CHARACTERIZATION WITH ^3H -HALOPERIDOL OF THE DOPAMINE RECEPTOR IN THE RAT KIDNEY PARTICULATE PREPARATION

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Accepted June 30, 1980

Abstract—The dopamine receptor of rat kidney particulate preparation was identified and characterized by the use of ^3H -haloperidol binding. Binding of ^3H -haloperidol to the kidney particulate preparation was slow and saturable. The dissociation constants (K_D) were 0.41 nM and 5.88 nM, respectively, according to the model of two classes of independent binding sites. Maximal binding of high affinity site was obtained with 166 fmole/mg protein which was about 40% of the total receptor density. A wide variety of neuroleptics at specifically low concentrations in nanomolar range inhibited the ^3H -haloperidol binding. There was an excellent correlation between the affinity of numerous neuroleptics for the kidney particulate preparation and that for the brain striatum.

Dopamine has been shown to dilate the renal vasculature when administered to dogs as well as humans. Several lines of pharmacological evidence have indicated that the renal vascular effect of dopamine is mediated through a specific dopamine receptor (1-6). Recently we provided the evidence that there was a dopamine-sensitive adenylate cyclase system in rat renal vascular tissue (7). This assumption was further supported by findings in rat kidney perfusion experiments (8). Thus, it was expected that the renal vascular dopamine receptor was somewhat similar to the striatal dopamine receptor.

The development of receptor binding techniques has greatly assisted in the identification, localization and characterization of neurotransmitter receptors in the central nervous system. This approach has been of considerable interest in the case of the dopamine receptor. Indeed, it has been reported from a number of laboratories that ^3H -haloperidol binds with high affinity in a saturable manner to membranes prepared from striatum, and the potency of a number of neuroleptic drugs which inhibits the high affinity binding of this ligand closely matches their clinical efficacy (9-11). Therefore, in the present study, the ^3H -haloperidol binding was carried out to elucidate the biochemical characteristics of renal dopamine receptors.

MATERIALS AND METHODS

Preparation of the rat kidney vascular tissue: The rat kidney particulate preparation was prepared from male Wistar rats (weighing 150-200 g) according to the procedure described in a previous paper (7). The rat kidney particulate preparation was subjected to hypotonic exposure by homogenizing in 5 mM Tris-maleate buffer, pH 7.5 using a glass

homogenizer. The homogenate was sonicated with a Sonifier B-12 (Branson Sonic Power Company, setting No. 5) for 5 sec three times, and centrifuged ($1,000\times g$, 10 min). The supernatant was stored in small aliquots at -20° until use.

Binding assay of 3H -haloperidol: Unless otherwise stated, 0.4 nM 3H -haloperidol was incubated with constant shaking for 120 min with the rat kidney particulate preparation (1 mg protein) in 50 mM Tris-maleate buffer, pH 7.5, containing 0.1 mM EGTA and non-radioactive compounds of varying concentrations in a final volume of 10 ml. Following incubation, 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 spectrometry. The binding of 3H -haloperidol to rat kidney particulate preparation in the presence of 1 μM trifluoperidol was referred to as non-specific binding of 3H -haloperidol. Under the standard assay conditions in the presence of 0.4 nM 3H -haloperidol, the specifically bound radioactivity was about 80% of the total bound radioactivity. The specifically bound radioactive compound was verified to be an unchanged haloperidol as follows. The bound radioactivity was extracted from the glass fiber filter with toluene : ethanol (1 : 1) mixture containing non-radioactive haloperidol. After concentration under a stream of nitrogen gas at $40^{\circ}C$, the residue was subjected to thin layer chromatography (silica gel, Merck) in the two solvent systems of chloroform : ethanol (9 : 1) and chloroform : methanol : ammonia (95 : 4 : 1). Only one radioactive spot was found at the authentic haloperidol position in these two systems. Protein content was determined by the method of Lowry et al., (12) using bovine serum albumin as standard.

Drugs and chemicals: Tritium-labeled haloperidol (8.5–14 Ci/mmmole) was purchased from IRE (Belgium). All neuroleptics were donated by F. Hoffmann-La Roche (Basle). All other chemicals and materials were purchased from local commercial sources.

RESULTS

Establishment of experimental conditions for 3H -haloperidol binding: In a previous paper (7), we demonstrated the presence of a specific dopamine receptor, in the rat kidney particulate preparation composed of tubules, glomeruli and blood vessels. In the present work, this particulate preparation was further micronized : the kidney particulate preparation was intensely homogenized in a hypotonic buffer, sonicated, and centrifuged and the supernatant used for study.

To insure proper kinetic conditions and to optimize and maximize the specific 3H -haloperidol binding to the kidney vascular receptors, the effects of varying the protein and electrolyte composition of the medium were examined. The specific 3H -haloperidol binding was linear with tissue concentration up to 2 mg protein/assay, when the ligand concentration was varied from 0.2 to 2 nM. The amount of specifically bound 3H -haloperidol at 0.4 nM was somewhat higher in 50 mM Tris-maleate buffer (pH 7.5) containing 0.1 mM EGTA than in other buffer, such as Krebs-buffer (pH 7.5), 50 mM Na/K phosphate buffer (pH 7.5) or 50 mM Tris-HCl buffer (pH 7.5) containing 10 μM pargyline, 120 mM NaCl, 5 mM

KCl, 2 mM CaCl₂, 1 mM MgCl₂ and 0.1% ascorbic acid. The specific ³H-haloperidol binding displayed a fairly broad pattern in alkaline pH from 7.0 to 9.5 with a maximum at pH 8.5.

Time course of ³H-haloperidol binding to rat kidney particulate preparation: Specific binding of ³H-haloperidol to rat kidney particulate preparation was time- and temperature-dependent (Fig. 1a). At a concentration of 0.4 nM, ³H-haloperidol binding reached an equilibrium between 1.5 to 3 hr. Data presented in Fig. 1a were replotted in the Fig. 1b and a second order association rate constant $k_{+1}=1.03 \text{ nM}^{-1} \text{ hr}^{-1}$ was calculated. As shown in Fig. 1c, we examined the rate of dissociation using 1 μM haloperidol to displace specifically bound ³H-haloperidol. A first order dissociation rate constant k_{-1} of 0.15 hr⁻¹ was obtained. The ratio k_{-1}/k_{+1} (0.15 nM) of the rate constants at 37° provides an

FIG. 1. Time course of association and dissociation of ³H-haloperidol specific binding to rat kidney particulate preparation. Tissue preparations were incubated at 37°C or 4°C with ³H-haloperidol (0.4 nM) 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 μM trifluoperidol. At the arrow, haloperidol (1 μM) was added to a parallel set of tubes and both total binding and dissociation of the ³H-haloperidol: receptor complex was monitored for an additional 2 hr at 37°C or 4°C. b, pseudo-first order kinetic plots of initial ³H-haloperidol binding at 37°C. B is the amount of ³H-haloperidol specifically bound at time t, and Be is the amount of ³H-haloperidol specifically bound at equilibrium. The slope of this line is k_{ob} , equal to the observed rate constant for the pseudo-first order reaction. The second order association rate constant, k_{+1} is calculated from $k_{+1}=(k_{ob}-k_{-1})/[^3\text{H-haloperidol}]$ where k_{-1} is the first order rate constant for dissociation and [³H-haloperidol] is the concentration of ³H-haloperidol used in the experiment (0.4 nM). c, rate of dissociation of ³H-haloperidol specifically bound. Data of Fig. 1a were replotted semilogarithmically vs. time. Points shown are the means from two representative experiments.

FIG. 2. a, Binding of ^3H -haloperidol to rat kidney particulate preparation as a function of increasing concentrations of ligand. Tissue preparations were incubated with increasing concentrations of ^3H -haloperidol (0.1 to 16 nM) for 2 hr. Non-specific binding was determined in the presence of 1 μM trifluoperidol. b, Scatchard plot of ^3H -haloperidol binding. Data of Fig. 2a were replotted. Points shown are those from a typical single experiment performed in triplicate.

estimate of the dissociation constant (K_D) for the interaction of ^3H -haloperidol with the kidney binding sites.

Equilibrium studies of ^3H -haloperidol binding: As shown in Fig. 2a the specific binding of ^3H -haloperidol increased with increasing concentrations of free ^3H -haloperidol and was saturable. By contrast, the non-specific component of binding was not saturable with increasing concentrations of ^3H -haloperidol. The Scatchard plot displayed a curve that was resolved into two linear components, according to the method of Vallner et al. (13) with dissociation constants (K_D) of 0.41 nM and 5.88 nM, respectively (Fig. 2b). The means $\pm$ standard errors for similar experiments were 0.41 ± 0.02 (n=8) and 5.88 ± 0.40 (n=5), respectively. The calculated maximal number of high affinity and total binding sites (B_{max}) were 166 ± 8 (n=8) and 434 ± 18 (n=5) fmole/mg protein of rat kidney particulate preparation, respectively. The ratio of total ^3H -haloperidol binding to nonspecific binding was about 4 at a concentration of 0.4 nM ^3H -haloperidol, this amount being used for routine binding assays.

Inhibition of ^3H -haloperidol binding by drugs including neuroleptics: The inhibitory effects of a wide variety of neuroleptics on high affinity ^3H -haloperidol binding were then examined (Table 1). All neuroleptics examined inhibited the ^3H -haloperidol binding to rat kidney particulate preparation at low concentrations, in nanomolar ranges. The Hill

TABLE 1. IC₅₀ values and Hill coefficients for inhibition of specific ³H-haloperidol binding to rat kidney particulate preparation by drugs including neuroleptics

Drugs	Inhibition of ³ H-haloperidol specifically bound	
	IC ₅₀ (nM)	Hill coefficient
Haloperidol	0.77	0.86
Trifluoperidol	3.1	0.86
Fluphenazine	18	0.94
Prochlorperazine	58	1.2
Penfluridol	80	0.95
Flupenthixol	120	1.2
Octoclothepine	130	1.0
Chlorpromazine	140	0.81
Pimozide	170	1.1
Thiethylperazine	220	1.2
Triflupromazine	230	1.1
Thioridazine	600	0.93
Chlorprothixene	790	0.90
Metoclopramide	820	0.98
Propranolol	390	0.89

Tissue preparations were incubated with 0.4 nM ³H-haloperidol for 2 hr at 37°C in the presence of various concentrations of neuroleptics. IC₅₀ is the concentration of the agent giving 50% inhibition of specific ³H-haloperidol binding. Each value represents the mean from at least three experiments. The following compounds had IC₅₀ > 10 μM; dopamine, apomorphine, norepinephrine, phentolamine, 5-hydroxytryptamine, levallorphan and vasopressin (>0.1 Unit/ml). The buffer contained the antioxidant ascorbate (0.004%) in catecholamines and 5-hydroxytryptamine displacing experiments.

FIG. 3. Inhibition of specific ³H-haloperidol binding to rat kidney particulate preparation by several neuroleptics. Tissue preparations were incubated with 0.4 nM ³H-haloperidol for 2 hr at 37°C with various concentrations of neuroleptics. Points shown are the means from at least three experiments.

coefficients for these neuroleptics ranged between 0.81 and 1.2, indicating the lack of cooperativity between binding sites. Figure 3 shows the inhibitory effect of some neuroleptics on high affinity ^3H -haloperidol binding. Several neurotransmitters and their antagonists listed in Table 1 had no effect on ^3H -haloperidol binding to rat kidney particulate preparation at micromolar concentrations, except for propranolol, which was an effective inhibitor with an IC_{50} value of 390 nM. A weak inhibitory effect of propranolol on dopamine response was also observed in the dopamine-sensitive adenylate cyclase system, in the same tissue (7).

Comparison of the inhibitory effects of neuroleptics on binding to rat kidney particulate preparation with that to rat brain striatum: The ^3H -haloperidol binding to rat brain striatal P_2 fraction, prepared according to the method of Gray and Whittaker (14), was determined by the same method, except that the incubation time was 1 hr. The high affinity binding of ^3H -haloperidol ($K_D=0.46$ nM, $B_{\text{max}}=310$ fmole/mg protein), and the inhibitory effect of a number of neuroleptics to the binding were also observed (data not shown). The affinities of the neuroleptics for renal vascular ^3H -haloperidol binding sites correlated highly with those for brain striatal ^3H -haloperidol binding sites ($r=0.89$, $p<0.001$) (Fig. 4). There were also significant correlations between the affinity for these two binding sites and the clinical potency of neuroleptics as antipsychotic agents in humans, simply determined by the average daily dose (15) ($r=0.70$, $p<0.05$ in the rat kidney particulate preparation and $r=0.77$, $p<0.02$ in striatal tissue).

FIG. 4. Correlation of neuroleptic drug affinity for ^3H -haloperidol binding sites in rat kidney particulate preparation and that in rat striatum. The rat kidney particulate preparation or the rat striatal P_2 fractions were incubated with 0.4 nM ^3H -haloperidol for 2 hr (kidney) or 1 hr (striatum) at 37°C in the presence of various concentrations of neuroleptics. IC_{50} is the concentration of the agent giving 50% inhibition of specific ^3H -haloperidol binding. Points shown are the means from at least three experiments.

DISCUSSION

The present study demonstrates the specific, saturable and high-affinity dopamine receptors in the rat kidney particulate preparation by the use of ³H-haloperidol binding; (i) binding of ³H-haloperidol to the kidney particulate preparation was slow and saturable, (ii) dissociation constants (K_D) were 0.41 nM and 5.88 nM, respectively, according to the model of two classes of independent binding sites (iii) a wide variety of neuroleptics at low concentrations in nanomolar range inhibited ³H-haloperidol binding.

The association or dissociation rate of ³H-haloperidol binding to the rat kidney particulate preparation at 37°C was reportedly much slower than that to striatal tissue (10). One of the reasons for this slow rate may be the use of a lower concentration of the radio ligand (0.4 nM vs 2.0 nM). Such slow association or dissociation rate of the dopamine receptor was also observed in bovine anterior pituitary membranes when using ³H-dihydro-ergocryptine, a potent dopaminergic agonist, as radio ligand (16).

There were two distinct binding sites of ³H-haloperidol in the rat kidney particulate preparation. The receptor density of the high affinity binding site ($K_D=0.41$ nM) was 166 fmole/mg protein such being about 40% of the total receptor density. The binding characteristics of the high affinity binding sites were similar to those of dopamine receptors in the striatum, since there were excellent correlations between the activities of several neuroleptics in displacing ³H-haloperidol bound to the rat kidney particulate preparation and that to the rat striatal P_2 fraction. In a Scatchard analysis, the K_D for both rat kidney and rat striatum were in excellent agreement, and the receptor density of high affinity binding site to the renal vasculature was about 50% that seen in the striatal P_2 fraction. The biochemical characteristics of the low affinity ³H-haloperidol binding sites were not examined.

Recently, many investigators reported the existence of multiple dopamine receptors in the central nervous system (17–23). Keabian and Calne (24) classified two distinct dopamine receptors in the brain; one of them is designated as D-1 and is linked to adenylate cyclase and the other is designated as D-2 and is not associated with adenylate cyclase. Considering our previous result that there was a dopamine-sensitive adenylate cyclase system in rat renal vascular tissue (7), the receptors so identified in the present study could be mediated through dopamine receptors, particularly those associated with the adenylate cyclase system. Therefore, the dopamine receptors in the rat kidney particulate preparation may be D-1 receptors, according to the designation of Keabian and Calne (24).

Acknowledgement: We express our appreciation to Drs. T. Yamada, K. Nakamura and T. Okada for comments on the manuscript.

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