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[^{125}I]Spiperone is not a useful ligand for studying the CHAPS solubilized dopamine D-2 receptor from rat striatum

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The interaction of [^{125}I]spiperone and [^{3}H]spiperone with CHAPS solubilized preparations of rat striatal tissue has been compared. Only about 15% of [^{125}I]spiperone binding was displaced by sulpiride compared with about 67% of [^{3}H]spiperone binding. In the presence of (+)-butaclamol the displacement of [^{125}I]spiperone was twice that found with sulpiride suggesting an interaction with sites other than D-2 receptors. The specific binding of [^{125}I]spiperone was not saturable within the maximum concentration range that could be employed and its affinity for soluble preparations was far lower than that of [^{3}H]spiperone. Despite its very high specific activity [^{125}I]spiperone offers no advantage over [^{3}H]spiperone in the identification of dopamine receptors in soluble tissue preparations.

Attempts to purify dopamine D-2 receptors following detergent solubilization have been hampered by the low receptor yield obtained. An appropriate high affinity ligand radio-labelled to a high specific activity is required to identify such sites in solubilized preparations, but to date this has not been available. Commonly [^{3}H]spiperone is used to identify D-2 receptors in solubilized preparations (see for example Gorissen & Laduron 1979; Lew et al 1981; Lerner et al 1981; Hall et al 1983; Kilpatrick et al 1985). However, its restricted specific activity (15–30 Ci mmol^{-1}) only allows the accurate determination of receptor numbers in dense receptor preparations.

Recently, the interaction of [^{125}I]spiperone, with a specific acivity of 2200 Ci mmol^{-1}, with rat *striatal membrane preparations* was described (Gundlach et al 1984). Although the ligand identified dopamine D-2 receptors, it showed a low affinity compared with [^{3}H]spiperone and the non-specific component of binding was greater. So [^{125}I]spiperone offered no advantage over [^{3}H]spiperone for the study of D-2 receptors in membrane preparations. However, it was suggested that [^{125}I]spiperone might be a useful tool for identifying solubilized D-2 receptors because of its high specific activity. We have compared the interaction of [^{125}I]spiperone with that of [^{3}H]spiperone using solubilized preparations of rat striatum obtained using the zwitterionic detergent CHAPS (Kilpatrick et al 1985).

Methods

Solubilization of striatal tissue obtained from female Wistar rats (150 ± 10 g) was by using 3-((3-cholamidopropyl)dimethylammonio)-1-propane-sulphate (CHAPS; 5 mM) as described previously (Kilpatrick et al 1985). Solubilized tissue preparations were usually diluted to 50 volumes based on original tissue weight using 50 mM ice-cold Tris HCl buffer containing 1 mM EDTA, pH 7·4. In some experiments the dilution of soluble preparations was varied from 25–250 volumes. To 400 µl of the solubilized preparation was added 50 µl of a solution of either [^{125}I]spiperone (usually 0·05 nM; 2200 Ci mmol^{-1}; New England Nuclear), or [^{3}H]spiperone (usually 0·35 nM; 18 Ci mmol^{-1}; Amersham International). In equilibrium studies the concentration of [^{125}I]spiperone was varied between 0·01 and 0·80 nM and that of [^{3}H]spiperone from 0·1 to 10 nM. Since [^{125}I]spiperone is supplied as a lower concentration solution than [^{3}H]spiperone (125 nM and 56 µM, respectively), the use of concentrations higher than 0·8 nM results in the inclusion of large amounts of the ethanol vehicle to the samples. Non-specific binding of the ligands was defined by the incorporation of 50 µl of a solution of either (±)-sulpiride (Delagrange) or (±)-butaclamol (Ayerst Laboratories) to give final concentrations of 3×10^{-5} M and 10^{-5} M, respectively. Samples were incubated at 4 °C for 4 h. Bound ligand was then separated from free by the addition of 100 µl of activated charcoal (10% w/v) coated with bovine serum albumin (2% w/v). Free ligand was absorbed to the charcoal preparation followed by centrifugation for 1 min in a Beckman Microfuge B. Aliquots of the supernatant (400 µl) were taken for determination of radioactivity. For [^{3}H]spiperone, 5·0 ml of ES 299 scintillation cocktail (Packard) were added and samples counted for 4 min in a Packard Tri-Carb 460C scintillation spectrometer at an efficiency of approximately 45%. Radioactivity derived from [^{125}I]spiperone was assayed by counting for 40 s in an MSC 120-3 automatic gamma counter (J. & P. Engineering Ltd) at an efficiency approaching 100%. All assays were performed in triplicate. In each experiment [^{125}I]spiperone and [^{3}H]spiperone binding was performed using the same tissue preparation.

Results

Total binding of [^{125}I]spiperone (0·05 nM) to CHAPS solubilized preparations was 13 300 ± 570 d min^{-1} (mean ± s.e.m.; n = 7); in the presence of 3×10^{-5} M (±)-sulpiride, residual binding was 11 600 ± 400 d min^{-1}. The association of specific binding was

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complete within 4 h. The specific binding of [^{125}I]spiperone (0·05 nM; defined using 3×10^{-5} M (±)-sulpiride) was linearly related to the amount of protein present between 25 and 250 volumes dilution of original tissue weight. A 50 volume dilution was used in subsequent experiments.

A comparison of the characteristics of specific [^{125}I]spiperone (0·05 nM) and [^{3}H]spiperone (0·35 nM) binding to CHAPS solubilized preparations of rat striatal tissue is presented in Table 1. At a single ligand concentration, the specific binding of [^{3}H]spiperone (0·35 nM; defined using 3×10^{-5} M (±)-sulpiride) was in excess of 65% of total binding. However, specific binding of [^{125}I]spiperone (0·05 nM; defined using 3×10^{-5} M (±)-sulpiride) was less than 15% of total binding. In the presence of 10^{-5} M (+)-butaclamol some 75% of total [^{3}H]spiperone (0·35 nM) binding was displaced but only 28% of total [^{125}I]spiperone (0·05 nM) binding. The low specific to non-specific ratio of [^{125}I]spiperone was apparently not due to an increased non-specific component of binding since the per cent of added ligand that was non-specifically bound did not vary between [^{3}H]spiperone (0·35 nM) and [^{125}I]spiperone (0·05 nM) (Table 1). Ideally, for direct comparison, the same concentration of [^{3}H]- and [^{125}I]spiperone would have been employed, however, the low concentration of [^{125}I]spiperone and low specific activity of [^{3}H]spiperone make this impractical. Nevertheless, the data in Table 1 indicate that only a small proportion of [^{125}I]spiperone binding is to D-2 receptors, even in concentrations up to 0·8 nM.

Table 1. A comparison of [^{3}H]- and [^{125}I]spiperone binding to CHAPS solubilized preparations of rat striatal tissue. Except for equilibrium saturation calculations, the final concentration of [^{3}H]spiperone was 0·35 nM and [^{125}I]spiperone was 0·05 nM. Apparent K_d and B_{max} values were calculated by computer curve fitting; 6–8 separate concentrations of ligand were used varying from 0·11–10 nM [^{3}H]spiperone and 0·01–0·80 nM [^{125}I]spiperone. The numbers of separate experiments performed are in parentheses.

	[^{3}H]Spiperone	[^{125}I]Spiperone
Specific activity (Ci mmol^{-1})	18	2200
K_d (nM)	0·33 ± 0·09 (3)	2·70 ± 0·1 (3)
B_{max} (pmol g tissue^{-1})	7·60 ± 0·50 (3)	2·86 ± 0·70 (3)
Correlation coefficient of Eadie-Hofstee analysis	0·95 ± 0·01 (3)	0·60 ± 0·10 (3)
% Total binding displaced by 3×10^{-5} M (±)-sulpiride	66·70 ± 2·40 (7)	14·50 ± 1·70 (7)
% Total binding displaced by 10^{-5} M (±)-butaclamol	74·10 ± 3·79 (3)	27·70 ± 3·20 (4)
Non-specific binding as a % of counts added	7·10 ± 0·50 (7)	7·5 ± 0·90 (7)

Equilibrium saturation analysis of specific [^{125}I]spiperone (0·1–10 nM) binding by computer curve fitting revealed saturable high affinity binding to a single population of binding sites. Over the concentration range employed the specific binding of [^{125}I]spiperone to CHAPS solubilized preparations did not saturate. In these experiments, specific binding of [^{125}I]spiperone varied with concentration between 20 and 7% of total binding. The K_d and B_{max} values derived from these experiments revealed the presence of a smaller number of lower affinity binding sites than observed for [^{3}H]spiperone. A K_d value could be derived for [^{125}I]spiperone by performing self inhibition experiments at a single [^{125}I]spiperone concentration. However such results would not select for D-2 receptors but may include 5-HT and spirodecanone sites with which spiperone will interact (Withy et al 1981; Howlett et al 1979). Furthermore, accurate assessment of displacement data is complex with such a low specific component.

Discussion

In rat striatal membrane preparations [^{125}I]spiperone was shown to interact with D-2 sites but with a low affinity (Gundlach et al 1984). Similarly, in the CHAPS-solubilized preparations a portion of the total binding of [^{125}I]spiperone can be displaced by the specific D-2 receptor antagonist sulpiride (Jenner & Marsden 1981). Approximately twice the amount of displacement of [^{125}I]spiperone occurred in the presence of (+)-butaclamol. (+)-Butaclamol interacts with both dopamine and 5-HT receptors (Enna et al 1976), suggesting [^{125}I]spiperone may bind to both these sites. In contrast, sulpiride displaced most [^{3}H]spiperone binding and only a small further displacement of binding was observed in the presence of (+)-butaclamol. So, [^{125}I]spiperone shows only a relatively low proportion of binding to dopamine sites and may interact with other receptors, although a full pharmacological analysis was not attempted because of the small component of specific binding.

Equilibrium analysis of [^{125}I]spiperone binding was hampered by the lack of saturation of specific binding. The form in which [^{125}I]spiperone is commercially available prevents the use of higher ligand concentrations. However, the limited analysis undertaken showed the ligand to have a much lower affinity for D-2 receptors than [^{3}H]spiperone. It appears that [^{125}I]spiperone labels a smaller number of binding sites than [^{3}H]spiperone. However, this may be artificial, either reflecting the lack of saturation or a higher rate of dissociation during the prolonged separation procedure necessary to assay binding to soluble receptor preparations, owing to its low affinity for the binding sites.

As in the membrane preparation, [^{125}I]spiperone does not offer an advantage over [^{3}H]spiperone for the identification of D-2 receptors in CHAPS solubilized preparations of rat striatal tissue. Despite the high specific activity of [^{125}I]spiperone, its low specific binding, the lack of receptor saturation, its low affinity and the implication that it may also identify sites other than dopamine receptors, argue against its use in the identification of D-2 sites in soluble preparations of rat striatum.

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