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QUANTITATIVE AUTORADIOGRAPHY OF [^{3}H]SULPIRIDE BINDING SITES IN RAT BRAIN*

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A technique has been developed to investigate [^{3}H]sulpiride binding in rat brain sections using quantitative autoradiography and tritium-sensitive film. Binding was saturable and reversible with very low nonspecific binding. [^{3}H]Sulpiride bound to an apparent single population of sites in striatum with a K_d of 3.2 nM and B_{max} of 447 fmol/mg protein. Binding sites were localized in the lamina glomerulosa of the olfactory bulb, nucleus accumbens, olfactory tubercle, striatum and substantia nigra.

Sulpiride is a substituted benzamide compound used widely in Europe as an antipsychotic drug [5, 10] which produces less extrapyramidal disturbances than other neuroleptics [14]. Sulpiride is considered to be an atypical neuroleptic drug since it possesses properties both similar to and different from the 'classical neuroleptics' such as phenothiazine, butyrophenone and thioxanthine antipsychotic drugs. Like the classical neuroleptics, sulpiride is a potent antiemetic drug [5]; it increases prolactin secretion [9] and selectively increases striatal and mesolimbic dopamine synthesis and tyrosine hydroxylase activity [11, 16]. Sulpiride, however, is less potent than the classical neuroleptic drugs when competing for striatal binding sites [4, 8], at blocking amphetamine- or apomorphine-induced hyperactivity, antagonizing apomorphine-induced stereotypies [12] and producing a cataleptic response [7]. Since sulpiride does not inhibit dopamine-stimulated adenylate cyclase activity [2, 19] and blocks the inhibiting action of dopamine on prolactin release from the pituitary, it has been proposed to be a selective D_2 antagonist [6], making it a valuable tool for investigations of dopaminergic systems.

Specific binding of [^{3}H]sulpiride to striatal membranes has been demonstrated us-

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ing filtration and centrifugation techniques [17, 20]. We report here the use of quantitative autoradiography to study the kinetic and saturation characteristics as well as the regional distribution of [^{3}H]sulpiride binding sites in rat brain.

The quantitative autoradiographic receptor binding technique used was similar to that described previously [13]. Male Sprague–Dawley rats (Charles River, Wilmington, MA; Strain Crl: CD(SD)BR), 150–175 g, were decapitated and their brains were quickly removed, blocked, mounted on a microtome chuck with Lipshaw embedding matrix and frozen under powdered dry ice. Twenty micrometer coronal brain sections were cut on a Harris cryostat and thaw-mounted onto gelatin-coated slides.

Sections were washed two times for 5 min in ice-cold 50 mM Tris-HCl (pH 7.7 at 4°C). For saturation studies, sections were incubated for 60 min at 22°C with various concentrations (0.1–40 nM) of [^{3}H](−)-sulpiride (72 Ci/mmol; New England Nuclear, Boston, MA) in 50 mM Tris-HCl (pH 7.7) containing 120 mM NaCl. Due to rostral-to-caudal variations in receptor density, specific binding for each concentration of tritiated ligand was determined four times at 280 μm intervals, and the average amount bound in these sections was taken as the representative amount bound for Scatchard analysis. In regional distribution investigations, sections were incubated for 60 min with a concentration of 10 nM [^{3}H]sulpiride. For kinetic studies, sections were incubated with 5 nM [^{3}H]sulpiride for 0–60 min. Nonspecific binding was determined in the presence of 1 μM unlabeled (±)-sulpiride (Delagrange International Paris, France) or 1 μM (+)-butaclamol (Ayerst Labs., Montreal, Canada). After incubation, sections were rinsed two times for 5 min in ice-cold 50 mM Tris-HCl (pH 7.7 at 4°C). For dissociation studies sections received the two standard buffer rinses followed by immersion in a large volume ('infinite dilution') of incubation buffer at 22°C for 0–60 min. Slides were blown dry with warm air, placed in x-ray cassettes with radioactive standards prepared as described elsewhere [13] and apposed to Ultrofilm [^{3}H](LKB). After a 30-day exposure at 4°C, the film was developed in Kodak D-19 for 4 min at 21°C, fixed and dried. The film was placed in a photographic enlarger and the optical densities of areas of the film were determined with a computer-assisted microdensitometer [1]. Forty readings were averaged from the striatal sections per tritiated ligand concentration and radioactivity per μg protein was determined by a regression analysis which compared film densities produced by the sections with those produced by the standards [13].

Optimal binding with this method was obtained under conditions in which sodium ions were included in the incubation buffer which is consistent with previous studies [18]. Blockade of [^{3}H]sulpiride binding by butaclamol was stereospecific; 1 μM (+)-butaclamol displaced 85% of the total binding of 30 nM [^{3}H]sulpiride while (−)-butaclamol had no detectable effect on the total binding. Specific binding of [^{3}H]sulpiride to brain slices represented 85–90% of total binding which compares favorably to that obtained using membrane preparations, 39% [17] and 80% [21],

and synaptic membrane preparations, 55% [20]. Regional distribution studies showed restricted localization of [^{3}H]sulpiride binding to dopaminergic areas. Specific [^{3}H]sulpiride binding was limited to the lamina glomerulosa of the olfactory bulb, caudate-putamen, nucleus accumbens, olfactory tubercle and substantia nigra (Fig.

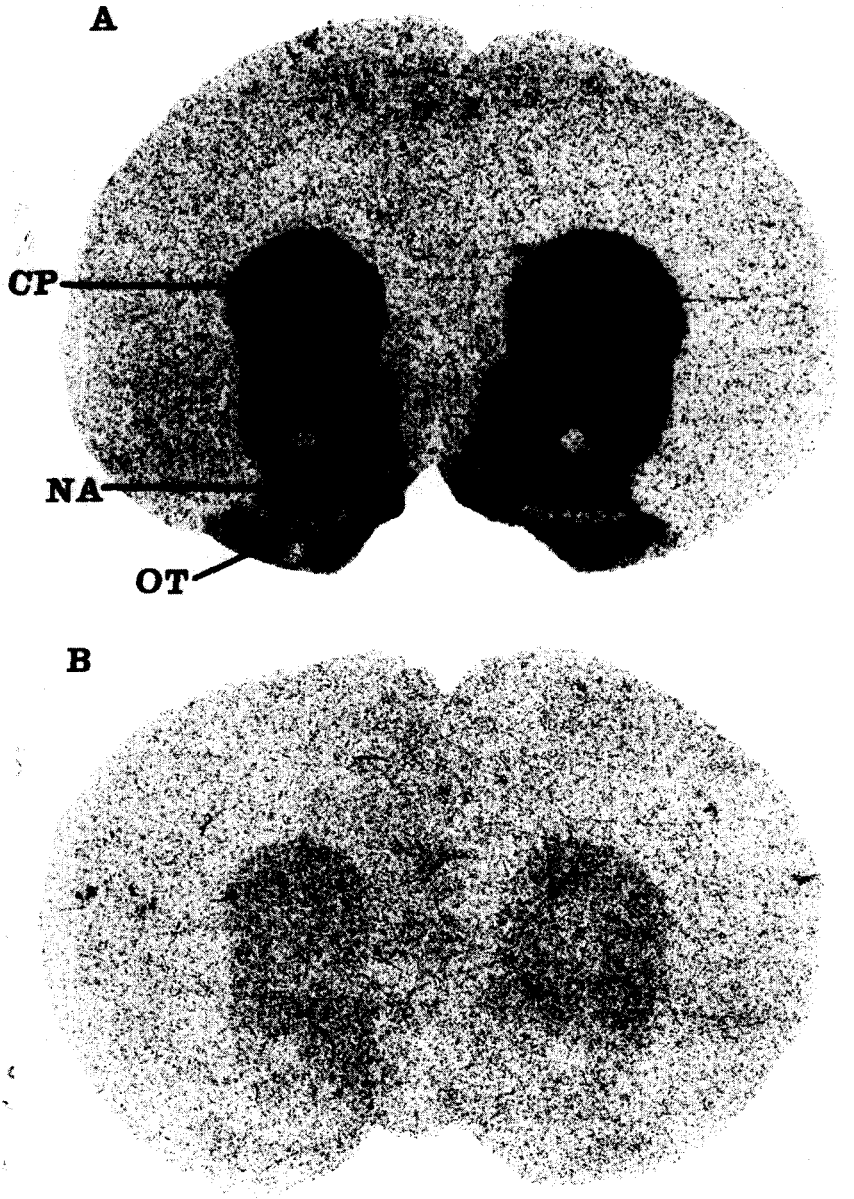

Fig. 1. Autoradiographs of [^{3}H]sulpiride binding. A: total binding in caudate-putamen (CP), nucleus accumbens (NA) and olfactory tubercle (OT). B: nonspecific binding of adjacent section as defined by the presence of 1 μM unlabeled sulpiride. [^{3}H]sulpiride concentration was 14.3 nM.

1). The highest density of binding was in the striatum and the lowest in the substantia nigra. No binding was detectable in non-dopaminergic areas such as the thalamus and cerebellum (Table I).

Binding of [^{3}H]sulpiride to brain slices was saturable and reached equilibrium by 30 min. The binding remained at equilibrium for at least 90 min. Scatchard analysis of [^{3}H]sulpiride binding sites in the caudate revealed an apparent single homogeneous population of binding sites with a K_d of 3.2 ± 0.4 nM and a B_{max} of 447 ± 14.0 fmol/mg protein, $n = 7$ (Fig. 2A). Hill coefficients were not different from 1 suggesting an apparent single set of binding sites. Association studies revealed an association rate constant (K_1) of $5.5 \pm 0.02 \times 10^7$ $M^{-1} \cdot min^{-1}$, $n = 3$. Dissociation studies revealed a dissociation rate constant (K_{-1}) of 0.15 ± 0.04 min^{-1}, $n = 3$. Both association and dissociation curves were monophasic suggesting a single set of sites (Fig. 2B). The estimated K_d value calculated from the ratio K_{-1}/K_1 was 2.6 nM which was in good agreement with our K_d value of 3.2 nM determined from equilibrium saturation studies.

This report demonstrates that kinetic, saturation and regional distribution data on [^{3}H]sulpiride binding can be obtained using quantitative autoradiographic techniques. The half time of dissociation of [^{3}H]sulpiride from striatal binding sites was 5 min at 22°C which is consistant with a $t_{1/2}$ of 4 min at 25°C reported for a membrane preparation filter binding technique [21]. [^{3}H]Sulpiride binds to the brain sections in a specific, saturable and reversible manner with a K_d of 3.2 nM and a B_{max} of 447 fmol/mg protein which is consistent with those described by others: $K_d = 7.4$ nM, $B_{max} = 240$ fmol/mg protein [20]; $K_d = 5.6$ nM, $B_{max} = 640$ fmol/mg protein [21]. Our data suggest that [^{3}H]sulpiride binds to a single high-affinity site. The possibility that a low-affinity site may appear at higher ligand concentrations than those used in this study is currently under investigation.

The [^{3}H]sulpiride binding sites are selectively located in the lamina glomerulosa

TABLE I

REGIONAL DENSITY OF [^{3}H]SULPIRIDE BINDING SITES

Relative density of [^{3}H]sulpiride binding sites in various brain regions was determined using a spot densitometer as described in text. Sections taken every 200 μm throughout the brain were incubated with 10 nM [^{3}H]sulpiride in the presence and absence of 1 μM sulpiride to define specific binding. Values reported are the average values taken from two brains which varied less than 10%.

Region	Specific binding (fmol/mg protein)
Lamina glomerulosa	111
Striatum	297
Nucleus accumbens	224
Olfactory tubercle	170
Substantia nigra	36
Cerebellum	0
Thalamus	0

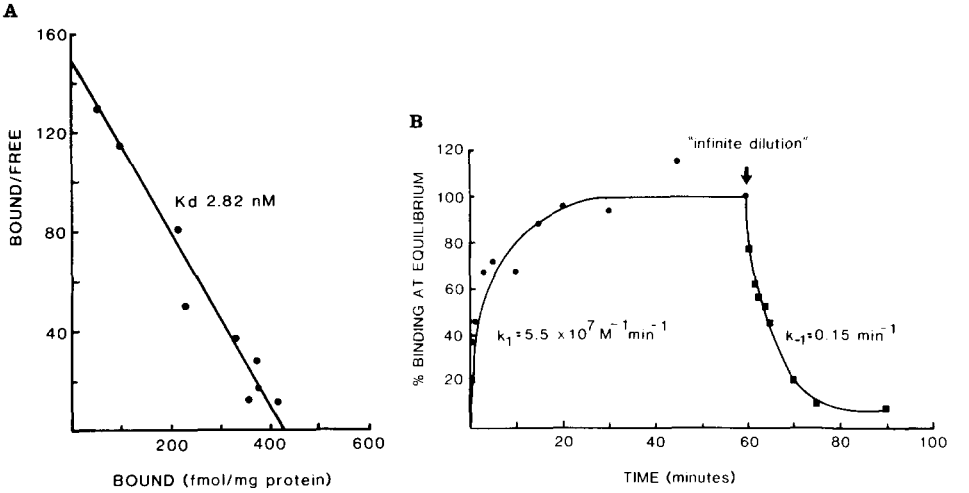

Fig. 2. A: a representative Scatchard plot of [^{3}H]sulpiride binding in striatum ($B_{max} = 423$ fmol/mg protein, $r = 0.98$). Ligand concentrations between 0.5 and 40 nM were used in this experiment. Autoradiography was performed as described in text. Each point represents specific binding (the average of 40 readings in the striatum minus readings from adjacent sections incubated in presence of 1 μM sulpiride). The experiment has been replicated 7 times. B: a representative association and dissociation plot of [^{3}H]sulpiride binding in striatum. The concentration of [^{3}H]sulpiride was 6 nM. Points represent specific binding. Tissue was incubated 0–60 min for association studies (circles), and additional sections were rinsed as described in text for dissociation studies (squares). The experiment has been replicated 3 times.

of the olfactory bulb, caudate putamen, nucleus accumbens, olfactory tubercle and substantia nigra. This regional distribution contrasts with the regional distribution of [^{3}H]spiroperidol binding sites as demonstrated by similar in vitro autoradiographic techniques [15], which include hippocampus and amygdala in addition to those areas labeled by [^{3}H]sulpiride in our study. As both sulpiride and low concentrations of spiroperidol are considered specific ligands for the D_2 receptor, the discrepancies seen in the regional distribution may suggest that sulpiride labels a subpopulation of D_2 or spiroperidol receptor sites. Alternatively [^{3}H]sulpiride may be the more selective D_2 ligand while the [^{3}H]spiroperidol binding in the hippocampus and amygdala may be due to interaction with non-D_2 sites for which [^{3}H]sulpiride has low affinity. This possibility is supported by the work of Zahnisher and Dubocovich who demonstrated two classes of sites for [^{3}H]spiroperidol, one of which was identical to the single site they demonstrated for [^{3}H]sulpiride [21]. It is also possible that there are regions of low-density sulpiride binding sites in the brain not detected at our present film exposure period. Longer film exposure periods are being examined.

It is of interest to note that the restricted distribution pattern of these receptors in caudate-putamen, nucleus accumbens and olfactory tubercle resembles the

acetylcholinesterase positive staining in these same areas, which has been cited by Heimer as one piece of evidence supporting his ventral striatal concept [3]. Heimer anatomically defines the olfactory tubercle and nucleus accumbens 'ventral striatum'. He suggests, based on embryological, cytological, histochemical and hodological evidence that the olfactory tubercle and nucleus accumbens are functionally related striatal structures which provide a means by which components of the limbic system may influence the striatum. The high density of [^{3}H]sulpiride binding sites in nucleus accumbens, olfactory tubercle and caudate-putamen suggests a relationship between these structures. [^{3}H]Sulpiride binding may prove to be a useful histochemical tool in examining the anatomical relationships of the striatal system.

We have demonstrated that saturation, kinetic and regional distribution data on [^{3}H]sulpiride binding sites may be obtained using quantitative autoradiographic techniques. The use of [^{3}H]sulpiride with this technique has the advantage of very low nonspecific binding even at high ligand concentrations (85% specific binding at 40 nM). In addition it allows for the examination of binding sites in highly circumscribed regions of the brain.

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