Br. J. Pharmac (1983), **78**, 005–007

A comparison of some of the properties of the [^{3}H]-sulpiride binding site in soluble and native membrane preparations of dog striatum

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[^{3}H]-sulpiride binding sites in dog striatum have been solubilised using CHAPS detergent. Solubilised sites were similar to those in native membrane preparations in terms of *Bmax*, *Kd* and sensitivity to dopamine and dopamine receptor antagonists. Binding of [^{3}H]-sulpiride to the solubilised binding site was dependent upon the presence of sodium ions and was inactivated by the sulphydryl group reagent N-ethylmaleimide.

Introduction Sulpiride is a substituted benzamide having neuroleptic properties. Binding studies, using [^{3}H]-sulpiride have suggested that this ligand is a useful label for central dopamine receptors (Woodruff & Freedman, 1981; Freedman, Poat & Woodruff, 1981). The binding of [^{3}H]-sulpiride to striatal membranes is dependent upon sodium ions (Theodorou, Hall, Jenner & Marsden, 1980; Freedman & Woodruff, 1981) and is inhibited by the sulphydryl group reagent N-ethylmaleimide, suggesting the presence of a single SH group at the binding site (Freedman, Poat & Woodruff, 1982). In the present study, we have achieved the solubilization of sulpiride binding sites from dog brain striatal membranes and have compared the properties of the solubilised receptor with those of the membrane bound receptor.

Methods Striata were obtained immediately after the death of mongrel dogs of either sex, and were frozen in liquid nitrogen for up to two weeks. Crude synaptosomal membranes were prepared as previously described (Templeton & Woodruff, 1982). In some experiments the membranes were pretreated with N-ethylmaleimide (Freedman *et al.*, 1982; Freedman, Templeton & Woodruff, 1982) before measuring [^{3}H]-sulpiride binding. For soluble preparations, the crude synaptosomal membrane fraction was re-suspended in 5 mM CHAPS buffer (3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulphonate) (Calbiochem) containing 1 mM dithiothreitol (approximately 2 g wet weight original tissue per 15 ml) and kept stirring, on ice, for 60 min. The sample was then centrifuged at 105,000 *g* for 60 min and the supernatant decanted. As with membrane-bound preparations, some experiments were carried out following treatment of the supernatant for 30 min at room temperature with N-ethylmaleimide. Binding of [^{3}H]-sulpiride to the solubilised site was assessed by a modification of the method used by Lew, Fong & Goldstein (1981) for studying spiperone binding. Two hundred microlitres of supernatant were incubated in the presence or absence of displacing drugs, with [^{3}H]-sulpiride, (1.37–35 nM) in a final volume of 510 µl, for 60 min, on ice. To allow separation of free and receptor bound [^{3}H]-sulpiride, the soluble receptors were precipitated by the addition of 100 µl of a solution containing 0.5 mg bovine gamma globulin and 1 ml 30% polyethylene glycol (6000). Samples were mixed and kept on ice for a further 10 min, then filtered under vacuum using millipore filters (HAWP0024). The filters were dissolved in 1 ml 3-methyoxyethanol, 10 ml scintillation fluid added, and radioactivity estimated with correction for quenching.

Results Solubilisation of ^{3}H-binding sites was assessed by the ability of the binding component to pass through millipore filters (HAWP0024). Also, soluble material prepared by centrifugation at 105,000 *g* for 60 min showed no decrease in binding capacity if subjected to a further 60 min spin. (Apparent maximum number of binding sites, in original supernatant was 161 fmol/mg protein and respun supernatant was 157 fmol/mg protein, $n = 3$). Saturation plots of the binding of [^{3}H]-sulpiride to membrane bound and solubilised receptors (specific binding being defined by 1 µM S-(−)-sulpiride) yielded *Kd* values of 10.8 nM and 15.9 nM and *Bmax* of 152.3 fmol/mg protein and 178.8 fmol/mg protein, respectively ($n = 6$). Protein in the soluble preparation was 1.1–1.2 mg/ml and 2.2–2.5 mg/ml in the membrane preparation. Thus the results suggest that the receptors have been solubilised rather than purified. *Ki*

0007-1188/83/010005-03-$01.00

Table 1 Binding characteristics of [^{3}H]-sulpiride to native membrane and soluble preparations of dog striatum

	Membrane preparation	*Soluble preparation*
Displacing ligand *Ki* (nM):		
Fluphenazine	0.4	0.7
cis-Flupenthixol	5.7	30.2
Sulpiride	8.5	12.0
Dopamine	83.6	138.5
Kd for [^{3}H]-sulpiride (nM)	10.8	15.9
Bmax for [^{3}H]-sulpiride (fmol/mg protein)	152.3	178.8
Na^+-sensitive	Yes	Yes
NEM-sensitive	Yes	Yes

Canine striatal membranes, and the soluble preparation produced from these membranes, were incubated with [^{3}H]-sulpiride and various concentrations of displacing ligand. Incubation was for 10 min at 37°C for membrane preparations and 60 min at 0°C for soluble preparations. *Ki* values were calculated from the Cheng & Prussof (1973) equation, using 10 nM and 15 M [^{3}H]-sulpiride for native membrane preparations and soluble preparations respectively. *Ki* values were derived from at least 2 experiments using at least 5 ligand concentrations, each in triplicate. *Kd* and *Bmax* were obtained by Scatchard analysis; each value is from 3 or 4 experiments using 5 ligand concentrations, each in triplicate. NEM = N-ethylmaleimide.

values for displacement of [^{3}H]-sulpiride by various ligands were calculated (Table 1) using the method of Cheng & Prussof (1973) from IC_{50} values (the concentration of unlabelled ligand required to displace 50% of the specific [^{3}H]-sulpiride binding). The results indicate that the CHAPS solubilised receptor has a similar degree of sensitivity to those ligands as does the native membrane receptor.

[^{3}H]-sulpiride binding to membrane preparations decreased on lowering the Na^+ concentration. A 50% restoration of specific [^{3}H]-sulpiride binding could be achieved at 4.8 mM Na^+ (EC_{50}). Similarly, [^{3}H]-sulpiride binding to the soluble preparation was sensitive to the concentration of Na^+ ions in the buffer. However, it was not possible to calculate an EC_{50} value in this case, since below 20 mM Na^+ no specific binding could be detected,while above 60 mM Na^+ binding was close to normal control levels.

The specific binding of [^{3}H]-sulpiride to the native membrane preparation from dog striatum was inhibited by preincubation of the membranes for 30 min at 37°C with N-ethylmaleimide. The IC_{50} value (concentration causing 50% inhibition of specific binding) for N-ethylmaleimide was 2.3 mM. This is similar to the IC_{50} in rat striatum of 0.84 mM (Freedman *et al.*, 1982). The binding of [^{3}H]-sulpiride to the soluble preparation from dog striatum was similarly inhibited by preincubation of the sample with N-ethylmaleimide. Thirty minutes preincubation at room temperature with 2 mM N-ethylmaleimide completely abolished the specific binding of sulpiride. Thus, the soluble receptor appears more sensitive to the alkylating agent than the membrane bound receptor (30 min preincubation at 37°C with 2 mM N-ethylmaleimide reduced specific binding by only 41% in the membrane preparation). This may be due to the lack of stability of the soluble receptor.

Discussion We have previously produced evidence that sulpiride labels striatal dopamine receptors. The present results show that the sulpiride binding site in dog striatum retains viability when solubilised with CHAPS detergent. Furthermore, the solubilisation appears to be quantitative since the *Bmax* for soluble and membrane preparations were similar (178.8 and 152.3 fmol/mg protein, respectively). The affinity constant for the soluble binding site, and the *Ki* values obtained for displacement of [^{3}H]-sulpiride binding by a number of dopamine receptor ligands, were similar to the corresponding values obtained in experiments on the site in native membranes. This strongly suggests that, during solubilisation, the properties of the binding site are retained. It is of particular interest that binding of sulpiride to the soluble receptor is dependent on Na^+ ions. This situation is similar to that found for the membrane bound receptor, implying that this Na^+ requirement is basic to sulpiride binding. Likewise, the preliminary indications of sensitivity of the sulpiride binding site to alkylation by N-ethylmaleimide in soluble preparations is important, since it suggests that the sulphydryl group affected by N-ethylmaleimide forms an integral part of the active site, being solubilised as part of it. If these sulphydryl groups were at

some distance on the membrane from the active site, it is unlikely that the soluble receptor would be sensitive to N-ethylmaleimide. Future experiments will delineate the role of sulphydryl groups in the binding of dopamine receptor ligands in more detail and will investigate whether the guanine nucleotide binding protein is also solubilised as part of, or together with, the [^{3}H]-sulpiride binding site.

W.W.T. is a Parkinson's Disease Society Postdoctoral Fellow. We thank Dr J. Hyttel, Lundbeck for *cis*-flupenthixol and Chemitechna Ltd for [^{3}H]-sulpiride.

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(*Received October 18, 1982.*)