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RAPID COMMUNICATION

pH DEPENDENCE OF SULPIRIDE BINDING TO D_2 DOPAMINE RECEPTORS IN BOVINE BRAIN

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The D_2 dopamine receptor is important for many of the physiological actions of dopamine as well as being a key site of action of anti-parkinsonian and anti-schizophrenic drugs. The receptor has recently been purified[1-3] and cDNA species corresponding to the receptor have been cloned[4-6]. An important question about this receptor concerns the mechanism of binding of ligands to the receptor. If this can be understood in detail, including defining the amino acid side chains that contact ligands, this will be important for both understanding how the receptor functions and for drug design.

An indirect approach to this is to examine the pH dependence of ligand binding to the receptor which may enable inference to be made about the amino acids involved. For the ligand [^{3}H]spiperone, binding is optimal around pH 6-8 but is reduced strongly by reduction in pH below 5.5 with a half maximal reduction being seen at pH 5.2[7]. In the same study it was found that whereas a reduction of pH from 7.4 to 6 had little effect on the binding of classical dopamine antagonists e.g. spiperone, domperidone, to D_2 dopamine receptors, the binding of substituted benzamides e.g. sulpiride, was substantially reduced by this pH change. In the present communication we have examined the detailed pH dependence of sulpiride binding to the D_2 dopamine receptor.

(-) Sulpiride binding to the D_2 dopamine receptor from bovine caudate nucleus was assayed by competition with [^{3}H]spiperone. Bovine caudate nucleus membranes were prepared and [^{3}H]spiperone binding assays were performed as described in [8,9] except that a wide pH range buffer was used (citric acid (28.5mM), diethylbarbituric acid (28.5mM), boric acid (28.5mM), KH_2PO_4 (28.5mM) $MgSO_4$ (0.8mM), NaCl (110mM)) and 0.3μM mianserin was included in all assays to prevent binding of [^{3}H]spiperone to $5HT_2$ serotonin receptors.

(-)-Sulpiride/[^{3}H]spiperone competition curves were determined at different pH values over the range pH 5.5-8.5. IC_{50} values were converted to K_i values as in [9] and K_i values are given in Table 1. Competition data fitted single binding site models in each case. A pKa of 7.3 was derived for the ionising group on the receptor that affects (-)-sulpiride binding using equation (1) derived in [7].

$$K_i\ (obs) = K_i\ (1 + \frac{[H^+]}{K_a}) \quad - \quad (1)$$

[K_i (obs) and K_i are dissociation constants for (-)-sulpiride binding to the receptor at a given pH (K_i(obs)) and at high pH (K_i) when the ionising group on the receptor is fully deprotonated. K_a is the dissociation constant of the ionising group on the receptor.]

Table 1 pH dependence of the binding of (-)-sulpiride to D_2 dopamine receptors

pH	5.5	6.0	6.5	7.0	7.5	8.0	8.5
K_i (μM)	4.8∓1.5	0.98∓0.40	0.30∓0.14	0.12∓0.03	0.093∓0.016	0.053∓0.016	0.11∓0.10
n	3	4	3	3	3	3	3

(-)-sulpiride/[^{3}H]spiperone competition data were obtained as described in the text at different pH values. IC_{50} values were converted to K_i values as in ref. 9 and data are expressed as mean ∓ SD for n experiments.

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Control experiments (data not shown) have shown that the effects observed are not on sulpiride itself, for example affecting its solubility or stability. Also the effects cannot be on the ionisation state of sulpiride which has a pKa of 9.12[10].

The results show clearly that the D_2 dopamine receptor of bovine brain has different pH dependencies for binding the butyrophenone spiperone (pKa 5.2) and the substituted benzamide, sulpiride (pKa 7.3). This suggests that the two drugs have different modes of binding to the receptor. There is much evidence that the substituted benzamides bind rather differently to the D_2 dopamine receptor compared with the other drugs. For example their binding is markedly dependent on Na^+ ions[11] unlike that of other antagonists and whereas generally antagonist binding to the receptor is entropy driven, the binding of substituted benzamides is mostly enthalpy driven[12] and may involve a conformational change in the receptor. These results argue for different modes of binding of the different classes of antagonists. Analogous observations have been made for the muscarinic acetylcholine receptor[13] with different classes of antagonists showing different pH dependencies so there may be a general pattern emerging for these kinds of G-protein linked receptor.

The ligand binding site of the receptor is thought to be formed from the putative transmembrane spanning regions of the amino acid sequence but we cannot at present define the amino acids involved in receptor-ligand interaction. The group that affects [^{3}H]spiperone binding (apparent pKa 5.2) may be an aspartic acid residue and there is evidence from mutagenesis studies for an important conserved aspartic acid residue in ligand binding to β-adrenergic[14] and muscarinic acetylcholine receptors[15]. This would correspond to Asp 114 of the D_2 dopamine receptor and independent evidence has been provided elsewhere for the importance of a carboxyl group in the binding of [^{3}H]spiperone to the D_2 dopamine receptor using the agent dicyclohexylcarbodiimide which irreversibly affects [^{3}H]spiperone binding[7].

The pKa value of the group affecting (-)-sulpiride binding (7.3) would be most consistent with a histidine residue and indeed there is a histidine residue at the active site (His365)[4]. This could interact with the benzamide drugs either via a hydrogen bond or perhaps via a hydrophobic interaction. Another possibility is that the benzamide drugs interact with a carboxyl group at the receptor site but that this is different from the one cited above with which spiperone interacts. The pKa (7.3) is rather high for a carboxyl group but it may be that the environment of the group is perturbed, perhaps by the presence of a second ionising group. There is a second aspartic acid residue (Asp80) within the binding site which could be a candidate for the carboxyl group with which the

benzamides interact and the pKa could be raised by the proximity of Asp114. A further alternative is that the ionising group with which the benzamides interact is a cysteine whose pKa has been lowered by the environment of the receptor binding site. Further experimentation should help decide among these possibilities.

In summary then these data suggest that butyrophenones such as spiperone and substituted benzamides such as sulpiride interact with different groups at the active site of the D_2 dopamine receptor. The drugs bind in different modes to the receptor which must therefore contain separate but overlapping binding sites for the two classes of drug. Understanding the precise interactions involved that generate this selectivity will be important for drug design.

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