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Functional Characterization of a Rat Dopamine D-2 Receptor cDNA Expressed in a Mammalian Cell Line

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SUMMARY

We recently cloned a complementary DNA for the rat dopamine D-2 receptor, making it possible to create cell lines expressing this receptor. A cell line (LZR1) was created by transfecting the D-2 cDNA (RGB-2) into mouse fibroblast Ltk$^-$ cells. LZR1 cells, previously described as L-RGB2Zem-1 cells, express a high density of D-2 receptors, whereas the wild-type cells do not. A number of agonists competitively and stereoselectively inhibited the binding of [^{3}H]spiroperidol to the expressed D-2 receptors in a GTP-sensitive manner. The potency of dopamine was decreased by the addition of GTP. NaCl and GTP together caused a further decrease in potency and increased the Hill slope for inhibition of radioligand binding by dopamine almost to 1.0. Pretreatment of cells with pertussis toxin inhibited high affinity binding of dopamine and prevented further inhibition of binding by GTP. The NaCl-induced decrease in affinity was not prevented by pertussis toxin treatment. Dopamine reduced forskolin-stimulated adenylate cyclase activity by 27% in membranes prepared from LZR1 cells. Inhibition by dopamine was blocked by (+)-butaclamol or prior treatment of intact cells with pertussis toxin. Other dopamine receptor agonists stereoselectively inhibited adenylate cyclase activity. These data indicate that the RGB-2 cDNA directs the expression of a dopamine D-2 receptor capable of interacting with guanine nucleotide-binding proteins and inhibiting adenylate cyclase activity. Furthermore, the RGB-2 cDNA provides a means of creating many cell lines that will be useful tools for the biochemical and pharmacological characterization of dopamine D-2 receptors.

DA receptors have been classified into two subtypes, based on functional and pharmacological profiles (1). DA D-2 receptors are characterized functionally by their ability to inhibit adenylate cyclase activity (2). Activation of D-2 receptors also inhibits calcium channels (3, 4), increases potassium conductance (5), and may inhibit accumulation of inositol phosphates (6, 7). One factor that has impeded research on the regulation and functional characteristics of DA receptors has been the lack of cell lines that express the receptors. One cell line, derived from a prolactin-secreting tumor, has recently been described in which DA inhibits adenylate cyclase activity and prolactin secretion (8).

We recently cloned a rat brain cDNA, designated RGB-2, that has significant homology with β_2-adrenergic receptors and other receptors that interact with G proteins. Three lines of evidence indicate that the RGB-2 cDNA encodes the DA D-2 receptor. 1) The deduced amino acid sequence of the protein suggests the existence of the seven membrane-spanning domains that are typical of receptors coupled to G proteins (9). 2) The distribution of messenger RNA that hybridizes with the cDNA parallels the distribution of the D-2 receptor. 3) When the RGB-2 cDNA is transfected into cells that lack high affinity binding of the D-2-selective ligand [^{3}H]spiroperidol, the transfected cells express binding sites for the radioligand with a pharmacological profile that is characteristic of D-2 receptors (10).

The cloning of a D-2 receptor cDNA makes it possible to express DA receptors in cell lines in which the effects of receptor activation can be readily determined. We previously described the binding of [^{3}H]spiroperidol and other D-2 antagonists to a line of cells derived by transfection of mouse L cells with the RGB-2 cDNA under the control of the mouse metallothionein promoter (10). We also reported that, under the assay conditions used previously, the binding of DA to LZR1 membranes was not responsive to GTP. We now demonstrate that the D-2 receptor encoded by the RGB-2 cDNA is functional with respect to the sensitivity of the binding of agonists

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ABBREVIATIONS: DA, dopamine; G_i, inhibitory guanine nucleotide-binding protein; 3-PPP, 3-(3-hydroxyphenyl)-*N*-*n*-propylpiperidine; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid; G protein, guanine nucleotide-binding protein; Ltk$^-$ cells, L cells deficient in the enzyme thymidine kinase.

to guanine nucleotides and NaCl and the ability of agonists to inhibit adenylate cyclase activity.

Experimental Procedures

Materials. [α-^{32}P]ATP (10–50 Ci/mmol) and [^{3}H]cyclic AMP (31.9 Ci/mmol) were purchased from New England Nuclear (Boston, MA), and [^{3}H]spiroperidol (95 Ci/mmol) was purchased from Amersham (Arlington Heights, IL). GTP, DA, cyclic AMP, 3-isobutyl-1-methylxanthine, ATP, pertussis toxin, and forskolin were purchased from Sigma Chemical Co. (St. Louis, MO). Quinpirole, LY181990 (Lilly Laboratories), bromocriptine (Sandoz Research Institute), and (+)- and (−)-3-PPP (Astra) were generous donations.

Transfection. The full RGB-2 cDNA was cloned into the plasmid pZem3 (11). The cDNA and the vector were made compatible by partially filling in the *BglII* site on the vector and a *SalI* site on the cDNA adaptor. This plasmid was co-transfected with the plasmid pRSVneo into mouse Ltk$^-$ cells by a $CaPO_4$ precipitation technique (12). Transfectants were selected in 350 μg/ml G418, isolated, and screened for expression of RGB-2 mRNA by Northern blot analysis. The subclone LZR1, which was selected on the basis of high expression of RGB-2 mRNA, was partially characterized previously as L-RGB2Zem-1 (10).

Tissue culture. Cells were plated at a density of 20,000 cells/cm^2 in 150-mm diameter Falcon tissue culture plates (Beckton Dickinson, Lincoln Park, NJ), subcultured by replacement of the growth medium with trypsin-EDTA (0.1% trypsin, 0.02% EDTA in phosphate-buffered saline) or fed on day 3, and harvested on day 5 or 6. Cells were grown in Dulbecco's modified Eagle's medium (Sigma), which was supplemented with 5% fetal bovine serum and 5% iron-supplemented calf bovine serum (Hyclone, Logan, UT), in an atmosphere of 10% CO_2/90% air at 37°. Cells were lysed by replacement of the growth medium with ice-cold hypotonic buffer (1 mM Na-HEPES, pH 7.4, 2 mM EDTA). After swelling for 10–15 min, the cells were scraped from the plate and centrifuged at 24,000 × *g* for 20 min. The resulting crude membrane fraction was resuspended with a Brinkmann Polytron homogenizer, at setting 6 for 10 sec, in Tris-isosaline (50 mM Tris-HCl, pH 7.4, 0.9% NaCl) and stored at −70° for receptor binding experiments, or was resuspended in Tris-isosaline, centrifuged again at 24,000 × *g* for 20 min, and resuspended in Tris-isosaline for immediate use in adenylate cyclase experiments.

Receptor binding assay. The membrane preparation was thawed, centrifuged at 24,000 × *g* for 20 min, and resuspended in Tris-isosaline except where indicated. Aliquots of the membrane preparation were added to duplicate assay tubes containing (final concentrations) 50 mM Tris-HCl, pH 7.4, 0.9% NaCl, 0.025% ascorbic acid, 0.001% bovine serum albumin, [^{3}H]spiroperidol, and appropriate drugs. (+)-Butaclamol (2 μM) was used to define nonspecific binding, which was typically less than 10% of total binding at concentrations of radioligand near the K_D value. Assays were carried out, in duplicate, in a volume of 2 ml for saturation analyses or 1 ml for inhibition analyses. Incubations were initiated by the addition of 15–50 μg of protein, carried out at 37° for 50 min, and stopped by the addition of 10 ml of ice-cold wash buffer (10 mM Tris, pH 7.4, 0.9% NaCl) to each assay. The samples were filtered through glass fiber filters (Schleicher & Schuell No. 30) and washed with an additional 10 ml of wash buffer. The radioactivity retained on the filters was counted using a Beckman LS 1701 scintillation counter. Data were analyzed by nonlinear regression, using the data analysis programs Enzfitter and CDATA. In competition experiments, K_I values were calculated from experimentally determined IC_{50} values by a modification (13) of the method of Cheng and Prusoff (14). Averages for K_I values are the geometric means. When averages include an experiment in which the Hill coefficient was substantially less than unity, affinity values are described as $K_{0.5}$. Statistical comparison of one- and two-site fits was carried out using the *F* ratio test for weighted residuals, as described by Munson and Rodbard (15). In experiments designed to assess the affect of GTP and NaCl on the binding of DA, fresh tissue was used. Cells were harvested, centrifuged, and resuspended in Tris-Mg^{2+} (50 mM Tris, pH 7.4, 4 mM $MgCl_2$). Tissue was incubated for 25 min at 37° in this buffer before recentrifugation. The resuspended protein was added to assays that contained Tris-Mg^{2+} with no added NaCl or GTP, Tris-Mg^{2+} with 100 μM GTP, or Tris-Mg^{2+} with 120 mM NaCl and 100 μM GTP.

Adenylate cyclase assay. The conversion of [α-^{32}P]ATP to [^{32}P] cyclic AMP was determined essentially as described by Salomon *et al.* (16). Membranes (50–100 μg of protein) that were resuspended in Tris-isosaline were added, in a volume of 0.1 ml, to an assay of 0.2 ml containing 50 mM Tris·HCl, pH 7.4, 5 mM cAMP, 1 mM 3-isobutyl-1-methylxanthine, 1 mM $MgCl_2$, 0.5 mM EGTA, 0.25 mM ATP, 30 μM GTP, approximately 2 × 10^6 cpm of [α-^{32}P]ATP, and various drugs. Assays, which were carried out in triplicate, were initiated by warming to 25° and terminated after 20 min by cooling to 0° and then addition of trichloroacetic acid (100 μl of a 30% solution) to each assay. [^{3}H] Cyclic AMP (approximately 30,000 cpm) was added to each assay as an internal standard. The assay volume was brought up to 1 ml with water, and tubes were centrifuged for 10 min at 2000 × *g*. Cyclic AMP in the supernatant was isolated by sequential chromatography on columns containing Dowex AG50W-X4 resin and neutral alumina. The 2-ml eluate from each column of alumina was dissolved in 10 ml of Bio-Safe II (RPI, Mount Prospect, IL) for liquid scintillation counting. Dose-response curves for inhibition of adenylate cyclase activity by agonists were analyzed by nonlinear regression, using the program Enzfitter. The data were fit to the equation:

$$E = (100 - E_{max})/(1 + (A/EC_{50})^n) + E_{max}$$

where E is the amount of enzyme activity, expressed as percentage of total stimulated activity, A is the concentration of agonist, EC_{50} is the concentration of agonist causing half-maximal inhibition of enzyme activity, E_{max} is the enzyme activity observed in the presence of maximally inhibiting concentrations of agonist, expressed as the percentage of total stimulated activity, and n is a slope factor. Averages for EC_{50} values are the geometric means. Protein concentration was determined by the method of Peterson (17).

Results

Inhibition of radioligand binding by agonists. The apparent affinity of D-2 receptors for several agonists and related compounds was determined in the presence of GTP and NaCl (Fig. 1; Table 1). Bromocriptine was the most potent of the six compounds tested, with a mean K_I value of 2.4 nM, whereas LY181990, the inactive enantiomer of the D-2-selective agonist quinpirole, was the least potent. Four of the six drugs appeared to be agonists, as determined by consistent inhibition of adenylate cyclase activity (Table 1; see also Fig. 3). Inhibition of radioligand binding by the four agonists was assessed in the absence of GTP/NaCl (Table 1). DA was approximately 15-fold more potent in the absence than in the presence of GTP/NaCl, and the mean Hill coefficient was increased from 0.65 to 0.93 by GTP/NaCl. Addition of GTP/NaCl decreased the potency of quinpirole and (+)-3-PPP by approximately 2.5- to 5-fold, whereas the potency of bromocriptine was not altered by the presence of GTP/NaCl.

The effect of GTP and NaCl on interactions of agonists with D-2 receptors was analyzed in greater detail by assessing inhibition of [^{3}H]spiroperidol binding by DA with no additions, in the presence of GTP alone, and in the presence of GTP/NaCl (Table 2; Fig. 2). In the absence of GTP and NaCl, inhibition curves for DA could be fit best by assuming the presence of two classes of binding sites ($p < 0.001$ in each of 10 experiments for the comparison of two-site versus one-site fits). One class of high affinity sites, representing 58 ± 3% of the total number of receptors, had a mean K_I for DA of 0.052 μM. The second

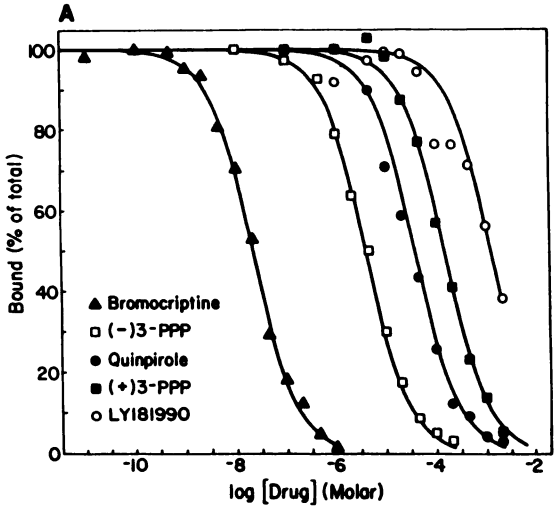

Fig. 1. Inhibition of radioligand binding by agonists. Results are plotted as specific binding, expressed as a percentage of specific binding in the absence of competing drug, versus the logarithm of the concentration of competing drug. Membranes were prepared from LZR1 cells, as described in the text. *Curves* from a single experiment are shown for inhibition of the binding of [^{3}H]spiroperidol by agonists. Each drug was tested twice. In this experiment, the free concentration of [^{3}H]spiroperidol was 230 pM, and the K_D value for [^{3}H]spiroperidol was 60 pM. K_I values and Hill coefficients in this experiment were 5 nM and 1.05 for bromocriptine, respectively, 790 nM and 0.89 for (−)-3-PPP, 8 μM and 1.0 for quinpirole, 31 μM and 1.05 for (+)-3-PPP, and 0.3 mM and 0.72 for LY181990.

class, representing 42 ± 3% of the total number of receptors, had a mean K_I value of 2.5 μM. The presence of 100 μM GTP decreased the likelihood of improving goodness-of-fit by assuming two classes of binding sites (in five experiments, p ranged from <0.001 to >0.05). The mean $K_{0.5}$ value for DA was increased from 0.20 μM to 0.95 μM by GTP, and Hill coefficients were increased from 0.65 to 0.82. Addition of 120 mM NaCl resulted in a further increase in the $K_{0.5}$ value to 3.2 μM and of the mean Hill coefficient to 0.93.

Effect of pertussis toxin on binding of DA. In some experiments, cells were treated for 16 hr with pertussis toxin before quantification of inhibition of radioligand binding by DA. The potency and Hill slope of the binding of DA were similar in the presence and absence of GTP in membranes from pertussis toxin-treated cells and were also similar to the respective values in the presence of GTP in control cells (Fig. 2; Table 2), indicating that pertussis toxin inhibited high affinity binding of DA. Addition of NaCl increased the K_I for DA to 6.3 μM and also increased the mean Hill coefficient (Table 2).

Inhibition of adenylate cyclase by agonists. In freshly prepared membranes from LZR1 cells, but not in membranes from wild-type Ltk$^-$ cells, DA caused a concentration-dependent attenuation of forskolin-stimulated adenylate cyclase activity. Maximal inhibition was 27% of total activity, with an EC_{50} value of 624 nM (Fig. 3A; Table 1). Quinpirole was approximately as efficacious as DA; that is, the maximal inhibition induced by quinpirole was similar to that induced by DA. LY181990 did not consistently inhibit enzyme activity (Fig. 3B; Table 1), indicating that D-2 receptor-mediated inhibition of adenylate cyclase activity was stereoselective. Similarly, (−)-3-PPP did not have detectable agonist activity, whereas (+)-3-PPP did. Bromocriptine, with an Ec_{50} value of 45 nM, was the most potent agonist. Bromocriptine and (+)-3-PPP were less efficacious than DA; thus, the drugs appeared to be partial agonists.

TABLE 1

Inhibition of radioligand binding and adenylate cyclase activity by agonists

The apparent affinity of six drugs for D-2 receptors on LZR1 cells, determined by inhibition of the binding of [^{3}H]spiroperidol (0.2 nM) in the presence of 100 μM GTP/120 mM NaCl (+GTP/NaCl), is shown, as well as the concentration of each drug that caused half-maximal inhibition of adenylate cyclase (EC_{50}). The binding of drugs that were agonists, as determined by inhibition of enzyme activity, was also determined in the absence of GTP/NaCl (−GTP/NaCl), at a radioligand concentration of 0.4 nM. The binding affinity value is referred to as $K_{0.5}$, because Hill slopes from some experiments were substantially less than unity. Affinity values, expressed as μM, are the geometric means, with 95% confidence intervals given in parentheses beneath the means. The Hill slope (n) is expressed as the mean ± standard error. Maximal inhibition of adenylate cyclase activity observed (Max) is expressed as the mean ± standard error of the percentage of inhibition of total activity in the presence of 10 μM forskolin. The number of experiments is given in parentheses as the last line for each condition. Enzyme activity was not consistently inhibited by LY181990 or (−)-3-PPP.

Drug	Binding				Adenylate cyclase	
	+GTP/NaCl		−GTP/NaCl			
	$K_{0.5}$	n	$K_{0.5}$	n	EC_{50}	Max
	μM		μM		μM	%
Dopamine	3.2 (2.1–4.5) (10)	0.93 ± 0.03	0.2 (0.1–0.40) (8)	0.65 ± 0.02	0.6 (0.3–1.4) (3)	27 ± 3
Quinpirole	8.8 (7.6–10.3) (2)	1.05 ± 0.05	1.2 (0.8–1.6) (3)	0.75 ± 0.02	0.7 (0.4–1.3) (3)	28 ± 2
LY181990	279 (227–341) (2)	0.72 ± 0.02				
Bromocriptine	0.0024 (0.0006–0.0096) (2)	1.02 ± 0.03	0.002 (0.0018–0.0024) (2)	1.24 ± 0.14	0.045 (0.015–0.138) (3)	17 ± 1
(+)-3-PPP	33 (30–37) (2)	1.08 ± 0.02	14 (11–19) (3)	0.83 ± 0.08	4.0 (3.1–5.2) (3)	16 ± 3
(−)-3-PPP	0.87 (0.73–1.04) (2)	0.89 ± 0.01				

TABLE 2

Modulation of DA binding affinity by GTP, NaCl, and pertussis toxin

Inhibition of the binding of [^{3}H]spiroperidol by DA in the presence of no additions, 100 μM GTP, or GTP and 120 mM NaCl was determined in membranes prepared from control cells or cells treated with pertussis toxin. All assays were carried out in the presence of 4 mM $MgCl_2$. Each inhibition curve was analyzed assuming the presence of one and two classes of binding sites and the weighted residuals were compared. Only one condition (control, no additions) was consistently improved by a two-site model. The number of independent experiments for each condition is given in parentheses. Affinity values, in μM, are referred to as $K_{0.5}$ because some curves had Hill coefficients substantially less than unity. The 95% confidence interval is given in parentheses after each geometric mean. Hill slopes (n) are expressed as mean ± standard error. Data for control tissue in the presence of no additions or GTP/NaCl are the same as in Table 1.

Condition	$K_{0.5}$	n
	μM	
Control		
No additions, one site (10)	0.20 (0.12–0.35)	0.65 ± 0.02
No additions, K_H	0.05 (0.04–0.10)	
No additions, K_L	2.5 (1.6–4.0)	
GTP (5)	0.95 (0.58–1.60)	0.82 ± 0.05
GTP/NaCl (8)	3.2 (2.1–4.5)	0.93 ± 0.03
Pertussis toxin-treated		
No additions (6)	1.0 (0.91–1.1)	0.86 ± 0.02
GTP (4)	1.6 (1.2–2.0)	0.87 ± 0.02
GTP/NaCl (3)	6.3 (5.4–7.4)	0.97 ± 0.06

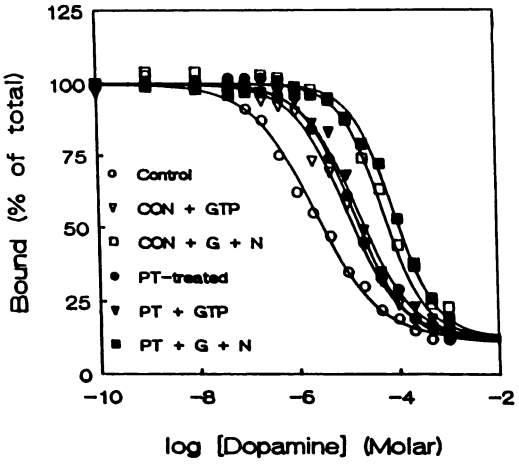

Fig. 2. Effect of GTP, NaCl, and pertussis toxin on inhibition of [^{3}H] spiroperidol binding of DA. Results shown are from one experiment, in which the data are plotted as total binding of [^{3}H]spiroperidol, expressed as a percentage of total binding in the absence of DA, versus the logarithm of the concentration of DA. The concentration of [^{3}H]spiroperidol was 0.4 nM. Membranes from control cells (*CON*) or cells treated with pertussis toxin (*PT*) (50 ng/ml for 16 hr) were used to determine the effect of GTP (*G*) and NaCl (*N*) on the binding of DA.

Inhibition of adenylate cyclase activity in LZR1 cells by 10 μM DA was prevented by including 10 μM (+)-butaclamol in the assay (Fig. 4), indicating that inhibition by DA is receptor mediated. Also, treatment of LZR1 cells with pertussis toxin (50 ng/ml of growth medium for 16 hr) blocked DA-inhibited enzyme activity in membranes prepared from the cells (Fig. 4), suggesting that inhibition of enzyme activity by DA is mediated by G_i. Interestingly, forskolin-stimulated adenylate cyclase activity in membranes from pertussis toxin-treated cells was approximately 2.2-fold greater than activity in control membranes.

Discussion

As reported previously, Ltk$^-$ cells transfected with a rat D-2 receptor cDNA express a high density of DA D-2 receptors

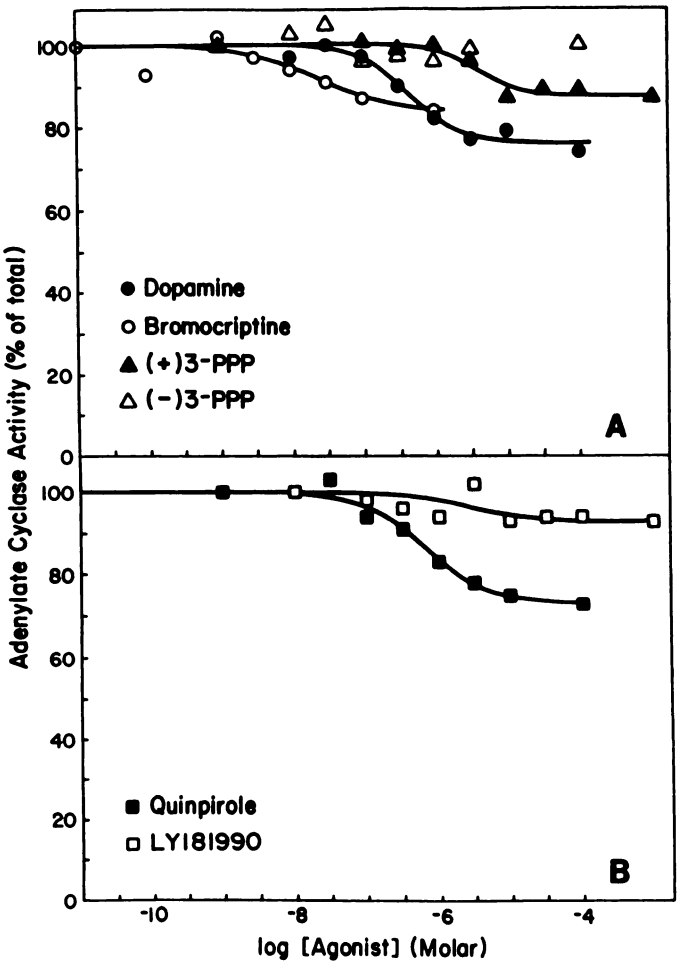

Fig. 3. Inhibition of adenylate cyclase activity in LZR1 cells. Agonists were tested for inhibition of adenylate cyclase activity in membranes prepared from LZR1 cells. Approximately 50 to 100 μg of protein were used in each assay. Results are shown as [^{32}P]cyclic AMP/mg of protein/min, expressed as a percentage of total activity in the presence of 10 μM forskolin. Representative dose-response curves are shown for six drugs, each tested at least 3 times. Data are plotted as enzyme activity versus the logarithm of the concentration of drug. No curve is plotted for the data for (−)-3-PPP, because no inhibition was observed. In the experiments shown in this figure, basal and forskolin-stimulated activity ranged from approximately 0.8 to 1.5 and 8.5 to 15.8 pmol/mg of protein/min, respectively.

(10). We have characterized one subclone of these cells, designated LZR1, that stably expresses D-2 receptors at a density of 750 to 1000 fmol/mg of protein.

The apparent affinity of D-2 receptors on LZR1 cells for several agonists and related drugs was determined by inhibiting the specific binding of [^{3}H]spiroperidol with the drugs in the presence of GTP/NaCl. Bromocriptine was an extremely potent agonist, with a K_i value of 2 nM. The potency of quinpirole, like that of DA, was between 1 and 10 μM. The binding of agonists was stereoselective, because LY181990 was much less potent than its active enantiomer quinpirole and (−)-3-PPP was more potent than (+)-3-PPP. These data extend our previous investigation of the binding of antagonists to D-2 receptors on LZR1 cells (10).

Physiological effects of stimulation of D-2 receptors appear to be mediated by a G protein, G_i, that inhibits adenylate cyclase activity (18). High affinity binding of agonists is thought to represent a ternary complex composed of agonist,

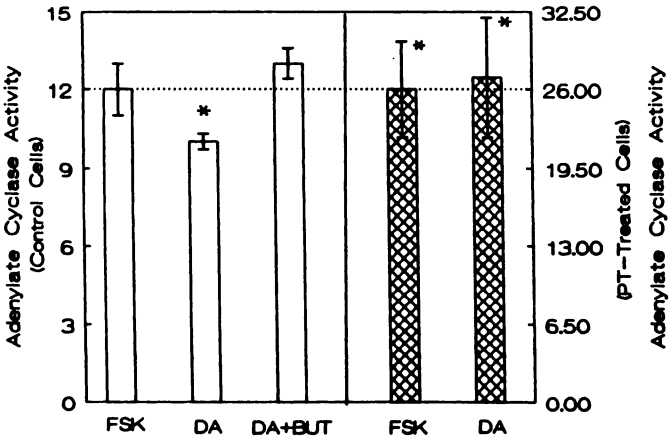

Fig. 4. Blockade of DA-sensitive adenylate cyclase. Results shown are means of three (control cells) or five (pertussis toxin-treated cells) experiments ± standard error, expressed as [^{32}P]cyclic AMP produced (pmol/mg of protein/min). Forskolin (*FSK*) was present in all the experiments shown, together with 10 μM DA or DA and 10 μM (+)-butaclamol (*BUT*), as indicated. Some cells were treated with pertussis toxin (*PT*) before harvesting for determination of enzyme activity. Basal activity in control and pertussis toxin-treated cells was 1.2 ± 0.07 and 1.5 ± 0.1 pmol/mg of protein/min, respectively. Total forskolin-stimulated activity in control cells (*FSK*) was 11.9 ± 1.0 pmol/mg of protein/min. *$p < 0.05$ compared with forskolin-stimulated activity in control cells, as determined by a *t* test for paired means.

receptor, and the α subunit of G_i ($G_{i\alpha}$), and inhibition of agonist binding to D-2 receptors by GTP represents GTP-induced uncoupling of D-2 receptors from $G_{i\alpha}$. To evaluate the ability of D-2 receptors encoded by the RGB-2 cDNA to couple to G proteins, the potency of several agonists was determined in the absence of GTP/NaCl and the results were compared with those obtained in the presence of GTP or GTP/NaCl. In preliminary studies using LZR1 cells, we found that, in our standard assay buffer containing 120 mM NaCl and no added Mg^{2+}, the binding of DA was not sensitive to GTP (10), although under the same conditions the binding of DA to rat striatal membranes is inhibited by GTP (data not shown). There were three possible explanations for the lack of sensitivity to GTP in LZR1 membranes. 1) LZR1 cells, derived from Ltk^- cells, could lack the appropriate G protein. 2) The RGB-2 cDNA could encode only a binding subunit of the D-2 receptor. This possibility seemed unlikely because of the similarity between the predicted primary structure of the protein encoded by RGB-2 and other receptors coupled to G proteins. 3) It could be that the ionic conditions of the binding assay were not appropriate for formation or destabilization of the ternary complex. In the studies described here, ionic conditions were varied to increase the likelihood of observing GTP-sensitive binding. To maximize high affinity agonist binding, tissue was preincubated with $MgCl_2$, and $MgCl_2$ was included in the assay buffer (19). Under these conditions, addition of GTP/NaCl decreased the potency of DA, quinpirole, and (+)-3-PPP for D-2 receptors in membranes from LZR1 cells and increased the slope of the inhibition curve relative to experiments carried out without addition of GTP/NaCl. As has been reported previously, the binding of bromocriptine was not inhibited by GTP (20). It has been hypothesized that the peptide side chain of ergopeptines such as bromocriptine contributes substantially to the binding energy of the compounds in a manner independent of coupling to a G protein, resulting in the observed lack of sensitivity to GTP (20). It is interesting that the ionic requirements for formation and destabilization of the ternary complex seem to be more stringent in membranes from LZR1 cells than in membranes from rat striatum.

Because GTP/NaCl induced a shift in the potency of DA that was greater than for the other agonists tested, DA was used for a more detailed analysis of the interactions of the cloned D-2 receptor with G proteins. In the absence of GTP, DA bound to high and low affinity classes of binding sites. GTP caused a partial loss of high affinity binding of DA, resulting in steeper dose-response curves that were shifted to the right relative to curves obtained in the absence of GTP. NaCl enhanced the effect of GTP, further decreasing the potency of DA and raising the mean Hill coefficient close to unity. The finding that NaCl enhances the inhibitory effect of GTP is in general agreement with previous work (19, 21).

We carried out similar experiments in membranes from cells that had been treated with pertussis toxin. Treatment with pertussis toxin abolished GTP-induced inhibition of DA binding but did not prevent inhibition by NaCl. When the data from control and pertussis toxin-treated membranes are considered together, as shown graphically in Fig. 2, the six conditions fall into three groups. Group 1 is represented by control membranes with no additions. Under these conditions, DA consistently binds to high and low affinity classes of sites. Group 2 consists of membranes from control cells in the presence of GTP and from pertussis toxin-treated cells in the presence or absence of GTP. Binding of DA under these conditions is indistinguishable from binding to the low affinity class of sites in group 1. Group 3 consists of curves determined in the presence of GTP/NaCl using membranes from control or pertussis toxin-treated cells. Under these conditions, DA inhibits [^{3}H]spiroperidol binding to a single class of sites with lower affinity than in groups 1 and 2. Thus, pertussis toxin treatment abolished the GTP effect by inhibiting high affinity binding of DA, but toxin treatment did not prevent inhibition by NaCl.

Sodium decreases the affinity of many G_i-coupled receptors for agonists (22, 23). The location of a binding site for sodium that could cause an allosteric effect on ligand binding affinity has not been determined. For α_2-adrenergic receptors, the sodium-binding site may be on the receptor itself, rather than on a separate membrane component, because sodium regulates the affinity of agonists in solubilized preparations (23). Our observation that NaCl decreased the affinity of D-2 receptors for DA in pertussis toxin-treated cells is consistent with the possibility that sodium binds directly to the receptor or to a third membrane component (24).

Inhibition of adenylate cyclase activity by DA D-2 receptors is a well characterized phenomenon (2, 25, 26). Inhibition of adenylate cyclase activity by several drugs was assessed in membranes from LZR1 cells. Maximally effective concentrations of DA and the D-2-selective agonist quinpirole decreased forskolin-stimulated enzyme activity by almost 30%. Inhibition of enzyme activity by DA was blocked by the D-2 antagonist (+)-butaclamol. The most potent agonist tested, bromocriptine, appeared to be a partial agonist, as reported by others (26, 27). Inhibition of adenylate cyclase by agonists was stereoselective, because LY181990, the dextrorotatory enantiomer of quinpirole, had little or no efficacy. As has been reported previously, the partial agonist (+)-3-PPP is a stronger agonist than (−)-3-PPP, although (−)-3-PPP binds to D-2 receptors with higher

affinity (28, 29). We observed no inhibition of adenylate cyclase activity by (−)-3-PPP in membranes from LZR1 cells. The EC_{50} values determined for inhibition of adenylate cyclase activity by agonists were similar to the K_l values determined in assays of ligand binding in the absence of GTP (Table 1). This similarity is consistent with the hypothesis that inhibition of adenylate cyclase activity is mediated by the binding of agonists to D-2 receptors in a high affinity state induced by formation of the ternary complex, as has been proposed for inhibition of enzyme activity by DA in the anterior pituitary (30).

DA did not inhibit adenylate cyclase activity in membranes from LZR1 cells that had been treated with pertussis toxin. Because pertussis toxin-catalyzed ADP-ribosylation of G_i prevents G_i-mediated inhibition of adenylate cyclase, this finding, together with the observation that pertussis toxin treatment inhibits high affinity binding of DA, is consistent with the hypothesis that D-2 receptors interact with $G_{i\alpha}$ in the transfected LZR1 cells. As has been observed for stimulation of adenylate cyclase activity by isoproterenol after pertussis toxin treatment of other cell types (31), treatment of intact LZR1 cells with pertussis toxin potentiated the ability of forskolin to stimulate adenylate cyclase activity, suggesting that in some cell lines G_i normally acts to attenuate forskolin- and hormone-stimulated adenylate cyclase activity.

We have characterized a cell line, transfected with the RGB-2 cDNA, that stably expresses a high density of D-2 receptors. With this cell line, it was determined that the cDNA encodes a DA D-2 receptor that interacts productively with a G protein, probably G_i, to inhibit adenylate cyclase activity. It seems likely that the RGB-2 cDNA would direct the expression of a functional D-2 receptor in almost any type of cell. For example, GH_4C_1 cells, derived from a rat pituitary tumor (32), are prolactin-secreting cells that lack DA receptors, even though lactotrophs in the rat anterior pituitary express D-2 receptors. Transfection of the RGB-2 cDNA into GH_4C_1 cells results in the expression of a D-2 receptor with functional characteristics that are similar to those described here (33). Cell lines created by transfection of a D-2 receptor cDNA will be useful in the study of mechanisms of action and regulation of D-2 receptors.

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