of adenylyl cyclase (Enjalbert & Bockaert, 1983), or inhibition of inositol phospholipid metabolism (Simmonds & Strange, 1985). In order to understand fully the mechanism of action of the D_2-receptor, it will be necessary to isolate and characterize the various components of the D_2-receptor system (Strange, 1983) and reconstitute them in a defined environment.

A monoclonal antibody directed against the D_2-receptor would be of great value for studies on the receptor, particularly in purification and cellular localization of receptors. Immunisation of mice with caudate nucleus membranes, solubilized D_2-receptor preparation and D_2-receptor partially purified on wheatgerm agglutinin-agarose and the subsequent use of hybridoma technology has been employed in an attempt to raise a monoclonal antibody directed against the D_2-receptor (Strange et al., 1986). In no case was such an antibody obtained. Another approach, which avoids the problems inherent in isolation and purification of the receptor, is through the production of anti-idiotypic antibodies. In this approach, antibodies are raised against a D_2-receptor-specific drug. Antibodies (anti-idiotypes) raised against the first antibody may be anti-receptor antibodies (for a review, see Farid & Lo, 1985). In the present report we have attempted to use this strategy with monoclonal antibodies using the D_2-receptor-selective drug haloperidol in order to extend our previous work with polyclonal antibodies (Juszczak & Strange, 1985).

Haloperidol was derivatized either via the carbonyl group using carbamoylmethylamine (type I haloperidol) or via the hydroxyl group using succinyl dichloride (type II haloperidol). Both derivatives were coupled to albumin and used to immunize mice. Hybridoma technology was applied and seven hybridomas secreting monoclonal antibodies (designated type I or type II according to the derivative used) specific for haloperidol have been obtained; the properties of some of the monoclonal antibodies are given in Table 1. Both type I and type II monoclonal antibodies bind [^3H]haloperidol with high affinity but differ in their selectivity for binding of other substances, as judged by the ability of these substances to displace [^3H]haloperidol binding. Type I monoclonal antibodies seem to be specific for haloperidol, whereas type II monoclonal antibodies can bind a range of substances, with a substantial affinity for the butyrophenones, droperidol, haloperidol and spiperone, and a weak but significant affinity for apomorphine, (+)-butaclamol and dopamine.

Immunization of rabbits with purified type I monoclonal antibody, gave antisera which at dilutions between 1/10 000 and 1/15 000 inhibited 50% of [^3H]spiperone binding to type I monoclonal antibody. However, these rabbit sera did not affect [^3H]spiperone binding to caudate nucleus membranes and thus were not anti-D_2-receptor antibodies.

In conclusion, monoclonal antibodies have been raised against haloperidol. These antibodies, in addition to binding haloperidol with high affinity, also bind other D_2-receptor-selective ligands with high affinity. Anti-idiotypic antibodies have been obtained directed against the anti-haloperidol antibodies, but these were not anti-D_2-receptor antibodies.

We thank the Wellcome Trust for financial support.


Received 16 June 1986

### Purification of D_2-dopamine receptors from bovine brain

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D_2-dopamine receptors exist at high densities in the anterior pituitary and in the brain, particularly in the striatum. In the anterior pituitary, D_2-receptors mediate the inhibition of prolactin release via the inhibition of adenylyl cyclase (Caron et al., 1978; Enjalbert & Bockaert, 1983), whereas in the brain they are involved in motor function and control of motor behaviour. The D_2-receptor has also been shown to inhibit polyphosphoinositol metabolism (Simmonds & Strange, 1985). In order to fully elucidate its transduction mechanism pure receptor must be reconstituted into model systems. As a first step towards this aim we have isolated D_2-receptor binding from receptor purification using affinity chromatography.

The carbamoylmethyl oxime derivative of haloperidol was prepared (Juszczak & Strange, 1985) and immobilized on α-aminoadecy1-Sepharose (S. Worrall, R. A. Williamson & P. G. Strange, unpublished work). A mixed mitochondrial-microsomal membrane preparation of bovine caudate and substantia nigra was prepared as described by Withy et al. (1982). The D_2-receptors contained in the membranes were solubilized using the method of Hall et al. (1983) with the following reagents:

<table>
<thead>
<tr>
<th>Drug</th>
<th>K_I (nM)</th>
<th>K_a (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apomorphine</td>
<td>10^5</td>
<td>10^3</td>
</tr>
<tr>
<td>(+)-Butaclamol</td>
<td>10^5</td>
<td>10^3</td>
</tr>
<tr>
<td>(-)-Butaclamol</td>
<td>10^5</td>
<td>10^3</td>
</tr>
<tr>
<td>Dopamine</td>
<td>10^5</td>
<td>10^3</td>
</tr>
<tr>
<td>Droperidol</td>
<td>10^5</td>
<td>10^3</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>10^5</td>
<td>10^3</td>
</tr>
<tr>
<td>Mianserin</td>
<td>10^5</td>
<td>10^3</td>
</tr>
</tbody>
</table>

Table 1. Inhibition of binding of [^3H]haloperidol to mouse anti-haloperidol monoclonal antibodies

We thank the Wellcome Trust for financial support.
modification that for solubilization they were resuspended at 4 mg of protein/ml in 20 mM-Hepes buffer pH 7.4 (containing 0.3% cholate, 1mM-NaCl, 10 mM-EDTA, 1 mM-EGTA).

The solubilized preparation obtained after centrifugation was diluted 3:1 with 20 mM-Hepes buffer pH 7.4 (containing 10 mM-EDTA and 1 mM-EGTA) and incubated with the affinity matrix for 15-20 h at 4°C. The gel was then washed with 20-30 bed vol. of 20 mM-Hepes (containing 0.2% cholate, 1 mM-NaCl, 10 mM-EDTA, 2 mM-sodium ascorbate, and 0.04% phosphatidylcholine) over a 2 h period. Bound receptor was eluted over 48 h with 1 bed vol. of 1 mM-metoclopramide in wash buffer. This eluate was subjected to further purification on a wheatgerm lectin-Sepharose column as described by Abbott & Strange (1985). Protein concentrations were generally determined using the method of Lowry et al. (1951) as modified by Wheatly & Strange (1983), except for the wheatgerm lectin column eluates which were assayed using the Amidoschwarz procedure of Schaffner & Weissmann (1973). Receptor-binding activity was assayed using [3H]spiperone as described in Hall et al. (1983).

In a series of experiments 73% of the applied binding activity (8696 ± 631 fmol, mean ± s.E.M., n = 7) was retained on the affinity column, the remainder being recovered in the run through and wash fractions. In contrast, of the applied protein (85.9 ± 3.7 mg, mean ± s.E.M.) more than 98% was recovered in these fractions. Only 2% of the protein eluted from the column using 1 mM-metoclopramide was retained and eluted from a wheatgerm lectin column (0.8 ± 0.1 µg/ml compared with 42.0 ± 3.2 µg/ml added). The recovery of binding activity in the wheatgerm lectin eluate was variable, with activity being recovered on four out of seven occasions. When activity was recovered, about 12% of the receptor binding retained by the haloperidol affinity column could be measured in the eluates, giving a purification of 1327 ± 280-fold (mean ± s.E.M., n = 4). Representative results for the purification procedure are given in Table 1.

The purification obtained by the present procedure is considerably below that required to produce a pure receptor preparation (about 100,000-fold needed if M, for receptor is 80,000). However, SDS/polyacrylamide-gel electrophoresis analysis of purified preparations (indicating a single major species of M, 80,000) suggests a considerably greater level of purification is being achieved than is apparent from the [3H]spiperone-binding activity data (S. Worrall, R. A. Williamson & P. G. Strange, unpublished work). This disparity presumably reflects instability of the receptor and the consequent loss of [3H]spiperone-binding activity in the eluates. Attempts are now being made to increase the stability of the eluted receptor.

We thank the M.R.C. and S.E.R.C. for financial support.


Received 16 June 1986

Interaction of serotonergic and adrenergic agonists on inositol phospholipid metabolism in rat frontal cortex slices

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Inositol phospholipid metabolism in nervous tissue has been shown to be stimulated by a number of monoamine neurotransmitters, such as histamine, acetylcholine analogues, noradrenaline and 5-hydroxytryptamine (serotonin) (Brown et al., 1984; Daum et al., 1984; Gonzales & Crews, 1984), as well as amino acid (Nicoletti et al., 1986) and peptide neurotransmitters (Downes, 1982). Recently, several studies have shown that co-addition of different receptor agonists to assays in vitro can affect the production of [3H]inositol phosphates by certain receptors. In some cases this receptor interaction was found to be approximately additive (Brown et al., 1984), whereas in others it was synergistic (Hollingsworth et al., 1986) or inhibitory (Simmonds & Strange, 1985; Nicoletti et al., 1986).

Using rat frontal cortex slices prelabelled with [3H]inositol, we have looked at the effect of 5-hydroxytryptamine and the α2-adrenoceptor agonist methoxamine, added individually or in combination, on [3H]inositol phosphate production. Rat frontal cortex slices were prepared, and incubated for 30 min in a shaking water bath, in Hepes-buffered Krebs solution (concentration in mM: NaCl 118, KC1 4.7, MgSO4 1.2, CaCl2 2.6, KH2PO4 1.2, glucose 11.7 and Hepes 20), pH 7.4 at 37°C, being gassed continuously with 95%O2/ 5%CO2. Gravity packed slices (50 µl) were pipetted into vials containing approx. 0.3 µl [3H]inositol in Hepes-buffered Krebs solution plus 8 mM-LiCl and 1 mM-ascorbate, and incubated for 45 min. At this time, either

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total protein (mg)</th>
<th>Total binding (fmol)</th>
<th>Specific activity (fmol/mg)</th>
<th>Purification (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble preparation</td>
<td>85.5</td>
<td>7547</td>
<td>88.3</td>
<td>1.0</td>
</tr>
<tr>
<td>Run through</td>
<td>73.8</td>
<td>689</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Wash</td>
<td>14.3</td>
<td>1105</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Eluate</td>
<td>0.6</td>
<td>N.D.*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WGL-Sepharose</td>
<td>0.008</td>
<td>1199</td>
<td>149875</td>
<td>1469</td>
</tr>
</tbody>
</table>

Table 1. Purification of D2-dopamine receptors from bovine brain

Cholate-solubilized D2-receptors (Soluble preparation) were applied to a haloperidol-Sepharose affinity column. Unbound receptor was recovered in ‘Run through’ and ‘Wash’ fractions and bound receptor was eluted with metoclopramide (‘Eluate’). Eluates were further purified on wheatgerm lectin(WGL)- Sepharose columns (Abbott & Strange, 1985). Receptor binding and protein were assayed as described, except for (*) where binding was not determined because metoclopramide was present.

Abbreviation used: EC90, concentration of agonist required to elicit 90% maximal response.

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