinkmann Polytron, and centrifuged for 15 min at 30,000 × *g*. Pellets were resuspended in 20 mM HEPES buffer (pH 7.4) containing 10 mM EDTA and 154 mM NaCl, homogenized, and incubated for 20 min at

37°. In experiments using cells that had been exposed to agonists and antagonists, the incubation buffer included 300 μM GTP. After centrifugation for 15 min at 30,000 × *g*, the tissue was resuspended in 50 mM Tris buffer (pH 7.4) containing 154 mM NaCl, for use in binding assays.

Binding assays with the D2 receptor antagonist [^{125}I]IBZM (0.05–5 nM) were initiated by the addition of 50 μl of tissue to 50 μl of 50 mM Tris buffer (pH 7.4) containing 10 mM EDTA, 154 mM NaCl, 0.004% BSA, [^{125}I]IBZM, and appropriate drugs. In experiments involving studies of the effects of GTP, the concentration of NaCl in the assay buffer was reduced to 120 mM, 1 mM $MgCl_2$ was included, and EDTA was omitted. In experiments using agonists, 300 μM GTP was routinely included. The [^{125}I]IBZM used in most experiments was prepared as previously described by Schonwetter *et al.* (31). The [^{125}I]IBZM used in recent experiments was supplied by New England Nuclear. Assays were incubated for 60 min at 37° and stopped by the addition of 5 ml of 10 mM Tris buffer (pH 7.4) containing 154 mM NaCl. Samples were filtered through glass fiber filters (Schleicher and Schuell no. 30) coated with protamine (2.5 mg/ml). Filters were washed three times with 5 ml of the same buffer and then dried under vacuum, and radioactivity retained on the filters was determined. Saturation binding data were transformed by the method of Scatchard (32). K_d and B_{max} values were estimated by linear regression analysis. Competition binding data were modeled to one-site and two-site equations using the NEWFITSITES program of the PROPHET software package available through the National Institutes of Health. IC_{50} values and Hill coefficients were obtained as described by Lin *et al.* (18). IC_{50} values were converted to K_i values with the Cheng and Prusoff equation (33). Protein was determined by the method of Lowry *et al.* (34).

Measurement of cAMP accumulation. cAMP accumulation in intact cells was measured using a modification of the method of Jones *et al.* (35). Cells at a density of 0.5–1 × 10^6 cells/ml were labeled with [^{3}H]adenine (1 μCi/ml) for 1 hr. Cells were washed with medium B (DMEM without phenol red, supplemented with 100 μg/ml BSA) and resuspended in medium B at 4 × 10^6 cells/ml. Assays were initiated by the addition of 10^6 cells to 250 μl of medium B containing IBMX (final concentration, 1 mM), ascorbate (final concentration, 70 μM), and drugs as indicated. Cells were incubated for 5 min at 37° in an atmosphere of 10% CO_2 in air. Assays were terminated by the addition of 1 ml of trichloroacetic acid (final concentration, 5%). Sequential chromatography on Dowex and alumina columns was used to separate [^{3}H]cAMP from [^{3}H]ADP and [^{3}H]ATP. Radioactivity was determined, and cAMP accumulation was expressed as the percentage of conversion of [^{3}H]ATP to [^{3}H]cAMP.

Measurement of prolactin secretion. Cells collected by centrifugation at 180 × *g* were resuspended in medium B at approximately 10^7 cells/ml. Aliquots (100 μl) of the cells were added to 200 μl of medium B containing drugs as indicated. Cells were incubated at 37° for 1 hr in an atmosphere of 10% CO_2 in air. Aliquots of the supernatant were removed and stored at −20° until prolactin content was determined by radioimmunoassay, essentially as described by Munemura *et al.* (25), using antiserum and prolactin standards supplied by the National Hormone and Pituitary Program (Baltimore, MD).

Results

Characterization of SUP1 cells. Cells were cultured from collagenase-dispersed 7315a tumors as described in Materials and Methods. Cells were cloned by limiting dilution, and several cell lines were established. In preliminary experiments, cell lines were screened for the presence of binding sites for [^{125}I]IBZM, a selective D2 receptor antagonist (31). One of the cell lines expressing binding sites, SUP1, was selected for use in subsequent experiments.

SUP1 cells were grown in suspension in medium that contained charcoal-treated fetal calf serum. Phenol red, a weak estrogen, was omitted from the medium. Cells settled to the bottom of tissue culture flasks and formed aggregates as they divided. Under the culture conditions used, the cells divided with a $t_{1/2}$ of approximately 30 hr. Individual cells appeared to be fairly small, with a diameter of 10–15 μm. Viability of triturated cells, as determined by trypan blue exclusion, was typically greater than 95%. The cells have been in culture for 2 years, and their characteristics have been stable over this period.

The expression of D2 receptors by SUP1 cells was investigated using the D2 receptor antagonist [^{125}I]IBZM. Specific binding of [^{125}I]IBZM to membranes prepared from SUP1 cells was saturable when nonspecific binding was defined with 2 μM (+)-butaclamol (Fig. 1). The data were best fit by a one-site model, with a K_d of 0.58 ± 0.05 nM and a B_{max} of 45 ± 5 fmol/mg of protein (24 determinations). Scatchard transformation of the data resulted in a linear plot, consistent with the existence of a single class of binding sites with a high affinity for [^{125}I]IBZM (Fig. 1, *inset*). Specific binding represented approximately 60% of total binding at the K_d for [^{125}I]IBZM.

The binding sites for [^{125}I]IBZM in SUP1 cells were pharmacologically characterized in competition experiments. When the binding of [^{125}I]IBZM to SUP1 membranes was inhibited by increasing concentrations of dopamine, the resulting competition curve had a Hill coefficient significantly less than 1 and was best fit by a two-site model (Fig. 2). In the presence of GTP, the competition curve was shifted to the right and was best fit by a one-site model, with a Hill coefficient of 1. In subsequent competition experiments using agonists, inhibition of the binding of [^{125}I]IBZM to membranes prepared from SUP1 cells was studied in the presence of 300 μM GTP (Fig. 3A). The resulting competition curves were best fit by a one-site model, with Hill coefficients of approximately 1. The order of potency of the agonists, bromocriptine > NPA > dopamine > 5-HT, was consistent with the order of potency of these drugs at D2 receptors. K_i values for these drugs were calculated, and the values obtained were similar to K_i values for these drugs at D2 receptors in the 7315a tumor and in rat striatum (Table 1).

Inhibition of the binding of [^{125}I]IBZM by the antagonists (+)-butaclamol, domperidone, sulpiride, and ketanserin resulted in monophasic competition curves that were best fit by a one-site model, with Hill coefficients of approximately 1 (Fig.

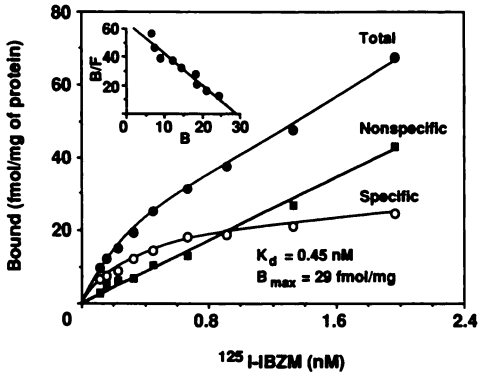

Fig. 1. Binding of [^{125}I]IBZM to membranes prepared from SUP1 cells. Increasing concentrations of [^{125}I]IBZM (0.12–2.0 nM) were incubated with membranes prepared from SUP1 cells. Nonspecific binding was defined with 2 μM (+)-butaclamol. Analysis of untransformed data indicated that [^{125}I]IBZM labeled a single class of sites (K_d = 0.45 nM, B_{max} = 29 fmol/mg of protein). Data shown are means of triplicate determinations in one of 24 similar experiments. ●, Total binding; ■, nonspecific binding; ○, specific binding. *Inset*, Scatchard transformation of specific binding. *B*, bound; *F*, free.

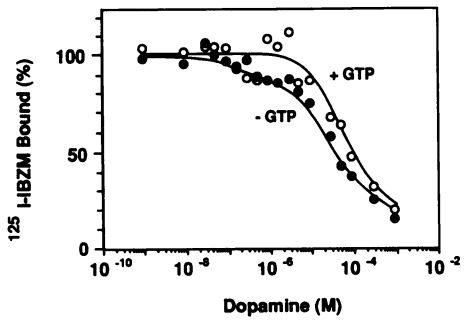

Fig. 2. GTP sensitivity of the inhibition of the binding of [^{125}I]IBZM by dopamine. The binding of [^{125}I]IBZM (0.66 nM) to membranes prepared from SUP1 cells was inhibited by increasing concentrations of dopamine in the absence (●) or presence (○) of 300 μM GTP. In the absence of GTP, the competition curve was best fit by a two-site model. The ratio of high affinity sites (IC_{50} = 160 nM) to low affinity sites (IC_{50} = 25 μM) was approximately 1:5. In the presence of GTP, the competition curve was best fit by a one-site model (IC_{50} = 36 μM; n_H = 1.0). Data shown are means of triplicate determinations in one of four similar experiments.

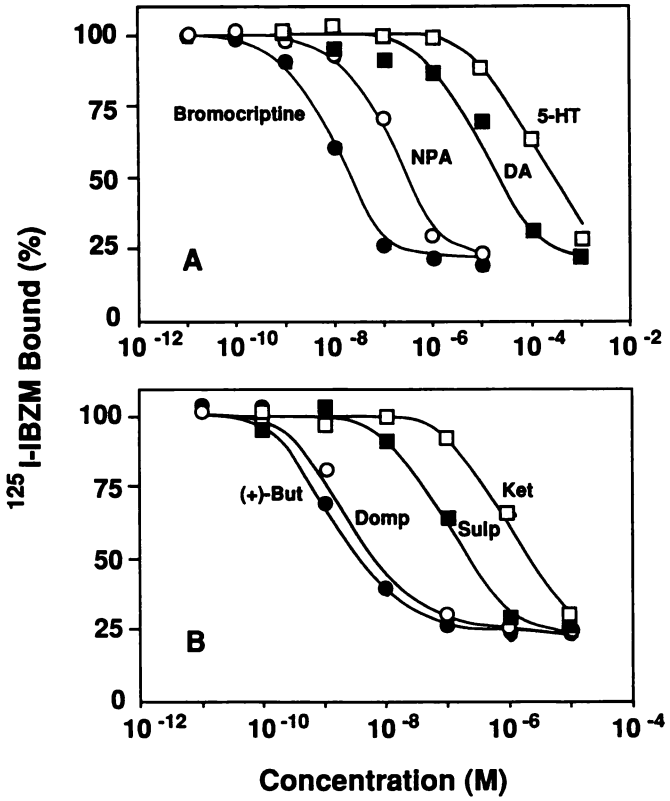

Fig. 3. Inhibition of the binding of [^{125}I]IBZM by competing ligands. A, The binding of 0.73 nM [^{125}I]IBZM to membranes prepared from SUP1 cells was inhibited by increasing concentrations of agonists in the presence of 300 μM GTP. The competition curves generated were best fit by a one-site model with a Hill coefficient of approximately 1. ●, Bromocriptine; ○, NPA; ■, dopamine; □, 5-HT. B, The binding of 0.28 nM [^{125}I]IBZM to membranes prepared from SUP1 cells was inhibited by increasing concentrations of antagonists. The competition curves generated were best fit by a one-site model. ●, (+)-Butaclamol; ○, domperidone; ■, sulpiride; □, ketanserin. Data shown are means of triplicate determinations in one of six similar experiments.

3B). The order of potency of these drugs, (+)-butaclamol > domperidone > sulpiride > ketanserin, was consistent with their order of potency at D2 receptors. The inactive stereoisomer (−)-butaclamol was 5000-fold less potent than (+)-butaclamol. K_i values calculated using the competition data were similar to values for these drugs at D2 receptors in the 7315a tumor and in rat striatum (Table 1).

TABLE 1

Pharmacological profile of the binding sites for [^{125}I]IBZM in SUP1 cells

K_i values for agonists and antagonists were calculated from IC_{50} values using the Cheng and Prusoff equation (33). Values shown are means ± standard errors (six determinations). Published K_i values for these drugs at D2 receptors in 7315a tumors and rat striatum are included for comparison.

	K_i		
	SUP1 cells	7315a tumor[a]	Striatum[a]
	nM		
Bromocriptine	4.9 ± 0.8	0.8	0.4
NPA	54 ± 17	18	6.5
Dopamine	8,800 ± 1,600	9,000	5,300
5-HT	45,000 ± 12,000	ND	ND
(+)-Butaclamol	1.7 ± 0.3	0.7	0.3
Domperidone	3.4 ± 0.5	1.2	0.3
Sulpiride	49 ± 13	70	24
Ketanserin	510 ± 170	490	300
(−)-Butaclamol	8,800 ± 2,300	ND	ND

[a] Lin *et al.* (18).
[b] ND, not determined.

The ability of D2 receptors in SUP1 cells to couple to the adenylyl cyclase second messenger system was investigated. In these experiments, cAMP accumulation in SUP1 cells was measured in the presence of 1 mM IBMX. Dopamine and the D2-selective agonist NPA caused monophasic, dose-dependent decreases in forskolin-stimulated cAMP accumulation in SUP1 cells (Fig. 4A). The maximal percentage of inhibition of cAMP accumulation was 23 ± 3% (25 determinations). The D2 receptor antagonists spiroperidol and sulpiride inhibited the effect of dopamine on cAMP accumulation more potently than did the α_2-adrenergic receptor antagonist yohimbine or the D1 receptor antagonist SCH23390 (Fig. 4B). Therefore, the ability of dopamine to inhibit cAMP accumulation in SUP1 cells appears to be mediated by an interaction with D2 receptors. SCH23390 did not potentiate the inhibition of cAMP accumulation produced by dopamine (data not shown).

To determine whether the inhibition of cAMP accumulation by dopamine was mediated by a G protein that is sensitive to pertussis toxin, SUP1 cells were exposed to pertussis toxin (25 ng/ml) for 24 hr, and the ability of dopamine to inhibit basal and forskolin-stimulated cAMP accumulation was measured. In control cells, the percentage of inhibition of basal cAMP accumulation by dopamine was similar to the percentage of inhibition of forskolin-stimulated cAMP accumulation (Fig. 5). However, in cells that had been treated with pertussis toxin, dopamine had no effect on basal or forskolin-stimulated cAMP accumulation (Fig. 5).

Exposure of SUP1 cells to dopamine resulted in inhibition of prolactin secretion. Prolactin released by SUP1 cells during a 1-hr period, in the presence and absence of forskolin, was measured by radioimmunoassay. Basal secretion of prolactin from SUP1 cells was approximately 14 ng/10^5 cells × hr (Fig. 6). In comparison, basal prolactin secretion from dispersed 7315a cells has been reported to be 3.5 ng/10^5 cells × hr, and basal secretion from cells dispersed from rat anterior pituitary was 48 ng/10^5 cells × hr (19). Prolactin secretion from SUP1 cells was stimulated when assays were carried out in the presence of 3 μM forskolin. Dopamine (100 μM) inhibited basal and forskolin-stimulated prolactin secretion from SUP1 cells (Fig.

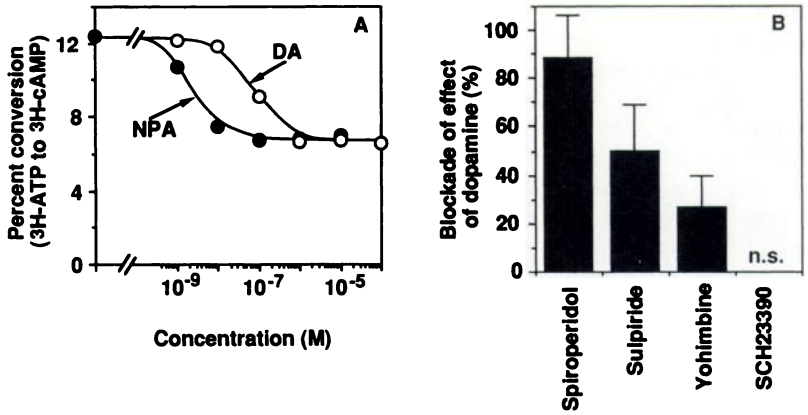

Fig. 4. Inhibition of cAMP accumulation in SUP1 cells by D2 receptor agonists. SUP1 cells, prelabeled by incubation for 1 hr with [^{3}H]adenine (1 μCi/ml), were incubated with drugs for 5 min in the presence of 1 mM IBMX and forskolin (10 μM). cAMP accumulation was expressed as the percentage of conversion of [^{3}H]ATP to [^{3}H]cAMP. A, Forskolin-stimulated cAMP accumulation was inhibited by increasing concentrations of dopamine (○) and NPA (●). The IC_{50} of dopamine was approximately 300 nM; the IC_{50} of NPA was approximately 5 nM. Data shown are means of triplicate determinations in one of four similar experiments. B, The ability of 1 μM spiroperidol, sulpiride, yohimbine, or SCH23390 to block the inhibition of cAMP accumulation produced by 100 μM dopamine was measured. The blockade produced by SCH23390 was not significant (*n.s.*) (−4 ± 9%). Data shown are means ± standard errors of data from three experiments.

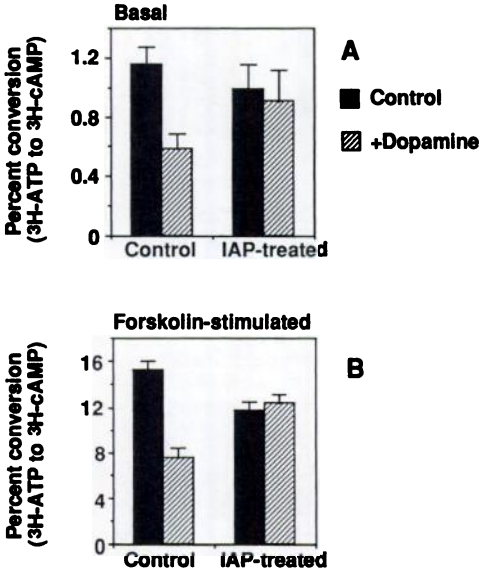

Fig. 5. Effect of pertussis toxin on the inhibition of cAMP accumulation by dopamine. SUP1 cells were exposed to pertussis toxin (*IAP*) (25 ng/ml) or vehicle for 24 hr. Cells were washed, and the effect of dopamine on basal (A) and 10 μM forskolin-stimulated (B) cAMP accumulation was measured. Data shown are means ± standard deviations of replicate determinations in one of three similar experiments.

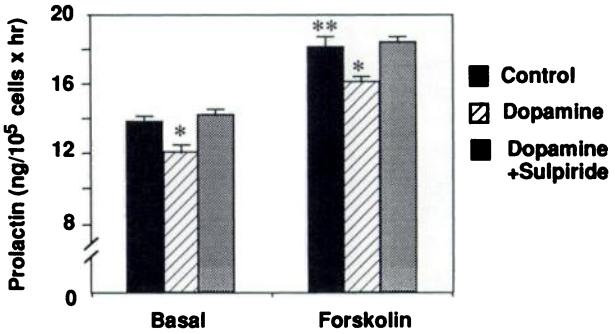

Fig. 6. Inhibition of prolactin secretion by dopamine. Aliquots of SUP1 cells were incubated for 1 hr in DMEM containing 100 μg/ml BSA, with or without forskolin (3 μM), dopamine (100 μM), and/or sulpiride (10 μM). Prolactin released into the medium was measured by radioimmunoassay. Shown are means ± standard errors of data from five samples in one of three similar experiments. *, $p < 0.05$ versus prolactin release in the absence of dopamine; **, $p < 0.05$ versus basal prolactin release.

6). The effect of dopamine on prolactin secretion was blocked by sulpiride (10 μM), suggesting that dopamine was acting through D2 receptors. In other experiments, SUP1 cells were grown for 3 days in medium that was supplemented with phenol red at the concentration present in normal DMEM (40 μM). These cells secreted 15–20% more prolactin than cells grown in the absence of phenol red, suggesting that SUP1 cells retained sensitivity to estrogen. Prolactin secretion from cells exposed to phenol red for this period of time was also inhibited by dopamine (data not shown).

Regulation of D2 receptors in SUP1 cells. The ability of dopamine to affect the density of D2 receptors in SUP1 cells was investigated. In initial experiments, cells were exposed to 100 μM dopamine or vehicle for 24 hr, and saturation binding of [^{125}I]IBZM was used to measure the density of D2 receptors

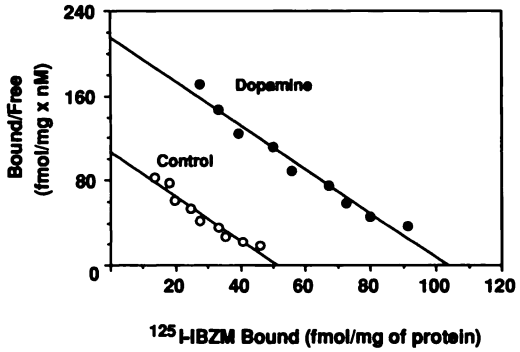

Fig. 7. Scatchard analysis of the binding of [^{125}I]IBZM to SUP1 cells after exposure of cells to dopamine. Dopamine (100 μM) or vehicle was added to flasks containing SUP1 cells. Cells were harvested 24 hr later, and membranes were prepared for binding assays. The specific binding of [^{125}I]IBZM (0.2–2.5 nM) was analyzed by the method of Scatchard. The following values were obtained from these plots: control cells, K_d = 0.48 nM; B_{max} = 51 fmol/mg of protein; treated cells, K_d = 0.48 nM; B_{max} = 103 fmol/mg of protein. Data shown are representative of those obtained in seven similar experiments.

(Fig. 7). The density of D2 receptors in SUP1 cells exposed to dopamine for 24 hr was significantly greater than the density of D2 receptors in control cells (Fig. 7). The affinity of the D2 receptors for [^{125}I]IBZM was not affected by the drug treatment. Exposure of SUP1 cells to dopamine for 24 hr had no effect on cell viability, as determined by trypan blue exclusion. However, the rate of cell growth was decreased by dopamine; after 24 hr of exposure to dopamine, the number of cells was approximately 85% of control values.

The apparent up-regulation of D2 receptors in SUP1 cells after exposure to dopamine could not be accounted for by the presence of residual dopamine in the membranes, because, as expected, the addition of dopamine to binding assays resulted in an increase in the K_d for [^{125}I]IBZM, with no change in the B_{max} (data not shown). When SUP1 cells were exposed to dopamine for periods of time ranging from 15 min to 21 hr, a time-dependent increase in the density of D2 receptors was observed (Fig. 8). The increase in the density of receptors was significant within 7 hr of exposure to dopamine. At no time was there a change in the affinity of the receptors for [^{125}I] IBZM.

In other experiments, cells were treated with the D2 receptor agonists dopamine and NPA and with the D2 receptor antagonist spiroperidol for 24 hr. Exposure of cells to dopamine or NPA resulted in an increase in the density of D2 receptors in SUP1 cells, with no change in the affinity of the receptors for [^{125}I]IBZM (Table 2). The effect of dopamine was blocked by spiroperidol (Table 2). The K_d of D2 receptors for [^{125}I]IBZM was increased slightly in membranes from cells that had been treated with spiroperidol, suggesting that residual spiroperidol was present in the membranes. However, spiroperidol had no effect on the density of D2 receptors in SUP1 cells.

To determine whether exposure of SUP1 cells to dopamine caused desensitization of D2 receptor-mediated responses, cAMP accumulation was measured in SUP1 cells that had been exposed to dopamine for 24 hr (Fig. 9). Basal (Fig. 9) and forskolin-stimulated cAMP accumulation was increased in cells pretreated with dopamine. The increase in cAMP accumulation was not due to the abrupt withdrawal of dopamine from the cells, because it was also observed in cells that had been exposed continuously to dopamine (data not shown). Dopamine retained the ability to inhibit cAMP accumulation in cells exposed to dopamine for 24 hr, suggesting that D2 receptors in these cells had not been desensitized (Fig. 9). The magnitude of the inhibition of cAMP accumulation by dopamine in pretreated cells (20 ± 3%; four experiments) was not significantly different from the magnitude of the inhibition by dopamine in control cells (14 ± 3%; four experiments).

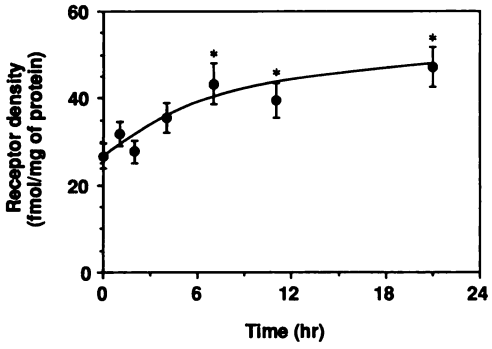

Fig. 8. Time course of the up-regulation of D2 receptors in SUP1 cells by dopamine. Dopamine (100 μM) was added to flasks of SUP1 cells so that cells were exposed to dopamine for 0, 1, 2, 4, 7, 11, or 21 hr. Cells were harvested, and membranes were prepared for binding assays. The density of D2 receptors was determined by Scatchard analysis of the saturation binding of [^{125}I]IBZM. Data shown are means ± standard errors of B_{max} values from four experiments. *, $p < 0.05$, compared with the density in cells not exposed to dopamine.

TABLE 2

Effects of dopamine, NPA, and spiroperidol on the density of D2 receptors in SUP1 cells

Cells were treated with dopamine (100 μM), NPA (10 μM), and/or spiroperidol (1 μM). Cells were harvested 24 hr later, and membranes were prepared for binding assays. D2 receptor densities and affinities were estimated from Scatchard plots of the binding of [^{125}I]IBZM. Data shown are means ± standard errors (four determinations).

Drug treatment	B_{max}	K_d
	fmol/mg of protein	*nM*
Control	64 ± 9	0.8 ± 0.2
Dopamine	146 ± 21*	0.9 ± 0.2
NPA	206 ± 44**	0.9 ± 0.1
Spiroperidol	68 ± 17	1.7 ± 0.7
Dopamine + spiroperidol	71 ± 10	1.6 ± 0.3

*$p < 0.01$, **$p < 0.01$ compared with control by ANOVA followed by Dunnet's post-hoc comparison.

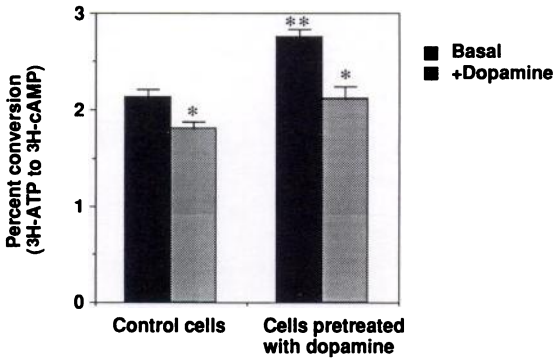

Fig. 9. cAMP accumulation in SUP1 cells after prolonged exposure to dopamine. SUP1 cells were exposed to 100 μM dopamine or vehicle for 24 hr. During the last hour of this treatment, the cells were labeled with [^{3}H]adenine. The incorporation of [^{3}H]adenine into ATP was similar in cells exposed to dopamine and in control cells. The ability of dopamine (100 μM) to inhibit cAMP accumulation in the presence of 1 mM IBMX was measured. Shown are means ± standard deviations of data from one of four representative experiments. *, $p < 0.01$ versus cAMP accumulation in the absence of dopamine; **, $p < 0.01$ versus basal cAMP accumulation in control cells.

Discussion

The objectives of the studies described in this report were to establish a clonal cell line that expresses dopamine D2 receptors and to investigate the effect of exposure to agonists on receptor expression. The SUP1 cell line was isolated from 7315a pituitary tumor cells adapted to tissue culture. The expression of D2 receptors by SUP1 cells was studied using [^{125}I]IBZM, a radioligand that is selective for D2 receptors (31). In saturation experiments, a single class of sites was labeled in membranes prepared from SUP1 cells. The potencies of competing compounds at the sites were consistent with their potencies at D2 receptors, indicating that the sites were D2 receptors. The relatively high affinity and low nonspecific binding of [^{125}I] IBZM made it possible to detect and characterize D2 receptors in SUP1 cells, although the density of D2 receptors in SUP1 cells (25 to 80 fmol/mg of protein) was somewhat lower than that found in 7315a tumors [80 fmol/mg of protein (18)].

D2 receptors in the anterior pituitary and brain are coupled through one or more G proteins to multiple second messenger pathways, including the inhibition of adenylyl cyclase activity (13). The potency of dopamine at binding sites labeled with [^{125}I]IBZM in SUP1 cells was decreased by GTP, suggesting that D2 receptors in SUP1 cells were also coupled to G proteins. Activation of D2 receptors in SUP1 cells by dopamine caused a concentration-dependent inhibition of cAMP accumulation in SUP1 cells. This inhibition was mimicked by NPA and was blocked by D2 receptor antagonists, including spiroperidol and sulpiride, indicating that the inhibition of cAMP accumulation was mediated by D2 receptors. The monophasic nature of the inhibition curve and the inability of the D1 receptor antagonist SCH23390 to enhance the inhibition suggested that SUP1 cells do not express D1 receptors that are coupled to stimulation of adenylyl cyclase activity. The results of these experiments suggest that D2 receptors on SUP1 cells are functionally coupled to at least one second messenger system.

SUP1 cells and dispersed 7315a tumor cells secrete prolactin spontaneously. Dopamine inhibits prolactin secretion from dispersed 7315a cells, but only after prior stimulation of secretion by a calcium ionophore (19). An abnormality in calcium metabolism has been hypothesized to exist in 7315a cells, accounting for the lack of an effect of dopamine on prolactin secretion (19). In contrast, dopamine interacted with D2 receptors in SUP1 cells and inhibited basal secretion of prolactin without prior stimulation of secretion by a calcium ionophore.

The choice of culture conditions used in the isolation of the SUP1 cell line was guided by published reports suggesting that steroids affect the density and/or function of D2 receptors. In particular, estrogens antagonize the effects of dopamine on lactotrophs (21). In primary cultures of cells from the anterior pituitary, estrogens decrease the ability of dopamine to inhibit adenylyl cyclase activity, without affecting the density of D2 receptors (24). However, a decrease in the density of D2 receptors in the anterior pituitary has been correlated with elevated estrogen levels during the estrus cycle of female rats (36). It is possible that antagonism of the function of dopamine by estrogens may be partially at the level of expression of D2 receptors. Therefore, cells dispersed from 7315a tumors were cultured in medium containing fetal calf serum that had been treated with charcoal to remove estrogens (30).

Estrogens stimulate prolactin secretion from lactotrophs *in vivo* and *in vitro* (37). Phenol red, the pH indicator present in most media, has weak estrogenic activity in some types of cells, including breast cancer cells (38) and lactotrophs (30, 39). SUP1 cells grown in the presence of phenol red secrete increased quantities of prolactin, suggesting that phenol red may have estrogenic activity in these cells. In view of possible estrogenic effects of phenol red, SUP1 cells have been grown and characterized in the absence of phenol red. However, some SUP1 cells have been maintained in medium containing phenol red, because it is possible that cells cultured for an extended time in the absence of phenol red will lose their responsiveness to estrogens (40). The ability of dopamine to inhibit prolactin secretion from SUP1 cells cultured for long periods of time in the presence of phenol red has not yet been investigated.

Exposure of SUP1 cells to dopamine led to increased expression of D2 receptors. An increase in the density of D2 receptors in SUP1 cells after exposure to an agonist was not anticipated, because in most systems exposure to agonists results in desensitization and eventual down-regulation of receptors. The magnitude of the increase was dependent on the time of exposure to dopamine, and there was no change in the affinity of the receptor for [^{125}I]IBZM after exposure of cells to dopamine. Furthermore, the increase in the density of receptors appeared to be mediated by an interaction with D2 receptors, because both dopamine and NPA caused an increase in receptor density, and spiroperidol blocked the increase. The magnitude of the increase in the density of receptors appeared to be greater in cells exposed to NPA than in cells exposed to dopamine, perhaps due to greater stability of NPA in the culture medium. It seemed possible that D2 receptors in cells exposed to dopamine for a prolonged period might be desensitized, despite the increase in receptor density. However, dopamine retained the ability to inhibit cAMP accumulation in SUP1 cells that had been exposed to dopamine for 24 hr. No desensitization of D2 receptor-mediated inhibition of adenylyl cyclase activity was observed. The percentage of inhibition of cAMP accumulation by dopamine was as great or greater in pretreated cells than in control cells.

The mechanism for the up-regulation of D2 receptors in SUP1 cells after exposure to agonists remains unknown. The possibility that an agonist present in the medium might constitutively down-regulate receptors, allowing an apparent up-regulation of receptors to be observed after treatment with a less efficacious agonist, was considered. This possibility is unlikely for three reasons. 1) Although the medium used to culture SUP1 cells was not defined, the serum used in the medium was treated with charcoal and would be expected to contain very low levels of endogenous catecholamines. 2) The agonists used in these studies, dopamine and NPA, are thought to be full agonists at D2 receptors. 3) Treatment with an antagonist would be predicted to block a constitutive down-regulation of receptors and cause receptor up-regulation. However, exposure of SUP1 cells to spiroperidol did not result in an increase in the density of D2 receptors. In contrast, spiroperidol antagonized the ability of dopamine to increase D2 receptor density.

It is possible that the effect of agonists on D2 receptor density in SUP1 cells involves an effect on intracellular levels of cAMP or some other second messenger. Monsma *et al.* (29) recently reported that differentiation of retinoblastoma Y-79 cells is accompanied by increased expression of D2 receptors. Because differentiation of these cells was achieved by serum deprivation and exposure to dibutyryl-cAMP, it was speculated that the D2 receptor gene might contain a cAMP-responsive element, so that exposure to cAMP would result in an increase in transcription of the D2 receptor gene (29). It might be predicted that, because D2 receptors in Y-79 cells are negatively coupled to adenylyl cyclase activity, activation of these receptors by agonists would result in down-regulation of D2 receptors. As yet, the effect of agonist exposure on D2 receptors in Y-79 cells has not been reported. In seeming contrast to this scenario, in SUP1 cells D2 receptors are negatively coupled to adenylyl cyclase activity, yet activation of these receptors leads to increased expression of D2 receptors. However, we have found that prolonged exposure of SUP1 cells to dopamine consistently leads to an increased level of adenylyl cyclase activity. In both Y-79 cells and SUP1 cells, therefore, increased cAMP levels may be associated with an increased expression of D2 receptors.

The mechanism for the increase in D2 receptor density after

exposure of SUP1 cells to dopamine might also involve differentiation of the cells. Differentiation of Y-79 cells following serum deprivation and exposure to dibutyryl-cAMP is associated with a decrease in cell division, attachment to plates, and expression of neuronal properties, including an increase in the density of D2 receptors (29). If receptors in Y-79 cells were down-regulated by exposure to dopamine, it would be informative to determine whether the drug treatment affected the state of differentiation of the cells. Interestingly, D2 receptor agonists seem to affect the cell cycle of SUP1 cells. Although 7315a tumors, from which SUP1 cells originated, are refractory to growth inhibition by D2 agonists (41), we have observed that exposure of SUP1 cells to dopamine leads to a decrease in the rate of cell division. These results suggest that exposure of SUP1 cells to D2 receptor agonists might cause these cells to differentiate or to alter the timing of cell cycle events. It seems possible that, in both Y-79 and SUP1 cells, the signals that cause cells to move out of the cell cycle also result in an increase in expression of D2 receptors.

In many studies of receptors for catecholamines, it has been shown that exposure to agonists leads to desensitization and to a decrease in receptor density. However, during the estrus cycle of female rats, there are cyclic changes in the density of D2 receptors and in dopamine levels in the pituitary (36, 42). The density of D2 receptors is positively correlated with dopamine levels, and it has been suggested that dopamine might induce expression of D2 receptors in the pituitary (36). It is possible that the ability of D2 receptor agonists to cause an increase in D2 receptor density in SUP1 cells may represent normal regulation of D2 receptors in the pituitary. Future studies will be directed toward determining whether steroid hormones affect D2 receptor expression in SUP1 cells and whether these hormones modulate the up-regulation of D2 receptors produced by exposure to agonist.

Several attempts to culture dispersed 7315a cells and establish permanent cell lines that maintain the expression of functional receptors have been described. For example, the P11 cell line derived from pituitary tumor 7315a expresses 5-HT_2 receptors but not D2 receptors (43). The MMQ cell line, which was also derived from pituitary tumor 7315a, appears to express D2 receptors, because dopamine inhibits cAMP accumulation in MMQ cells and prolactin release from the cells (27). Exposure to low concentrations of dopamine resulted in a small increase in cAMP accumulation in MMQ cells, an effect not seen in SUP1 cells. This suggests that MMQ cells may express D1 receptors or other receptors that are activated by dopamine and linked to stimulation of adenylyl cyclase activity, as well as D2 receptors. It would be interesting to determine whether exposure of MMQ cells to dopamine also leads to an up-regulation of D2 receptors.

In summary, SUP1 cells express a measurable density of functional D2 receptors, and the density of these receptors is increased after exposure of cells to D2 receptor agonists. SUP1 cells should provide a useful model system for future studies of the regulation of expression and function of D2 receptors in cultured cells.

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