EX VIVO ³H-SPIROPERIDOL BINDING TO RAT STRIATUM AND THE INHIBITORY EFFECTS OF NEUROLEPTICS

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Abstract—Neuroleptic drugs were given to rats and at different time intervals the animals were decapitated and ³H-spiroperidol binding to the striatal homogenates was determined *in vitro*. Two hours after an intraperitoneal administration of spiroperidol, perphenazine, haloperidol, chlorpromazine or thioridazine in doses of 0.03–30 mg/kg, ³H-spiroperidol binding to the striatal homogenates was inhibited dose-dependently with an ID₅₀ value of 0.11, 0.23, 1.1, 3.7 or 9.4 mg/kg, respectively. After a single intramuscular administration of fluphenazine enanthate (10 mg/kg), a long-acting neuroleptic drug, long-lasting inhibitory effect (over 4 weeks) on the binding was also observed. These results indicate that *ex vivo* ³H-spiroperidol binding method may be useful to measure the duration and the potency of anti-dopaminergic activities of drugs.

In vitro ³H-neuroleptics binding studies, because of their simplicity, sensitivity and specificity, are particularly suitable for investigating the biochemical properties of neuroleptic binding sites and for screening of neuroleptic drugs (1). With the *ex vivo* ³H-neuroleptics binding methods, it is possible to show directly whether the neuroleptics and/or their metabolites interact with dopaminergic receptors, and pharmacokinetic characteristics can be elucidated. The present study was carried out to demonstrate the validity of the *ex vivo* ³H-spiroperidol binding method.

MATERIALS AND METHODS

Pilot experiments to select the tissue preparation and the optimum ligand concentration for *ex vivo* study: Male Sprague-Dawley rats (180–250 g) were given saline or 3 mg/kg chlorpromazine (CPZ i.p.). Two hr

later, the rats were decapitated and the striata homogenized in 35 volumes of ice-cold water. Aliquots (3 ml) of the striatal homogenates were further centrifuged at 20,000×g for 20 min at 4°, and the pellets resuspended in 3 ml of ice-cold water. To assess the degree of CPZ incorporation among these fractions, striatal homogenates (0.5 ml, obtained from rat treated with CPZ), membrane fraction (0.5 ml, CPZ) or supernatant fraction (0.5 ml, CPZ) plus membrane fraction (0.5 ml, control) were incubated for 15 min at 37° with 62.5 pM ³H-spiroperidol, according to the method of Fields et al. (2) with slight modification.

In the kinetic study, high affinity ³H-spiroperidol binding to striatal homogenates was determined at five different ligand concentrations—0.031, 0.0625, 0.125, 0.25 and 0.5 nM. The incubation mixture (in triplicate) with a total volume of 10 ml

contained ^3H -spiroperidol at the required concentration, 50 mM Tris-maleate buffer (pH 7.5), 0.1 mM EGTA and 0.5 ml of tissue preparation (about 1.5 mg protein, the specific ^3H -spiroperidol binding has been proved to be almost linear with tissue concentration up to 1.5 mg protein/assay). After incubation for 15 min at 37° , the samples were rapidly filtered through Whatman GF/B glass fiber filters, under reduced pressure. The filters were washed three times with 5 ml of ice-cold buffer, and were subsequently counted by liquid scintillation spectrometer. The binding of ^3H -spiroperidol to rat striatal tissue in the presence of $1\text{ }\mu\text{M}$ trifluoperidol was referred to as the non-specific binding of ^3H -spiroperidol.

Effects of neuroleptics on *ex vivo* ^3H -spiroperidol binding: Male Sprague-Dawley rats (180–250 g) were given various doses of neuroleptics either i.m. or i.p. At different time intervals, the animals were decapitated, and the striata was homogenized in 35 volumes of ice-cold water. In routine studies, 0.5 ml of striatal homogenates was incubated with 62.5 pM ^3H -spiroperidol. Under the standard assay conditions in the presence of 62.5 pM ^3H -spiroperidol, the specifically bound radioactivity was about 90% of the total bound radioactivity.

Protein was determined by the method of Lowry et al. (3) using bovine albumin as the standard.

Chemicals used were as follows: chlorpromazine (Meiji), haloperidol (Dainippon), perphenazine (Yoshitomi), amitriptyline (Yamanouchi), fluphenazine enanthate (Nippon-Squibb), trifluoperidol, spiroperidol, thioridazine, clozapine (Hoffmann-La Roche), and 1-phenyl-4- ^3H -spiroperidol (spec. act. 25.64 Ci/mmol, New England Nuclear).

RESULTS

Pilot experiments to select the tissue preparation and the optimum ligand concen-

tration for *ex vivo* study: After administration of 3 mg/kg i.p. of chlorpromazine, *ex vivo* ^3H -spiroperidol binding was carried out both in the whole homogenates and in the membrane fraction at the ligand concentration of 62.5 pM (Fig. 1). About 40% inhibition of ^3H -spiroperidol binding was observed in both preparations, but the binding to striatal membrane fraction obtained from saline-treated rats was slightly inhibited by addition of the supernatant fraction of chlorpromazine-treated animals. This means that most of the chlorpromazine was retained in membrane fractions after injection of this drug and only

Saline, Chlorpromazine (3 mg/kg, i.p. 2 hr)		
Rat striatal homogenates (water $\times 35$)		
20,000 $\times$ g, 20 min		
	membrane	sup
	water	
Fraction	^3H -Spiroperidol Specifically Bound (cpm/assay)	% Inhibition
Sal (hom)	2667	—
CPZ (hom)	1611	40
Sal (mem)	2134	—
CPZ (mem)	1310	39
Sal (mem)+ Sal (sup)	2043	—
Sal (mem)+ CPZ (sup)	1872	8

Fig. 1. Effect of chlorpromazine administration on ^3H -spiroperidol binding to striatal preparations. The striata was dissected from saline (Sal) or 3 mg/kg chlorpromazine (CPZ) treated rat 2 hr after the i.p. administration. Striatal homogenates (hom), membrane fraction (mem), or membrane fractions plus supernatant fraction (sup) were incubated for 15 min at 37° with 62.5 pM ^3H -spiroperidol in a total volume of 10 ml containing 50 mM Tris-maleate buffer, pH 7.5, 0.1 mM EGTA. Non-specific binding was determined in the presence of $1\text{ }\mu\text{M}$ trifluoperidol. Each value represents the mean from a typical single experiment performed in triplicate.

Fig. 2. Saturation curve and Scatchard plot of ^3H -spiroperidol binding to saline or 3 mg/kg chlorpromazine (i.p., 2 hr) treated rat striatal homogenates. The striatal homogenates were incubated with increasing concentrations of ^3H -spiroperidol (0.031–0.5 nM) for 15 min at 37° . Points shown are those from a typical single experiment performed in triplicate.

a small amount of the drug remained or was liberated into the supernatant fraction. Figure 2 shows the saturation curves of ^3H -spiroperidol binding to striatal homogenates of saline or chlorpromazine treated animals. The Scatchard analysis in the preparation obtained from both treated rats indicated a single component of binding with apparent dissociation constants (K_D) of 0.05 nM (control) and 0.11 nM (CPZ) and the inhibition of binding observed was of a competitive nature, making the lower ligand concentration the more sensitive. Based on the above mentioned pilot experiments, the whole homogenates were chosen as tissue preparations, and the ligand concentration was set at 62.5 pM (in typical case, total binding: 2800 cpm, non-specific binding; 200 cpm) in further studies.

Effects of neuroleptics on *ex vivo* ^3H -spiroperidol binding: As shown in Fig. 3, 2 hr after a single i.p. administration of spiroperidol, perphenazine, haloperidol, chlorpromazine or thioridazine in doses of 0.03–30 mg/kg, the dose-dependent inhibitions on

Fig. 3. Inhibition of *ex vivo* ^3H -spiroperidol binding to rat striatum by several neuroleptics. The striata were dissected from neuroleptic drug treated rats 2 hr after an i.p. administration of the drug. Striatal homogenates were incubated with 62.5 pM ^3H -spiroperidol for 15 min at 37° . Points shown are means $\pm$ S.E.M. from at least five animals.

^3H -spiroperidol binding were observed and their ID₅₀ values (50% inhibition of ^3H -spiroperidol specifically bound) were calculated to be 0.11, 0.23, 1.1, 3.7 or 9.4 mg/kg, respectively.

Duration of anti-dopaminergic potency of chlorpromazine or fluphenazine enanthate in rat striatum as judged by *ex vivo* ^3H -spiroperidol binding: Following a single i.p. administration of chlorpromazine (10 mg/kg), rats were decapitated at various time intervals and ^3H -spiroperidol binding to their striatal homogenates was determined (Fig. 4-a). The maximal inhibition of specific ^3H -spiroperidol binding by chlorpromazine was attained within the first 2 hr, after which a rapid decrease was seen. Figure 4-b shows the inhibitory effect of a long-acting neuroleptic drug, fluphenazine enanthate, pretreatment (10 mg/kg, i.m.) on specific ^3H -spiroperidol binding to the striatal homogenates. Maximal inhibition of specific ^3H -spiroperidol binding by this drug was attained within the 3rd day, then declined gradually and about 50% of peak inhibition was still

Fig. 4. Time course of inhibitory effect of *ex vivo* ^3H -spiroperidol binding to striatum by chlorpromazine (a) or fluphenazine enanthate (b). *Ex vivo* ^3H -spiroperidol binding to striatum was determined at various times after a single administration of chlorpromazine (i.p.) or fluphenazine enanthate (i.m.). Points shown are the means $\pm$ S.E.M. from at least five animals.

observed on 28th day. The ID₅₀ value at 7 day was calculated to be 2.9 mg/kg.

DISCUSSION

The results presented here indicate that *ex vivo* ^3H -spiroperidol binding is a simple method and a useful tool to assess the biochemical profile of numerous neuroleptic drugs, as the potency and the duration of anti-dopaminergic activities of drugs can be easily and rapidly measured. The inhibitory potencies of short-acting neuroleptics in *ex vivo* ^3H -spiroperidol binding correlated well with their reported clinical antipsychotic

efficacy and also with their increasing potencies of homovanillic acid in the striatum (4). Among the neuroleptics tested, however, haloperidol showed a weak inhibitory effect on *ex vivo* ^3H -spiroperidol binding in comparison with its clinical potency or its *in vivo* pharmacological activity. In previous work, we found that the binding sites of haloperidol were, in part, different from those of spiroperidol (5). In our study, the neuroleptics we tested all inhibited *ex vivo* ^3H -spiroperidol binding significantly, but promethazine, a phenothiazine without neuroleptic activity, and amitriptyline, a tricyclic antidepressant, did not exert any inhibition at a relatively high dose of 10 mg/kg (i.p., 2 hr, data not shown).

A typical application of this *ex vivo* ^3H -spiroperidol binding would be for the pharmacokinetic studies of long-acting neuroleptics. The inhibitory profile of *ex vivo* ^3H -spiroperidol binding is in good agreement with the pharmacokinetic (6) or the *in vivo* pharmacological profile (7).

The synthesis and turnover of dopamine are increased acutely by typical short-acting neuroleptics in a manner roughly proportional to their clinical efficacy (8, 9). Scatton et al. (10) reported, however, that striatal dopamine turnover was increased by the acute administration of neuroleptics, but this effect disappeared with repeated administration of neuroleptics. These authors postulated that this phenomenon might be involved in the tolerance formation of extrapyramidal side effects in patients treated chronically with neuroleptics. In the study of a long-acting neuroleptic drug, fluphenazine enanthate, the attenuation of increased homovanillic acid contents in the striatum was also observed 6 days after the administration (11). The explanation for the lack of tolerance to the inhibitory effect on our *ex vivo* ^3H -spiroperidol binding of fluphenazine enanthate probably lies in the estimation of

biologically active drug and/or the metabolites in striatum, since the inhibition of binding observed was of a competitive nature (data not shown). Therefore, this system might be a useful tool for measuring the duration and potency of anti-psychotic activity but not for measuring the possible tolerance formation of extrapyramidal syndromes. A few days after cessation of chronic neuroleptic treatments, a simple *in vitro* ³H-spiroperidol binding to the striatum in these animals may assist in understanding mechanisms related to tolerance formation of extrapyramidal syndromes.

It has been suggested that the blockade of dopamine receptors in the mesolimbic or the mesocortical systems by neuroleptics may be more closely related to their antipsychotic activities than the action of these drugs on striatal dopaminergic receptors (12, 13). In the present study, however, we examined the *ex vivo* ³H-spiroperidol binding to rat striatal homogenates, since it has been recently reported that ³H-spiroperidol apparently binds to serotonin receptors in the rat frontal cortex, although not in the striatum (14, 15).

Laduron et al. (16) and Owen et al. (17) reported the preliminary *ex vivo* ³H-spiroperidol binding to brain membrane fraction. Our results support these findings. The *ex vivo* ³H-haloperidol binding was also reported by Burki (18). However, it seems that ³H-spiroperidol is a more suitable ligand for the study of dopamine receptors *ex vivo* than ³H-haloperidol because of its higher specific radioactivity, its higher affinity to the binding sites and its higher specific/non-specific binding ratio.

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