Attempts to obtain anti-(D₂ dopamine receptor) antibodies via the anti-idiotypic route

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Five stable hybridomas have been obtained that secrete monoclonal antibodies against the D₂-dopamine receptor-selective drug spiperone. Each monoclonal antibody has been characterized in terms of its ability to bind a range of dopamine-receptor-selective ligands. One monoclonal antibody has been purified by Protein A affinity chromatography and used to immunize mice. Anti-idiotypic antisera and one hybridoma secreting an anti-idiotypic monoclonal antibody were obtained and shown to inhibit [³H]spiperone binding to the anti-spiperone antibody used for immunization. Neither the antisera nor the anti-idiotypic monoclonal antibody, however, inhibited binding of [³H]spiperone to D₂-dopamine receptors.

INTRODUCTION

Antibodies are useful tools in studying membrane-bound receptors, allowing valuable information to be obtained regarding their structure and function. Thus, for example, monoclonal antibodies have been generated against many parts of the nicotinic acetylcholine receptor and used as probes of its structure and function (Lindstrom, 1984). In addition, Schoch *et al.* (1985) and Pfeiffer *et al.* (1984) have prepared monoclonal antibodies against the γ -aminobutyrate/benzodiazepine and glycine receptors respectively as probes of the structures.

However, membrane-bound receptors generally exist at very low concentrations and purification procedures are frequently difficult. These problems have meant that the production of either polyclonal or monoclonal antibodies to receptors is rarely an easy task. As a result of these difficulties some workers have used an alternative route to obtain anti-receptor antibodies which bypasses the need to immunize animals with the purified receptor. This alternative method, known as the 'anti-idiotypic approach' [reviewed in Haber (1983), Strosberg *et al.* (1981) and Venter *et al.* (1984)], is based on the hypothesis that, regardless of functional differences, macromolecules of the same specificity will show structural homologies in their binding sites. Thus, there may be similarities between the antigen-binding site of an antibody directed against a receptor-selective ligand and the ligand-binding site of the relevant receptor. Therefore, antibodies directed against the variable region of the anti-ligand antibody (anti-idiotypic antibodies) might also react with the receptor. Several groups have successfully used this approach to generate anti-receptor antibodies; for example, Schreiber *et al.* (1980), Homcy *et al.* (1982) and Guillet *et al.* (1984) have made antibodies to the β -adrenergic receptor, Ng & Isom (1984) and Glasel & Myers (1985) have made antibodies to the opiate receptor and Cleveland *et al.* (1983) have made antibodies to the nicotinic acetylcholine receptor by this route.

We have been studying the D₂-dopamine receptor for

several years, using a variety of biochemical techniques (Strange, 1983*a,b*). Antibodies directed against the receptor protein would help in further characterization of the receptor. Initial attempts to obtain such antibodies by immunization of animals with very impure receptor preparations were unsuccessful and therefore we decided to use the anti-idiotypic approach by initially making monoclonal antibodies to the dopamine antagonist spiperone and then anti-idiotypic antibodies to the anti-spiperone antibodies. We report here the successful production of both polyclonal and monoclonal anti-idiotypic antibodies. These anti-idiotypic antibodies did not, however, recognize the D₂-dopamine receptor.

MATERIALS AND METHODS

Chemicals

Aminopterin, 8-azaguanine, 2,2'-azinobis-(3-ethyl-benzthiazolinesulphonic acid), haemocyanin (from *Limulus polyphemus*), penicillin, streptomycin, Protein A-Sepharose, sheep anti-(mouse IgG)-peroxidase conjugate, thymidine and hypoxanthine were all from Sigma. The sheep anti-(mouse immunoglobulin) kit was from Serotec. All other chemicals were obtained as described in Hall *et al.* (1983) or Juszczak & Strange (1985) or were from commercial sources and were of the highest purity available.

Cell lines and media

NS-1 myeloma cells were kindly given by Dr. E. Billett (Department of Biochemistry, University of Nottingham, U.K.). The cells were maintained in RPMI 1640 medium supplemented with 10% foetal calf serum (both from Gibco). The growth medium contained additionally penicillin (50 units/ml), streptomycin (50 μ g/ml) and 8-azaguanine (0.1 mM). The cells were grown at 37 °C in a humidified atmosphere containing 10% CO₂.

Preparation of albumin-spiperone conjugates

Spiperone was coupled to human serum albumin with a water-soluble carbodiimide by the method described in

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Juszczak & Strange (1985). The number of spiperone molecules coupled per albumin molecule was determined from the absorption at 250 nm compared with control samples.

Immunizations

Balb/c mice (2–3 months old) received an intraperitoneal injection of 100 µg of albumin–spiperone [coupling ratio of spiperone:albumin 4:1 (mol/mol)] in Freund's complete adjuvant (Gibco), and 3 weeks later the mice received a booster intraperitoneal immunization of 100 µg of a second sample of albumin–spiperone (coupling ratio 16:1) in Freund's incomplete adjuvant (Gibco).

Anti-idiotypic antisera were produced by injecting the monoclonal antibody AS-1 in several different forms (see Table 2). Four Balb/c mice (mice 1–4) were given an intraperitoneal injection of 50–100 µg of the purified antibody emulsified in adjuvant as described previously. Alternatively, the purified AS-1 was first coupled to haemocyanin by using the procedure described by Reth *et al.* (1979) and then 10–20 µg of the conjugate was used to immunize the mice (mice 5 and 6) as described before. A further immunization procedure was to immunize mice (mice 7 and 8) with the hybridoma cells that secreted AS-1 [fixed in 1% (v/v) glutaraldehyde]. The hybridoma was shown to have the antibody in the membrane by a direct binding assay using [³H]spiperone (results not shown). The protocol for the latter immunization was the same as that described by Guillet *et al.* (1984) except that the mice were immunized intraperitoneally rather than intravenously.

Fusion of immune spleen cells with NS-1 cells

Fusions were performed essentially as described by Oi & Herzenberg (1980). Approx. 2×10^7 NS-1 cells were fused with approx. 2×10^8 immune spleen cells using 50% poly(ethylene glycol) 1500 (BDH Chemicals). The fused cell suspension was dispensed into four 96-well tissue culture plates (Northumbria Biologicals) containing a feeder layer of cells from the peritoneum of a rat. Selection of hybridoma cells was performed in medium containing hypoxanthine, aminopterin and thymidine. Approx. 2–3 weeks after a fusion the culture supernatants were tested for the presence of appropriate antibodies.

Growth of positive hybridomas

Positive hybridomas were expanded into Petri culture dishes and cloned at least twice by the limiting dilution method (Oi & Herzenberg, 1980). Monoclonal antibody was generated in culture supernatant by growing the cells beyond the point at which they begin to die. Antibody could be purified from the culture supernatant by using Protein A affinity chromatography as described in Oi & Herzenberg (1980). Briefly, the antibody was taken up by the adsorbent at pH 8.6 and, following a thorough wash with buffer (sodium phosphate, 50 mM, pH 8.6), was eluted in 0.2 M-glycine/HCl, pH 2.8, and neutralized immediately with saturated Na₂HPO₄.

Radioimmunoassay

Culture supernatants were incubated with [³H]spiperone (1 nM approx.) in a final volume of 0.5 ml of buffer [sodium phosphate (10 mM), NaCl (154 mM), NaN₃ (0.1%) and gelatine (0.1%) (pH 7.4)].

After 16 h at 4 °C or 1 h at 25 °C, when equilibrium

was attained, free [³H]spiperone was removed with a charcoal/albumin mixture as described in Wheatley & Strange (1983) and bound radioactivity was determined by liquid-scintillation spectrometry. Putative anti-idiotypic samples (sera or culture supernatants) were assayed by their ability to inhibit [³H]spiperone binding in the radioimmunoassay after a preincubation with the monoclonal anti-spiperone antibodies (16 h, 4 °C).

Isotypic determination

Isotypic determination of the monoclonal antibodies was performed by using an enzyme-linked immunosorbent assay. Sheep antisera directed against mouse immunoglobulin sub-classes were adsorbed onto poly(vinyl chloride) microtitre plates (Flow Laboratories, Rickmansworth, Herts, U.K.) by incubating 100 µl (1:500 dilution) for 16 h at 4 °C in each well. The plates were washed three times by successively filling and emptying the wells with buffer [sodium phosphate (20 mM) and NaCl (150 mM) (pH 7.4) containing 0.05% Tween 20 (PBS/Tween)]. Remaining protein binding sites were blocked by incubating with 150 µl of the PBS/Tween containing 0.1% bovine serum albumin. After washing as before, 50 µl of culture supernatant was added (6 h, 20 °C) and then, after a further wash, bound mouse antibodies were detected by the addition of 50 µl of a 1:500 dilution of a sheep anti-(mouse immunoglobulin)–peroxidase conjugate (16 h, 4 °C). The plates were then washed three times with PBS/Tween and three times with PBS alone and the bound enzyme was detected by adding 100 µl of the substrate 2,2'-azinobis-(3-ethylbenzthiazolinesulphonic acid) [1 mg/ml in buffer (0.1 M-citric acid/0.2 M-sodium phosphate, pH 4.0) containing H₂O₂ (0.05%)]. The reaction was terminated after 2 h at 20 °C with 50 µl of a solution of (0.05%) NaN₃ in citric acid (0.1 M) and the A₄₀₅ was determined in a Titertek Multiskan MC spectrophotometer (Flow Laboratories).

D₂-dopamine receptor binding assays

[³H]spiperone binding to caudate nucleus membranes was performed as described in Withy *et al.* (1980, 1981) to assay for D₂-dopamine receptors. Sera and culture supernatant [concentrated 6-fold by (NH₄)₂SO₄ (50%) precipitation] were assayed for anti-(D₂-dopamine receptor) antibodies by preincubating either 10 µl or 100 µl respectively with the membranes (30 min, 25 °C) before the addition of radioligand.

Soluble D₂-dopamine receptors were obtained by solubilization of bovine caudate nucleus membranes with cholate as described in Hall *et al.* (1983) and assayed as in Wheatley & Strange (1983). Sera were assayed for anti-receptor antibodies by preincubating 25 µl with 425 µl of the soluble receptor preparation (16 h, 4 °C) before adding the radioligand. Concentrated culture supernatant was assayed similarly for anti-receptor antibodies except that 225 µl were preincubated with 225 µl of the soluble receptor preparation.

RESULTS

Production and characterization of monoclonal antibodies to spiperone

In order to obtain an immune response to spiperone the drug first had to be coupled to albumin. This was

Table 1. Properties of monoclonal anti-spiperone antibodies

Mouse monoclonal anti-spiperone antibodies were characterized as shown. IC_{50} values (concentrations of substance that inhibits 50% of the maximum [3H]spiperone binding) were determined as described by including the competing ligand in the radioimmunoassay for [3H]spiperone binding. The values shown are derived from full inhibition curves with points determined in duplicate and for AS-1 and AS-5 the data are the mean of two experiments. Typical affinities (K_i or corrected IC_{50} , nM) for binding to membrane-bound D_2 -dopamine receptors are: apomorphine, 76^a; (+)-butaclamol, 1.1^a; (–)-butaclamol, 1960^a; domperidone, 3.9^b; haloperidol, 4.5^c; mianserin, 2050^a; spiperone, 0.14^a; (+)-sulpiride, 2000^d; (–)-sulpiride, 81.5^d; values are taken from ^aWithy *et al.* (1981), ^bS. H. Simmonds & P. G. Strange (unpublished work), ^cM. Leonard & P. G. Strange (unpublished work), and ^dSeeman (1980). The titre of antibody was determined as the dilution of tissue culture supernatant that bound 50% of the maximum in the [3H]spiperone radioimmunoassay. The isotypes were determined as described in the Materials and methods section.

Property	Monoclonal anti-spiperone antibody				
	AS-1	AS-2	AS-3	AS-4	AS-5
IC_{50} (nM) of different ligands for inhibition of [3H]spiperone binding to monoclonal antibodies					
Apomorphine	> 10 ⁶	500000	350000	> 10 ⁶	> 10 ⁶
(+)-Butaclamol	335000	400000	200000	> 10 ⁶	> 10 ⁶
(–)-Butaclamol	282000	160000	100000	> 10 ⁶	> 10 ⁶
Domperidone	3000	2240	5600	10000	490
Haloperidol	265	1580	38000	–	1780
Mianserin	> 10 ⁶	800000	200000	> 10 ⁶	> 10 ⁶
Spiperone	6	9	6	10	3
(–)-Sulpiride	> 10 ⁶	–	–	–	–
(+)-Sulpiride	> 10 ⁶	–	–	–	–
Titre of antibody for [3H]spiperone binding	1:57	1:60	1:37	1:182	1:85
Heavy and light chain isotype					
Heavy chain	γ_{2b}	γ_1	γ_1	γ_1	γ_1
Light chain	κ	κ	κ	κ	κ

achieved via the carbonyl group following conversion to a carboxymethoxyloxime and then coupling to human serum albumin with a water-soluble carbodi-imide as in Juszczak & Strange (1985). Coupling ratios varied from 4:1 to 16:1 (spiperone:albumin, mol:mol). The coupled spiperone had an affinity for soluble D_2 -dopamine receptors approx. 30-fold lower than that of free spiperone (K_i spiperone, 2.3 nM; K_i albumin-spiperone, 65 nM).

The spleen of a mouse immunized with albumin-spiperone was removed 5 days after a booster immunization and the cells were fused with NS-1 myeloma cells. By this procedure five stable monoclonal hybridoma cell lines (designated with reference to the antibodies secreted as AS-1–AS-5) were produced that secreted antibody that would bind [3H]spiperone in a radioimmunoassay. The undiluted culture supernatants would bind essentially all of the radioligand in the radioimmunoassay. The titres of the culture supernatants (described as that dilution that would bind 50% of the maximum in the radioimmunoassay) are shown in Table 1. They ranged from 1:37 to 1:182, the differences probably reflecting the different concentrations of antibody. The affinity of a series of dopamine antagonists and agonists and the 5HT₂-serotonin antagonist mianserin were estimated by assessing their ability to inhibit [3H]spiperone binding to the monoclonal antibodies in the radioimmunoassay. The results in Table 1 show that all five monoclonal antibodies are quite similar in that they all had a high affinity for spiperone, they all recognised haloperidol and domperidone but they did not bind with high affinity

Fig. 1. Inhibition of [3H]spiperone binding to antibody AS-1 by anti-idiotypic serum from mouse 1

[3H]Spiperone binding to antibody AS-1 was determined by radioimmunoassay in the presence of different dilutions of serum from mouse 1 that had been immunized with AS-1. The serum and AS-1 were preincubated (16 h, 4 °C) before the addition of [3H]spiperone as described. Data are the means of duplicate determinations (variation generally < 10%).

(+)- or (–)-butaclamol, apomorphine or the 5HT₂-serotonin receptor antagonist mianserin. Additionally, AS-1 did not show any affinity for the stereoisomers of sulpiride. The results of the isotypic determination are shown in Table 1. All five monoclonal antibodies had the

Table 2. Titres of anti-idiotypic antisera obtained from immunization of mice with monoclonal anti-spiperone antibody AS-1

Titres were determined from full inhibition curves as in Fig. 1 with each serum being assayed in duplicate at the times shown (variation between determinations < 10%). The dilution of mouse serum that inhibited 50% of the maximum [³H]spiperone binding to AS-1 in the radioimmunoassay is given, for mice immunized with monoclonal antibody AS-1, the same antibody coupled to haemocyanin or the hybridoma secreting AS-1 fixed as described. ^aMaximum inhibition 10%; ^bno inhibition.

Mouse	Immunogen	Titre after:		
		Two injections	Three injections	Four injections
1	AS-1	—	1:140	1:255
2	AS-1	—	1:916	—
3	AS-1	^a	1:80	1:140
4	AS-1	1:1428	—	—
5	AS-1-haemocyanin	1:900	1:2214	—
6	AS-1-haemocyanin	1:2288	1:5633	—
7	AS-1 hybridoma	—	^b	—
8	AS-1 hybridoma	—	^b	—

Fig. 2. Inhibition of [³H]spiperone binding to antibody AS-1 by tissue culture supernatant from AID-1 hybridoma

[³H]Spiperone binding to antibody AS-1 was determined by radioimmunoassay in the presence of different dilutions of culture supernatant from hybridoma AID-1. AID-1 and AS-1 were preincubated (16 h, 4 °C) before the addition of [³H]spiperone as described. Data are the means of duplicate determinations (variation generally < 10%). In this experiment 50% inhibition of [³H]spiperone binding was obtained at a dilution of 1:67 and in a separate experiment 50% inhibition was obtained with a dilution of 1:54.

κ -light chain. AS-1 was of the γ_{2b} subclass, whereas AS-2, AS-3, AS-4 and AS-5 were all of the γ_1 sub-class.

Production and characterization of anti-idiotypic antibodies

As AS-1 was of the γ_{2b} sub-class it could be easily purified from other proteins in the culture supernatant by using Protein A affinity chromatography (Goding, 1978). Because of the ease of purification this antibody was chosen in an attempt to raise anti-idiotypic antibodies by a syngeneic route, i.e. Balb/c mouse antibody into a Balb/c mouse. The mice were immunized with one of three different preparations; either the monoclonal antibody AS-1 was injected by itself or it was coupled to haemocyanin to increase its immunogenicity or lastly the glutaraldehyde-fixed hybridoma cell line that secreted

Table 3. Effect of anti-idiotypic antiserum and monoclonal antibody (AID-1) on [³H]spiperone binding to anti-spiperone monoclonal antibodies AS-1-AS-5 and [³H]haloperidol binding to anti-haloperidol monoclonal antibody

The effect of antiserum from mouse 6 (dilution 1:100) and monoclonal antibody AID-1 (dilution 1:20 of culture supernatant) on [³H]spiperone binding to antibodies AS-1-AS-5 and [³H]haloperidol binding to an anti-haloperidol monoclonal antibody (AH) (R. Juszczak & P. G. Strange, unpublished work) were determined by radioimmunoassay as described. Data are the means of duplicate determinations (variation generally < 10%). Essentially similar results to those obtained with the antiserum from mouse 6 were obtained with the antiserum from mouse 2.

Antibody	Binding (% of maximal)	
	Antiserum (mouse 6)	Monoclonal antibody (AID-1)
AS-1	5.5	30.4
AS-2	110.8	98.9
AS-3	94.7	106.8
AS-4	96.4	96.7
AS-5	105.2	114.7
AH	101.3	98.5

AS-1 was used. Four mice received the first preparation (mice 1-4), two the second (mice 5 and 6) and two the third (mice 7 and 8). Titres were estimated for each antiserum at different times (being that dilution of serum that half-maximally inhibited [³H]spiperone binding to AS-1) (Fig. 1) and are shown in Table 2. Thus the highest titres were obtained in the mice (5 and 6) immunized with AS-1 coupled to haemocyanin, even though the mice received only small quantities of protein.

The spleen cells from three of these mice were used in separate fusions with NS-1 myeloma cells. In two cases (mice 2 and 4), although the fusion was successful, in that 80% of the wells in the microtitre plates contained growing hybridoma cells, none of the hybridomas was found to secrete anti-idiotypic antibody. In the third

Table 4. Effect of anti-idiotypic antiserum and monoclonal antibody AID-1 on [³H]spiperone binding to D₂-dopamine receptor

[³H]Spiperone binding to membrane bound D₂-dopamine receptors or cholate-solubilized receptors from bovine caudate nucleus was determined as in Withy *et al.* (1981) and Hall *et al.* (1983) but including a preincubation of receptors with anti-idiotypic antisera or monoclonal antibody (tissue culture supernatant concentrated 6-fold) as described in the Materials and methods section. Data are the means of triplicate determinations with variation generally less than 10%.

Anti-idiotypic serum	Specific [³ H]spiperone binding (% of maximal)	
	Membrane-bound receptors	solubilized receptors
Mouse 1	—	100.3
Mouse 2	—	95.0
Mouse 3	—	101.5
Mouse 4	—	90.2
Mouse 5	99.2	—
Mouse 6	93.2	—
Anti-idiotypic antibody AID-1	90.3	93.5

case, in addition to the booster immunization in Freund's incomplete adjuvant the mouse also received intraperitoneal immunisations 2 days and 1 day before the fusion without adjuvant according to the method of Stahli *et al.* (1980). This mouse (mouse 6) had the highest titre of anti-idiotypic antibody in its serum and one culture supernatant was detected as positive in the anti-idiotypic assay from approx. 300 wells that contained growing hybridomas. The cells from the positive well were grown and cloned to yield a stable monoclonal hybridoma cell line secreting the antibody designated AID-1. The anti-idiotypic effect of AID-1 in culture supernatant is shown in Fig. 2. It was of the γ_{2b} sub-class with a κ -light chain.

The anti-idiotypic antiserum from mouse 6 and the monoclonal antibody AID-1 could not inhibit [³H]spiperone binding to any of the other four monoclonal anti-spiperone antibodies or a monoclonal anti-haloperidol antibody (Table 3) developed by R. Juszczak & P. G. Strange (unpublished work). Thus the idiotypic determinant recognised on AS-1 is 'private', i.e. it does not occur on other monoclonal antibodies to spiperone or haloperidol.

Anti-D₂-dopamine receptor activity of anti-idiotypes

The antiserum from mice 1–4 and the culture supernatant containing AID-1 [concentrated 6-fold by (NH₄)₂SO₄ precipitation] were assayed for the presence of anti-D₂-dopamine receptor activity by their ability to inhibit [³H]spiperone binding to soluble receptors (Table 4). In addition the serum from mice 5 and 6 and the concentrated culture supernatant were assayed for their effects on [³H]spiperone binding to membrane bound receptors (Table 4). However, in none of these cases did the anti-idiotypes show any significant inhibition. Thus although both polyclonal and monoclonal anti-idiotypic

antibodies were produced to the monoclonal anti-spiperone antibodies they did not recognise the D₂-dopamine receptor.

DISCUSSION

The anti-idiotypic approach to producing anti-receptor antibodies is essentially a two-step process. Firstly antibodies are produced to a receptor-selective ligand with the aim of producing an antigen binding site that resembles the binding site of the receptor for the ligand. Thus the anti-ligand antibody can be considered equivalent to the receptor. The second step is to produce antibodies to the idio type of the anti-ligand antibodies. If the anti-ligand antibody has a similar specificity to the receptor the anti-idiotypic antibodies may cross-react with the receptor. Thus anti-receptor antibodies can in principle be made without needing to immunize animals with the purified receptor.

In this study five monoclonal antibodies were made to the D₂-dopamine receptor antagonist spiperone. In some respects the binding specificity of these monoclonal antibodies was similar to that of the D₂-dopamine receptor in that the antibodies would bind spiperone with a high affinity and would also recognize the dopamine antagonists domperidone and haloperidol. However, in other ways they were markedly different as they showed no discrimination between the stereoisomers of butaclamol and sulpiride and also could not interact with the agonist apomorphine. It is possibly better to regard the monoclonal antibodies to spiperone as recognizing structures related to spiperone whilst showing low affinity for unrelated structures.

The monoclonal antibody AS-1 was used to produce an anti-idiotypic response because it could be easily purified from culture supernatants by using a Protein A adsorbent. The highest titre anti-idiotypic responses were obtained when the purified AS-1 was coupled to haemocyanin before immunization, a result which is in agreement with Reth *et al.* (1979) and Phillips *et al.* (1984). No anti-idiotypic response was detected when the idio type-bearing hybridoma was used to immunize the mice, a result which is in contrast with the report of Guillet *et al.* (1984). One hybridoma was made that secreted anti-idiotypic antibody by using the spleen of a mouse that was immunized with the antibody-haemocyanin conjugate. However neither the antisera from mice 1–6 or antibody AID-1 showed any ability to recognize either membrane-bound or soluble D₂-dopamine receptors. The most probable reason for this result was that the anti-spiperone antibodies did not resemble the D₂-dopamine receptor sufficiently. It is important to mention that, even after coupling to albumin, spiperone retained its ability to bind to the D₂-dopamine receptor although the affinity was reduced. Thus the receptor-active part of spiperone would have been available to stimulate an immune response although it seems that the anti-spiperone antibodies generated did not recognize exactly that part of the molecule.

Several groups have successfully used the anti-idiotypic approach to generate anti-receptor antibodies (Schreiber *et al.*, 1980; Rockson *et al.*, 1980; Homcy *et al.*, 1982; Guillet *et al.*, 1984; Ng & Isom, 1984; Wasserman *et al.*, 1982; Glasel & Myers, 1985). The anti-ligand antibodies in these studies are often more similar to the particular receptor than is the case in the present study. Thus, for

example, Rockson *et al.* (1980) made an antiserum to the β -adrenergic antagonist alprenolol and purified a fraction of the antibodies that would recognize both agonists and antagonists and also showed some stereospecificity in the recognition of the stereoisomers of propranolol. Similarly, the antiserum made by Ng & Isom (1984) to morphine could bind opiate agonists and antagonists and showed some stereospecificity. However, the anti-ligand antibodies did not in all cases closely resemble the corresponding receptor. Thus for example the monoclonal antibodies to morphine reported by Glasel *et al.* (1983) did not strongly resemble the opiate receptor with respect to their binding affinities for a series of opiate ligands. Similarly the monoclonal antibody used by Guillet *et al.* (1984) [described in Chamat *et al.* (1984)] showed only very low stereospecificity and had an affinity for agonists much lower than that for antagonists, in contrast to the β -adrenergic receptor identified by Lefkowitz & Williams (1977). Therefore, even when the anti-ligand antibody does not closely resemble the receptor, the approach has been used successfully.

Anti-idiotypic antibodies have also been described from an alternative view as containing an 'internal image' of the ligand at their binding site (Jerne, 1974; Nisonoff & Lamoyi, 1981). Thus the anti-idiotypic antibody is viewed as having a structure within its binding site that is similar to the ligand and therefore it can essentially behave in a similar manner to the ligand. However this does not seem to be the case in this study, as the anti-idiotypic antibodies would not recognize several proteins that bind spiperone and related substances with a high affinity. Thus, although they recognised AS-1, they could not bind other monoclonal antibodies to spiperone or a monoclonal antibody to haloperidol. In addition they did not recognise the D_2 -dopamine, $5HT_2$ -serotonin or α_1 -adrenergic receptors (the present paper; W. M. Abbott & P. G. Strange, unpublished work) all of which are known to have a high affinity for spiperone (Leysen *et al.*, 1981).

There has been a recent report of the production of antibodies to spiperone and their anti-idiotypes which in preliminary results seemed to cross-react with the D_2 -dopamine receptor (Schreiber *et al.*, 1983). However, as the only definition of anti-receptor activity was the ability to bind homogenates of rat and calf striatum (an area rich in D_2 -dopamine receptors) but not muscle or cerebellum (areas with few or no D_2 -dopamine receptors) the results remain only tentative at present.

In conclusion we were unsuccessful in using the anti-idiotypic approach for raising anti-(D_2 dopamine receptor) antibodies in this study. The most probable reason was that the monoclonal antibodies generated to spiperone did not sufficiently resemble the D_2 -dopamine receptor and thus subsequent anti-idiotypes would not cross-react with the receptor. If the approach is to be applied successfully then the anti-ligand antibody would probably need to have a closer resemblance to the D_2 -dopamine receptor than those made in this study. With this in mind it would be of interest to produce antibodies to more selective D_2 -dopamine receptor ligands such as sulpiride or domperidone, or alternatively to dopamine agonists. Juszczak & Strange (1985) have raised polyclonal antibodies against haloperidol which showed a somewhat greater resemblance to the D_2 receptor than the antibodies generated in the present

paper. Anti-idiotypic antibodies that cross-reacted with D_2 -dopamine receptors were obtained in one study using these anti-haloperidol antibodies, indicating that this general approach may still be useful.

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