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SHORT COMMUNICATIONS

Dopamine D_2 receptor dissociation constant for spiperone: Identical values using ^{3}H-labeled agonist or ^{3}H-labeled antagonist

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A major difficulty in studying dopamine receptors has been that the same neuroleptic gives different dissociation constants according to the dopaminergic [^{3}H]ligand used. For example, the dissociation constant of spiperone at the dopamine D_2 receptor is 52 pM when obtained directly with [^{3}H]spiperone but is 910 pM when obtained indirectly by the competition of spiperone against [^{3}H]dopamine [1].

It was discovered recently, however, that dopamine has a higher affinity for the D_1 receptor than for the D_2 receptor [2]. That is, dopamine has a dissociation constant of 0.8 nM at the high-affinity state of the D_1 receptor and about 3–10 nM at the high-affinity state of the D_2 receptor [2].

This finding, therefore, indicated that [^{3}H]dopamine, particularly when used at about 1 nM, would preferentially label D_1 and not D_2 receptors. Thus, in order to label D_2 receptors, it would be best to use about 10 nM [^{3}H]-dopamine and, if necessary, to use SCH-23390 [2, 3] to occlude D_1 receptors.

Using this approach, therefore, in the present study the dissociation constant for spiperone at the D_2 receptor was found to be the same using [^{3}H]spiperone or [^{3}H]dopamine.

Homogenates of Sprague–Dawley rat brain striatum were prepared fresh for use on the same day; the tissues were not frozen. The striata were homogenized in a glass–teflon homogenizer (see Refs. 4 and 5 for method) in 9 vol. of 50 mM Tris–HCl (pH 7.4 at 20°), preincubated for 10 min at 37° (to metabolize endogenous dopamine), washed twice [4, 5] in a buffer containing 50 mM Tris-HCl (pH 7.4), 5 mM KCl, 4 mM $MgCl_2 \cdot 6H_2O$, 12 μM nialamide and 0.1% ascorbic acid, and, finally, rehomogenized using a Brinkmann Polytron (PT-10; 20 sec, setting 6). The final concentrations of [^{3}H]spiperone (24 Ci/mmole; New England Nuclear, Boston, MA) were 10–1000 pM, while those for [^{3}H]dopamine (38.9 Ci/mmole; New England Nuclear) were 0.1 to 50 nM (for saturation-type experiments) or 8 nM (for competition-type experiments). The final concentration of tissue was between 1.5 mg (for [^{3}H]-spiperone experiments) and 5 mg (for [^{3}H]dopamine experiments) of original wet weight of tissue per final total incubate volume of 1.5 ml. The suspensions were incubated to equilibrium for between 1.5 hr (for [^{3}H]dopamine) and 2 hr (for [^{3}H]spiperone) at 20° and then filtered with a cell harvester (Skatron, Lier, Norway), using two glass fiber filters [5]. Specific binding to D_2 receptors was defined as that binding which was inhibited by either 10 μM *S*-sulpiride [4] or 1 μM YM-09151-2 [6].

Figure 1 illustrates that spiperone inhibited the binding of [^{3}H]dopamine in two phases, as previously noted [1]. The [^{3}H]dopamine concentration of 8 nM was too low to label the low-affinity states (D_1^{low} or D_2^{low}), both of which have a micromolar affinity for dopamine [2]. Thus, spiperone in the nanomolar region inhibited the attachment of [^{3}H]dopamine to D_2^{high}, while spiperone in the micromolar region inhibited [^{3}H]dopamine from binding to D_1^{high}, as shown in Fig. 1. The spiperone K value (dissociation constant) at D_2^{high} was 39 ± 1 pM (mean ± S.E.M., N = 3), using 1 μM YM-09151-2 to define nonspecific binding [6], using 2.9 nM as the K for [^{3}H]-dopamine (obtained by saturation-type experiments in the presence of 100 nM SCH-23390), and using the SCAFIT program [1, 7, 8].

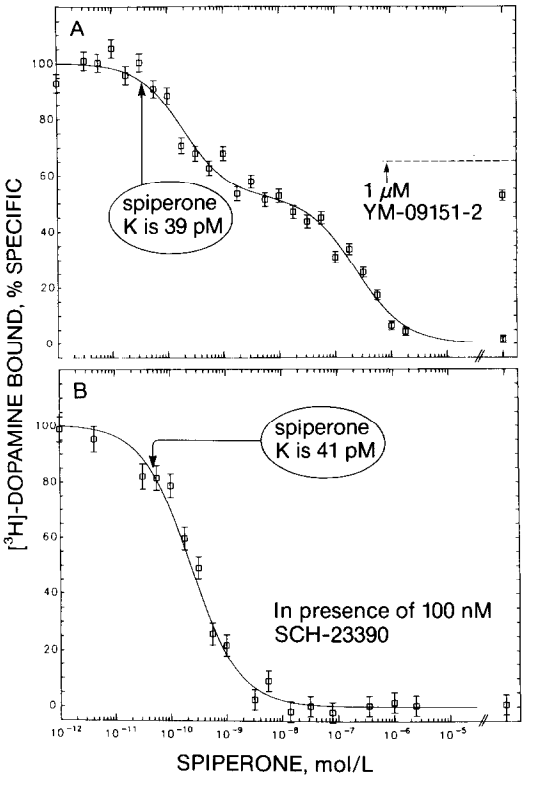

Fig. 1. Dopamine D_2 receptor dissociation constant for spiperone: Identical values using ^{3}H-labeled agonist or ^{3}H-labeled antagonist. The spiperone dissociation constant (K_D) was 39 pM, using 8 nM [^{3}H]dopamine and YM-09151-2 as baseline (panel A); the spiperone K_D was also 41 pM in the presence of SCH-23390 (panel B). Total binding was 15,000 dpm per filter (in A) of which 60% was specific, or 4000 dpm per filter (in B) of which 22% was specific. Lines were drawn by SCAFIT [1, 7, 8]. Specific binding was that inhibited by 1 μM (+)-butaclamol.

This value of 39 pM agreed precisely with that determined directly using [^{3}H]spiperone: 38 ± 3.5 pM (mean ± S.E.M., N = 13), an example of which is in Fig. 2. The findings indicate that the dissociation constants of the antagonist (spiperone), obtained by using a ^{3}H-labeled agonist or a ^{3}H-labeled antagonist, were identical.

In summary, the dissociation constant of spiperone at the dopamine D_2 receptor in the rat brain striatum was the same (between 37 and 41 pM) whether determined by spiperone/[^{3}H]dopamine competition experiments or by [^{3}H]spiperone saturation-type experiments.

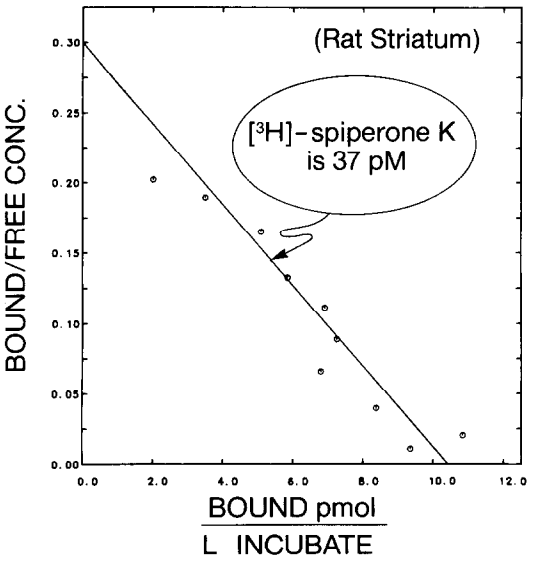

Fig. 2. Typical Scatchard analysis of data from a saturation-type experiment for [^{3}H]spiperone (10–1000 pM) binding to D_2 dopamine receptors in rat striatum. The K_D was 37 pM, as determined by the LIGAND program.

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The formation of the ultimate carcinogen of benzo(*a*)pyrene during non-enzymic lipid peroxidation

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B(*a*)P-7,8-diol, 9,10-epoxide exists in two stereoisometric forms, diol epoxide I (BPDE I) and diol epoxide II (BPDE II). These epoxides are widely believed to be the ultimate carcinogens formed from the polycyclic aromatic hydrocarbon benzo(*a*)pyrene [1]. The half-life of the diol epoxides under physiological conditions is 20–30 sec [2] and, as a consequence of rapid and spontaneous hydrolysis, each epoxide forms two distinct stereoisometric tetrols. B(*a*)P-7,8-diol, 9,10-epoxide is an obligatory intermediate in the formation of B(*a*)P tetrols from the pro-carcinogen B(*a*)P-7,8-diol [3], and analysis of the tetrols formed from the B(*a*)P-7,8-diol therefore demonstrates the intermediate formation of diol epoxides.

We have demonstrated previously that peroxidising polyunsaturated fatty acids can convert B(*a*)P to oxidised products [4] which are mutagenic [5] and in this investigation we present biochemical and biological evidence to suggest that B(*a*)P-7,8-diol is converted to this ultimate carcinogen in the presence of polyunsaturated fatty acids (PUFA) undergoing peroxidation catalysed by Fe^{2+}/ascorbate in the absence of any enzyme system.

Materials and methods

B(*a*)P-7,8-diol (ITT Research Institute, Chicago, U.S.A.) dissolved in dimethyl-sulphoxide was added at a final concentration of 20 μM and was incubated with ferrous sulphate (1 mM), ascorbate (1 mM) and arachidonic acid ($C_{20:4}$) or docosahexaenoic acid ($C_{22:6}$) (500 μM) dissolved in acetone, in phosphate buffer (0.025 M) at pH 7.4 for 30 min. Control incubations were carried out in parallel from which (a) docosahexaenoic acid ($C_{22:6}$) or (b) Fe^{2+}/ascorbate was omitted.

The oxidation products of B(*a*)P-7,8-diol were analysed by HPLC as follows: Unreacted B(*a*)P-7,8-diol and its oxidation products were extracted from the incubation mixture, twice, with 2 vol. of ethyl acetate. Extracts were pooled and 2 mg/100 ml of naphthoglucopyranoside (NG) was added as internal standard, then dried over anhydrous sodium sulphate. The samples were filtered through a 0.5 μm Millipore filter and evaporated to dryness under vacuum in a Speedvac rotary concentrator. The residue was redissolved in 250 μl methanol: H_2O (1:1) and 50 μl injected onto a DuPont Zorbax ODS column (25 cm × 4.6 mm i.d.). The DuPont model 8100 high pressure liquid chromatograph was linked to a Perkin Elmer series LS3 Fluorimeter as detector, fitted with an Apple IIe microcomputer. Products were eluted from the column using a flow rate of 1 ml/min with a linear gradient. Solvent composition was changed from 45% methanol in water to 75% methanol in water during 40 min and then increased to 100% methanol during 0.5 min. 100% methanol was pumped through the column for a further 30 min, to ensure complete elution of retentive tetrols or unreacted B(*a*)P-7,8-diol. The fluorimeter was set at excitation and emission wavelengths of 242 and 390 nm respectively. B(*a*)P tetrols were identified by comparison with authentic B(*a*)P tetrol standards (ITT Research Institute, Chicago, U.S.A.) and quantified by calculating peak areas (Chromatochart,