The glycosylation properties of D₂ dopamine receptors from striatal and limbic areas of bovine brain

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D₂ dopamine receptors from bovine brain (caudate nucleus and olfactory tubercle) have been solubilized using sodium cholate/NaCl and their glycoprotein properties studied in terms of their interaction with wheat-germ agglutinin-agarose (WGA-agarose). Under optimal conditions about 65% of the applied D₂ dopamine receptors bound to WGA-agarose and could be eluted with *N*-acetylglucosamine. The ability of receptors to adsorb to the affinity column was shown to be dependent on the cholate and salt concentrations used. Digestion of the membrane bound D₂ dopamine receptors with neuraminidase prior to solubilisation reduced the ability of the receptors to bind to WGA-agarose (50% of applied receptors bound) whereas digestion with *N*-acetylglucosaminidase did not significantly affect binding to WGA-agarose. Digestion with the two enzymes together resulted in a larger decrease in binding to WGA-agarose than was seen with the two enzymes alone (40% of applied receptors bound). Stepwise elution of bound receptors from the WGA-agarose columns using 2.5 mM- and 100-mM-*N*-acetylglucosamine showed that about 40% of the bound receptors interacted with WGA-agarose in a low-affinity manner, the remainder showing a high-affinity interaction. Neuraminidase treatment reduced the low-affinity population suggesting that the interaction of oligosaccharides bearing sialic acid with WGA-agarose is of lower affinity and that higher-affinity binding is via *N*-acetylglucosamine. These data are discussed in terms of the heterogeneity of carbohydrate moieties on the D₂ dopamine receptors within a brain region. In all the tests applied here, however, receptors from caudate nucleus and olfactory tubercle behaved identically so their glycosylation patterns must be very similar.

INTRODUCTION

Recently much work has been directed towards determining the numbers and functions of brain receptors for the neurotransmitter dopamine. The majority of available data suggests that dopamine mediates its effects through two receptor species (reviewed in Seeman, 1980; Strange, 1987). These are the D₁ dopamine receptor linked to stimulation of adenylate cyclase (Kebabian & Calne, 1979) and the D₂ dopamine receptor, which is linked to inhibition of adenylate cyclase (Cooper *et al.*, 1986), inhibition of inositol phospholipid metabolism (Simmonds & Strange, 1985; Enjalbert *et al.*, 1986; Pizzi *et al.*, 1987) and stimulation of K⁺ channels (Lacey *et al.*, 1987; Memo *et al.*, 1987). There have been a number of reports suggesting that D₂ dopamine receptors may be heterogeneous either within brain regions or between brain regions, e.g. striatal versus limbic (reviewed in Leonard *et al.*, 1987). We have examined such heterogeneity using ligand binding (Leonard *et al.*, 1987), and can find no evidence for differences at the level of antagonist binding. Some evidence did emerge from this work and from mechanistic studies (Stoof & Verheijden, 1986; Kelly & Nahorski, 1987) for differences in D₂ dopamine receptor mechanism between limbic and striatal regions of brain.

In common with many receptors the D₂ dopamine receptor is a glycoprotein (Lew & Goldstein, 1984; Kilpatrick & Caron, 1984; Abbott & Strange, 1985; Kilpatrick *et al.*, 1985; Grigoriadis *et al.*, 1988), and in

the present report we have investigated whether the glycosylation patterns vary within a brain region and between limbic and striatal brain regions. We have used the techniques of lectin affinity chromatography on wheat-germ agglutinin-agarose (WGA-agarose) and digestion with exoglycosidases to examine this.

MATERIALS AND METHODS

Materials

Crude soybean phosphatidylcholine, sodium cholate, *N*-acetylglucosamine, WGA, WGA-agarose (5–10 mg/ml packed gel), neuraminidase (from *Clostridium perfringens*) and *N*-acetylglucosaminidase (from Jack beans) were obtained from Sigma Chemical Company, Poole, Dorset, U.K. Other chemicals were obtained as described in Leonard *et al.* (1987) and Hall *et al.* (1983).

Solubilization of D₂ dopamine receptors

A mixed mitochondrial/microsomal preparation of bovine caudate nucleus or olfactory tubercle was obtained as described by Leonard *et al.* (1987) and diluted to a final protein concentration of 8 mg/ml in a 20 mM-Hepes buffer containing 1 mM-EDTA, pH 7.4 (Buffer I). For solubilization, the diluted membrane preparation was mixed at 4 °C with an equal volume of Buffer I containing 2 M-NaCl and 0.6% (w/v) sodium cholate, phenylmethanesulphonyl fluoride (0.1 mM final

Abbreviations used: WGA, wheat-germ agglutinin.

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concentration) having been added just prior to solubilization and the mixture was stirred gently at 4 °C for 60 min. The solubilized fraction (containing 1.8–2.0 mg of protein/ml) was obtained by centrifuging the mixture at 130 000 *g* for 60 min at 4 °C and taking 80–90 % of the clear supernatant.

Affinity chromatography on WGA–agarose columns

Affinity chromatography of solubilized D₂ dopamine receptors on WGA–agarose was performed using a similar method to that employed by Abbott & Strange (1985). Except where otherwise mentioned, 4 ml of soluble receptor preparation was added to columns containing 1.5 ml of packed gel, the flow was stopped and the mixture agitated by turning the column end-over-end for 90 min at 4 °C. Unbound receptors were collected in the flow through volume and two consecutive 10 ml washes of Buffer I containing 0.67 M-NaCl, 0.2 % (w/v) sodium cholate, 0.04 % (w/v) phosphatidylcholine and 2 mM-sodium acetate. To elute the bound receptors, 10 ml of 100 mM-*N*-acetylglucosamine (in the above buffer) was added to the columns, the flow was stopped and the mixture was agitated for a further 90 min at 4 °C. Before measurement of binding activity, the applied receptor and the run-through fraction were diluted in Buffer I to decrease the NaCl and sodium cholate to 0.67 M and 0.2 % (w/v) respectively.

[³H]Spiperone binding assays

Soluble receptors were assayed for [³H]spiperone binding, separating free and bound radioligand by a charcoal adsorption separation technique as described by Wheatley & Strange (1983). Assays were performed for 4 h or 16 h at 4 °C and no significant difference was seen between [³H]spiperone binding at these two times. Specific [³H]spiperone binding was defined as the difference in binding in parallel assays containing 1 μM-(+)- and (–)-butaclamol. In assays on WGA–agarose-column eluates, the concentration of *N*-acetylglucosamine varied depending on the particular eluate. In control experiments this sugar was found not to affect [³H]spiperone binding to D₂ dopamine receptors. Protein was determined as described by Leonard *et al.* (1987).

Exoglycosidase treatment of D₂ dopamine receptors

To cleave terminal *N*-acetylglucosamine or sialic acid residues from D₂ dopamine receptors, mixed mito-

chondrial/microsomal membrane preparations were incubated at 30 °C in the presence of *N*-acetylglucosaminidase or neuraminidase respectively (5 munits of enzyme/mg of protein) for 30 min, in buffer I. Following this treatment the D₂ dopamine receptors were solubilized and subjected to lectin-affinity chromatography as described above.

Quantification of sialic acid liberated by neuraminidase

To determine the amount of sialic acid released following neuraminidase treatment, the membranes were immediately chilled to 4 °C and centrifuged at 130 000 *g* for 60 min. A 200 μl sample of the clear supernatant was taken and the sialic acid was quantified by the method described by Warren (1959) and a molar absorption coefficient of 5.7 × 10⁴. In all cases sialic acid was determined for neuraminidase-treated membranes against not-treated controls.

Analysis of radioligand binding data

All radioligand binding data were analysed as described by Leonard *et al.* (1987).

RESULTS

Optimization of conditions for WGA–agarose-affinity chromatography

Elsewhere we have shown that, whereas solubilization of brain D₂ dopamine receptors is most efficient using 0.3 % sodium cholate/1 M-NaCl, the preparation may be rendered much more stable by dilution after solubilization (0.67–0.7-fold dilution; Strange & Williamson, 1988). Such a dilution would be desirable for performing WGA–agarose-affinity chromatography. Therefore, we first investigated the effects of such dilution on the adsorption of solubilized D₂ dopamine receptors to WGA–agarose and subsequent elution by *N*-acetylglucosamine (100 mM). D₂ dopamine receptors were assayed in these experiments by [³H]spiperone binding. [³H]Spiperone binding was unaffected by free WGA (results not shown; Abbott & Strange, 1985).

The results (Table 1) show that for the standard soluble preparation (0.3 % cholate/1 M-NaCl) approx. 68 % of the D₂ dopamine receptors are taken up and can be eluted from the WGA–agarose columns. Dilution of

Table 1. Effect of alteration of sodium cholate and NaCl on the interaction of D₂ dopamine receptors with WGA–agarose

D₂ dopamine receptors from bovine caudate nucleus were solubilized with 0.3 % sodium cholate/1 M-NaCl as described. The cholate and NaCl concentrations were adjusted to those stated in the Table and adsorption to WGA–agarose and elution with 100 mM-*N*-acetylglucosamine in buffer with reduced cholate and/or NaCl performed as described. The distributions of receptors in the various fractions were calculated as percentages of the recovered material, which in all cases represented greater than 90 % of that applied to the column. Results are expressed as mean ± S.D. (*n*).

Sodium cholate [% (w/v)]	NaCl (M)	Receptors remaining unbound (%)	Receptors specifically eluted by 100 mM <i>N</i> -acetylglucosamine (%)
0.3	1.0	32.1 ± 7.2 (3)	67.9 ± 7.2 (3)
0.1	1.0	86.0 ± 6.3 (5)	14.0 ± 6.3 (5)
0.3	0.33	60.5 ± 7.5 (3)	39.5 ± 7.5 (3)
0.1	0.33	95.8 ± 2.4 (6)	4.2 ± 2.4 (6)

Fig. 1. Analysis of proteins eluted from WGA-agarose under different conditions

Solubilized receptor preparation (4 ml of 0.3% cholate/1 M-NaCl, 5.33 ml of 0.225% cholate/0.67 M-NaCl, 12 ml of 0.1% cholate/0.33 M-NaCl) were applied to separate WGA-agarose columns (1.5 ml) as described. The columns were washed and eluted with 100 mM-*N*-acetylglucosamine (8 ml). Of each eluate, 3 ml was prepared for electrophoresis using chloroform/methanol precipitation (Wessel & Flugge, 1984). SDS/polyacrylamide-gel electrophoresis was carried out under reducing conditions according to the method of Laemmli (1970) and the gel stained with PAGE Blue 83. Lane 1, standard proteins (M_r 94000, 67000, 43000, 30000 and 20100 from top to bottom); Lane 2, 0.1% cholate/0.33 M-NaCl preparation; Lane 3, 0.225% cholate/0.67 M-NaCl preparation; Lane 4, 0.3% cholate/1 M-NaCl preparation.

the cholate and NaCl to 0.1% and 0.33 M respectively virtually abolishes adsorption of D_2 receptors to WGA-agarose and intermediate effects are observed if dilution

is restricted to one component only. The effect of dilution seems to be due to reduction of both the cholate and NaCl with the former having a greater effect. In preliminary experiments increased adsorption was achieved by raising the cholate and NaCl above the standard 0.3%/1 M (e.g. at 0.35% cholate/1.16 M-NaCl, 85% of the receptors bound to WGA-agarose), but under these conditions the preparation becomes very labile and difficult to work with.

As indicated above dilution of the standard preparation 0.67–0.7-fold considerably increases the stability of the preparation. This, however, reduces the adsorption of receptors to WGA-agarose to 25–30% so that it is necessary to perform all adsorptions to the WGA-agarose under standard conditions used for solubilization, 0.3% cholate/1 M-NaCl, for which a higher adsorption is seen. Elutions are, however, preferably carried out with the diluted concentrations to increase stability and for consistency all fractions are then adjusted to 0.2% cholate/0.67 M-NaCl before assay for [3 H]-spiperone binding.

These data establish that dilution of cholate and NaCl reduces adsorption of D_2 dopamine receptors to WGA-agarose. We investigated whether adsorption of other proteins is also reduced upon dilution (Fig. 1). The results show that although there are some differences in the proteins eluted under different conditions there is a general reduction in protein adsorption to WGA-agarose as the cholate and NaCl are diluted. Thus this is not an effect specific for the D_2 dopamine receptor.

We have investigated further the interaction of the D_2 dopamine receptor with WGA-agarose. In the standard experiments outlined above, 4 ml of receptor preparation was applied to a 1.5 ml WGA-agarose column resulting in approx. 65% uptake on to the column and elution with *N*-acetylglucosamine. If the amount of receptor preparation applied to the column was increased to 10 ml, then approx. 30% is taken up by the column and subsequently eluted, the remainder appearing in the run-through and wash fraction (Table 2). Reapplication of the run-through fraction to a similar column resulted in uptake of about 40% of the receptors. Thus under the conditions where 10 ml of receptor preparation is applied to 1.5 ml of WGA-agarose the capacity of the column is exceeded. Reapplication of the run-through fraction from a standard incubation, however, resulted in uptake of 6% or less of the receptors. Thus in a standard incubation the capacity of the column is not exceeded but there seems to be a population of receptors that are unavailable for uptake by the column. Quantitative analysis of the data of Table 2 indicates that, in all the experiments shown in that Table, this proportion represents about 35% of the starting soluble preparation. Although this could be taken to imply the existence of receptor variants with differing glycoprotein side-chains, this seems unlikely as further uptake can be achieved by increasing the cholate and NaCl concentration (see above). The adsorption of receptors is not, however, increased by increasing the incubation time on the WGA-agarose from 90 min to 16 h (results not shown), so the incomplete retention is not due to incomplete equilibration on to the column.

Application of receptor preparation solubilized from bovine olfactory tubercle to WGA-agarose resulted in essentially identical results, approx. 65% of the receptors being taken up under standard conditions (Table 4).

Table 2. Adsorption of D₂ dopamine receptors to WGA-agarose

D₂ dopamine receptors were solubilized from bovine caudate nucleus using 0.3% cholate/1 M-NaCl as described and applied to WGA-agarose columns (1.5 ml of packed gel) in varying loading ratios as indicated (lanes *a* and *b*). The run-through fractions (containing unbound receptors) from lanes *a* and *b* were applied to fresh columns (lanes *c* and *d*). Bound receptors were eluted with 10 ml of 100 mM-*N*-acetylglucosamine in buffer I containing 0.67 M-NaCl, 0.2% sodium cholate (w/v), 0.04% phosphatidylcholine (w/v) and 2 mM-sodium acetate. The distributions of receptors in the various fractions were calculated as percentages of the recovered material which in all cases represented more than 90% of the material applied to the column. Results are expressed as mean $\pm$ S.D.

	Material applied to WGA-agarose	Receptors remaining unbound (%)	Receptors bound and eluted by <i>N</i> -acetylglucosamine (%)	Number of experiments
(a)	Soluble receptor preparation (10 ml)	69.3 $\pm$ 4.1	30.7 $\pm$ 4.1	5
(b)	Soluble receptor preparation (4 ml)	33.6 $\pm$ 2.3	66.4 $\pm$ 2.3	3
(c)	Run-through fraction from (a) (10 ml)	63.0 $\pm$ 9.1	37.0 $\pm$ 9.1	3
(d)	Run-through fraction from (b) (4 ml)	94.4 $\pm$ 2.2	5.6 $\pm$ 2.2	3

Table 3. Comparison of [³H]spiperone-competition experiments for soluble D₂ dopamine receptor preparation and WGA eluates

Bovine caudate nucleus soluble receptor preparation and WGA-agarose eluates were incubated with [³H]spiperone ($\sim$ 1.0 nM) in the presence of various concentrations of displacing drugs for 4 h at 4 °C. Specific binding was defined as the difference in [³H]spiperone binding in the presence of 1 μ M (+)- and (-)-butaclamol. Competition-binding data were analysed as in Leonard *et al.* (1987) by computer non-linear least squares fitting to give *K_i* (inhibition constant) and *nH* (pseudo-Hill coefficient). Data values are mean $\pm$ S.D. for three experiments or mean $\pm$ range for two experiments. N.D., not determined.

Drug	Soluble preparation			WGA-agarose eluates		
	<i>K_i</i>	<i>nH</i>	<i>n</i>	<i>K_i</i>	<i>nH</i>	<i>n</i>
(+)-Butaclamol	10.4 $\pm$ 0.5 nM	0.94 $\pm$ 0.03	2	5.8 $\pm$ 0.9 nM	1.09 $\pm$ 0.09	2
Domperidone	21 $\pm$ 4 nM	0.94 $\pm$ 0.07	2	18 $\pm$ 3 nM	0.95 $\pm$ 0.02	2
Mianserin	26 $\pm$ 2 μ M	N.D.	3	14 $\pm$ 2 μ M	N.D.	2

Pharmacological analysis of eluates from WGA-agarose columns relative to starting soluble receptor preparations

[³H]Spiperone/antagonist competition experiments were performed and the data analysed as shown in Table 3 for WGA-agarose eluates and starting soluble receptor preparation. (+)-Butaclamol and domperidone competed with similar high affinity in both preparations and competition curves were characterized by pseudo-Hill coefficients close to one. The 5HT₂ serotonin receptor antagonist mianserin showed a homogeneous low-affinity competition curve indicating the absence of 5HT₂ serotonin receptor binding. Inhibition constant (*K_i*) values for the competing ligands are similar in the two preparations. Thus in the starting soluble receptor preparation [³H]spiperone binding is to a homogenous population of D₂ dopamine receptors whose properties are unchanged by adsorption to and elution from WGA-agarose.

Exoglycosidase treatment of D₂ dopamine receptors

Neuraminidase. This exoglycosidase enzyme was used to cleave specifically terminal sialic acid residues from membrane-bound glycoproteins. Preliminary control experiments examined the ability of the procedure used

to achieve complete digestion of sialic acid residues. When bovine caudate nucleus membranes were subjected to neuraminidase (5 munits/mg of protein) digestion, free sialic acid release reached a maximum level by 30 min (approx. 3.5 μ g/mg of protein) after which no additional release was seen. Addition of a second aliquot of neuraminidase failed to produce additional sialic acid release (results not shown). Thus in all subsequent experiments, membranes were treated with neuraminidase (5 munits/mg of protein) for 30 min as described, prior to receptor solubilization and application to WGA-agarose. [³H]Spiperone binding to D₂ dopamine receptors was unaffected by this treatment. Table 4 compares the binding of untreated and neuraminidase-treated receptor preparations to WGA-agarose columns. Neuraminidase treatment reduced the binding of D₂ dopamine receptors to WGA-agarose by about 15% in both caudate nucleus and olfactory tubercle. Thus it may be concluded that some of the carbohydrate portion of D₂ dopamine receptors from both brain regions studied contains terminal sialic acid residues.

***N*-Acetylglucosaminidase.** This enzyme was used to cleave terminal *N*-acetylglucosamine residues from mem-

Table 4. Effect of neuraminidase and *N*-acetylglucosaminidase on the adsorption of D₂ dopamine receptors to WGA-agarose

Bovine caudate nucleus or olfactory tubercle membranes were treated with neuraminidase or *N*-acetylglucosaminidase as described. Receptors were solubilized and applied to WGA-agarose (4 ml soluble preparation, 1.5 ml of packed gel) and unbound and bound fractions determined as the run-through and washes and the *N*-acetylglucosamine eluate respectively. The distribution of receptors in the various fractions were calculated as percentages of the recovered material which in all cases represented greater than 90 % of the applied material. Results are given as mean $\pm$ S.D. ($n = 3$).

Sample	Caudate nucleus		Olfactory tubercle	
	Receptors remaining unbound (%)	Receptors bound and eluted by <i>N</i> -acetylglucosamine (%)	Receptors remaining unbound (%)	Receptors bound and eluted by <i>N</i> -acetylglucosamine (%)
Control	33.6 $\pm$ 2.3	66.4 $\pm$ 2.3	34.1 $\pm$ 4.1	65.9 $\pm$ 4.1
Neuraminidase-treated	47.3 $\pm$ 0.5	52.7 $\pm$ 0.5	50.8 $\pm$ 3.7	49.2 $\pm$ 3.7
<i>N</i> -Acetylglucosaminidase-treated	38.1 $\pm$ 4.2	61.9 $\pm$ 4.2	39.0 $\pm$ 3.7	61.0 $\pm$ 3.7
Treated with both enzymes	56.7 $\pm$ 2.9	43.3 $\pm$ 2.9	63.0 $\pm$ 3.6	37.0 $\pm$ 3.6

brane-bound glycoproteins and the procedure used was essentially identical to that used for neuraminidase. The enzyme treatment did not affect [³H]spiperone binding to D₂ dopamine receptors. The results shown in Table 4 indicate that the enzyme treatment has no significant effect on subsequent binding of solubilized receptors to WGA-agarose, so that there are unlikely to be any accessible terminal *N*-acetylglucosamine residues on the carbohydrate portion of D₂ dopamine receptors.

Neuraminidase and *N*-acetylglucosaminidase. When the two enzyme treatments were combined the results (Table 4) showed a much greater effect on subsequent WGA-agarose binding than for either treatment alone. The combined enzyme digestion reduced binding by 25–30 % compared with a 15 % decrease (neuraminidase) and no significant effect (*N*-acetylglucosaminidase). This finding also suggests that the lack of effect of *N*-acetylglucosaminidase when used alone is not due to a technical problem, but that the enzyme is active under the conditions of the experiment.

Very similar results were obtained from the two brain regions for all the enzyme treatments suggesting that the glycosylation patterns in the two regions are similar.

Demonstration of high- and low-affinity binding sites for WGA-agarose on solubilized D₂ dopamine receptors

Although WGA has been shown to interact with oligosaccharides via sialic acid, *N*-acetylglucosamine and *N*-acetylgalactosamine residues (Bhavanandan & Katlic, 1979; Peters *et al.*, 1979), it seems that interactions via terminal sialic acid residues are of lower affinity compared with those through other residues such as internal *N*-acetylglucosamine sequences (Gallagher *et al.*, 1985).

In an attempt to see if low- and high-affinity binding sites exist for WGA on D₂ dopamine receptors, untreated and neuraminidase-treated soluble D₂ receptors were applied to WGA-agarose columns. The bound receptors were eluted in a stepwise fashion: firstly, the receptors bound with a low affinity were eluted with 10 ml of 2.5 mM-*N*-acetylglucosamine and then receptors bound with a high affinity were eluted with 10 ml of 100 mM-*N*-

Fig. 2. High- and low-affinity binding of D₂ dopamine receptors to WGA-agarose columns

Control membranes and neuraminidase-treated membranes of bovine caudate nucleus and olfactory tubercle were solubilized using cholate (0.3 %) and NaCl (1 M) and applied to WGA-agarose columns as described. After washing the columns, bound receptors were eluted in a stepwise manner with 10 ml of 2.5 mM-*N*-acetylglucosamine followed by 10 ml of 100 mM-*N*-acetylglucosamine. The percentage of applied receptors eluted are shown for control (unhatched) and neuraminidase-treated (hatched) membranes. The recovery of receptors in all experiments was greater than 90 %. The results are shown as mean $\pm$ S.D. for three experiments.

acetylglucosamine. Neither concentration of *N*-acetylglucosamine affected [^3H]spiperone binding to D_2 dopamine receptors. The results are shown in Fig. 2 and indicate that in each brain area, about 65% of the applied untreated receptors became bound to the WGA-agarose column. About 40% of these were seen to be of a low affinity, whilst the remaining 60% were bound with a high affinity. When neuraminidase-treated receptors from either brain area were applied to WGA-agarose columns, only 50% became specifically bound. Interestingly, only 15–20% of these appeared to be bound with a low affinity, whilst the remaining 80–85% appeared to be bound with a high affinity. These results indicate that both high- and low-affinity binding sites exist for WGA on D_2 dopamine receptors and that, removal of terminal sialic acid residues results in a selective reduction in the proportion of low-affinity binding sites. These findings suggest that the high-affinity binding component was due, at least in part, to the presence of *N*-acetylglucosamine residues. Once again, no clear differences were seen between the results obtained for receptors from bovine caudate nucleus or olfactory tubercle, suggesting that the glycosylation patterns of these receptors are essentially identical.

DISCUSSION

In this paper we have shown that solubilized D_2 dopamine receptors adsorb to WGA-agarose and that this interaction may be used to probe the nature of the carbohydrate moiety of the receptor. This work extends our previous study on lectin-affinity chromatography (Abbott & Strange, 1985), and those of others (Lew & Goldstein, 1984; Kilpatrick & Caron, 1984; Kilpatrick *et al.*, 1985). Thus the D_2 dopamine receptor is a glycoprotein like many other cell-surface receptors, e.g. β -adrenergic (Cervantes-Olivier *et al.*, 1985; Stiles *et al.*, 1984), muscarinic acetylcholine (Herron & Schimerlik, 1983; Shirikawa *et al.*, 1983) and opiate (Gioannini *et al.*, 1982).

The results reported here show that under all the tests applied the D_2 dopamine receptors from striatal regions (caudate nucleus) and limbic regions (olfactory tubercle) of bovine brain behave identically. The glycosylation patterns are indistinguishable under the present experimental conditions and differences in glycosylation are unlikely to contribute to any pharmacological differences observed between the two brain regions (see Introduction).

Two indications of heterogeneous interaction of D_2 dopamine receptors with WGA-agarose emerged from the present study. Firstly, it was found that under the standard conditions (0.3% sodium cholate/1 M-NaCl) about 35% of the receptors would not adsorb to WGA-agarose. This was not due to lack of equilibration with the lectin. In addition, under conditions where the column capacity was not exceeded, receptors that did not bind in a first application would not bind when reapplied to a fresh column. Therefore, 'unbinding' receptors do not equilibrate rapidly with 'binding receptors'. The proportion of receptors that binds can, however, be increased by raising the concentration of cholate and NaCl after solubilization, e.g. at 0.35% cholate/1.16 M-NaCl 85% of the applied receptor was bound. This suggests that the lack of full adsorption is not due to heterogeneity of the carbohydrate moieties on the

receptors and may rather be a function of the protein-detergent micellar form. This contention is further supported by the graded reduction in adsorption as the cholate and NaCl are reduced from 0.3% and 1 M respectively. Thus it seems likely that as the detergent and salt concentration are varied, the form of the protein-detergent micelle alters in such a way as to alter the accessibility of the carbohydrate moieties on the receptor to WGA-agarose. Effects of NaCl on the size of cholate micelles have been reported (Carey & Small, 1972).

Secondly, we have provided evidence for heterogeneity within the carbohydrate moieties of receptors that do bind to WGA-agarose. Other studies (Abbott & Strange, 1985; M. N. Leonard & P. G. Strange, unpublished results) demonstrated lack of interaction with concanavalin A-agarose, indicating that the D_2 dopamine receptor does not carry high mannose-type oligosaccharide chains. Binding of a glycoprotein to WGA-agarose generally indicates interaction through either terminal sialic acid residues or specific sequences containing *N*-acetylglucosamine (Gallagher *et al.*, 1985).

Neuraminidase digestion of D_2 dopamine receptors indicated a role for terminal sialic acid residues in the interaction of the receptor with WGA. Even after neuraminidase digestion, however, a large proportion of receptors could still bind to WGA-agarose indicating that sialic acid was not the only determinant in binding.

Digestion with *N*-acetylglucosaminidase did not affect adsorption to WGA-agarose suggesting that terminal *N*-acetylglucosamine residues are either absent or unavailable to the enzyme. When the receptors were digested with neuraminidase and *N*-acetylglucosaminidase together, a larger effect was seen than with the two enzymes used separately. This suggests that some of the receptors have terminal sialic acid residues and subterminal *N*-acetylglucosamine residues or that terminal *N*-acetylglucosamine residues are present, but obscured by sialic acid. In either case, prior removal of the sialic acid enables *N*-acetylglucosaminidase digestion to occur.

This heterogeneity was further examined in the experiments using high and low concentrations of *N*-acetylglucosamine to elute receptors bound to WGA-agarose. In other systems low-affinity interactions with WGA are via sialic acid and unfavourable *N*-acetylglucosamine configurations, whereas high-affinity interactions seem to be through specific sequences containing *N*-acetylglucosamine (Gallagher *et al.*, 1985). In the present experiments, high- (60%) and low- (40%) affinity binding populations of D_2 dopamine receptors were indeed observed and the low-affinity population was reduced by about 60% after neuraminidase treatment, supporting the idea that the low-affinity interaction is partly via sialic acid residues. A similar alteration in affinity upon neuraminidase treatment was seen for muscarinic acetylcholine receptors (Herron & Schimerlik 1983).

These results indicate substantial heterogeneity within the oligosaccharide chains of D_2 dopamine receptors and can be accommodated by postulating three populations of receptor each of which binds to WGA-agarose under control conditions. One population, constituting about 60% of those that bind to WGA does so with a high affinity and its binding is insensitive to neuraminidase digestion. By comparison with other work, this is likely to bear a specific sequence or sequences of *N*-acetyl-

glucosamine residues that confers high-affinity binding (Debray *et al.*, 1981; Yamamoto *et al.*, 1981; Gallagher *et al.*, 1985). About 60% of the low-affinity binding population bind via sialic acid residues as their binding is sensitive to neuraminidase. These are then likely to have oligosaccharides with terminal sialic acid residues (Hubbard & Ivatt, 1980; Hemming, 1982). Grigoriadis *et al.* (1988) have also provided evidence for terminal sialic acid residues on oligosaccharide chains linked to D₂ dopamine receptors. The remaining low-affinity binding population binds in a sialic acid-independent manner and so is likely to contain sequences of *N*-acetylglucosamine residues in a low-affinity binding configuration (Debray *et al.*, 1981; Yamamoto *et al.*, 1981).

Heterogeneity of oligosaccharides attached to receptors has also been described for nicotinic acetylcholine receptors (Salvaterra *et al.*, 1977), muscarinic acetylcholine receptors (Herron & Schimerlik, 1983), and β -adrenergic receptors (Stiles *et al.*, 1985; Cervantes-Olivier *et al.*, 1985).

In conclusion, D₂ dopamine receptors from bovine caudate nucleus and olfactory tubercle are glycoproteins with indistinguishable carbohydrate moieties. The receptors from either brain region bear heterogeneous oligosaccharide chains which contribute to high and low affinity interactions with WGA-agarose. Precise definition of the structural differences between the oligosaccharide chains awaits full purification of the receptors.

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