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Short communication

Relative affinities of dopaminergic drugs at dopamine D_2 and D_3 receptors

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Abstract

Quantitative autoradiography was used to evaluate the pharmacological profile of dopamine D_2-like receptors labeled by [^{125}I]iodosulpiride. Caudate/putamen, a brain region associated primarily with dopamine D_2 receptor mRNA, was used as a prototypical D_2 tissue; cerebellar lobule X (D_3 mRNA associated), as a D_3 tissue. 7-OH-DPAT ((±)-2-dipropylamino-7-hydroxy-1,2,3,4-tetrahydronaphthalene) exhibited selectivity for cerebellar receptors (24-fold), followed by quinpirole (6-fold). Haloperidol and domperidone were 4- and 18-fold more potent at striatal receptors, respectively. These data are in close agreement with that derived from dopamine D_2 and D_3 receptor-expressing cell lines.

Keywords: Dopamine D_2 receptor; Dopamine D_3 receptor; [^{125}I]Iodosulpiride; Receptor autoradiography; Striatum; Cerebellum; (Rat)

1. Introduction

The dopamine D_3 receptor, which has homology with dopamine D_2 receptor, has been proposed as potential antipsychotic target (Sokoloff et al., 1990). This hypothesis is based largely on the regional distribution of dopamine D_3 mRNA and binding sites in brain: the receptors appear to be expressed primarily in limbic brain regions with low levels of mRNA or binding sites in the caudate/putamen. This observation suggests that the receptor may be an appropriate site of action for antipsychotic drugs that might be free of extrapyramidal side effects (Sokoloff et al., 1990). Efforts are currently directed towards developing selective dopamine D_3 ligands as potential antipsychotic agents. Dopamine D_3 receptor-expressing cell lines have been widely utilized for this purpose.

We have demonstrated that dopamine receptors in the molecular layer of cerebellar lobule X, which appear to be colocalized exclusively with dopamine D_3 receptor mRNA (Bouthenet et al., 1991), exhibit guanyl nucleotide regulation and a pharmacological profile appropriate for the D_3 site (Levant and DeSouza, 1993; Levant et al., 1992a). Thus this brain area can serve as a prototypical dopamine D_3 tissue, lacking dopamine D_2 receptors, for the study of this binding site in brain tissue. In contrast, the caudate-putamen, which expresses substantially greater amounts of dopamine D_2 receptor mRNA and exhibits relatively little dopamine D_3 receptor binding proves useful as a prototypical dopamine D_2 tissue (Levant and DeSouza, 1993). Using these brain areas as prototypical tissues, it is possible to evaluate the selectivities of drugs for these receptors in brain tissue without a selective radioligand.

In previous studies, we have demonstrated that [^{3}H]quinpirole-labeled dopamine receptors in rat striatum and cerebellum exhibit differential pharmacological profiles which are similar to those reported for dopamine D_2 and D_3 receptors, respectively, expressed in Chinese hamster ovary (CHO) cells (Levant and DeSouza, 1993). However, in competition with

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[^{3}H]quinpirole, the relative D_2/D_3 selectivities of all compounds were shifted towards D_2 compared to that reported in the transfected cell lines. For example, (±)-2-dipropylamino-7-hydroxy-1,2,3,4-tetrahydronaphthalene (7-OH-DPAT) was only 5-fold more potent in competition for [^{3}H]quinpirole-labeled cerebellar receptors than striatal receptors (Levant and DeSouza, 1993) but was reported to be 78-fold more potent at CHO/D_3 than CHO/D_2 (Lévesque et al., 1992). One reason for this discrepancy may be the different in vitro assay systems used in these studies – i.e. different radioligands, buffers, assay conditions, etc.

This study is designed to extend the characterization of the pharmacological profile of dopamine D_3 receptors in rat brain and to evaluate the effect of in vitro assay conditions of the D_2/D_3 selectivity of dopaminergic drugs. Quantitative autoradiographic method using [^{125}I]iodosulpiride, the ligand used in the initial characterization of CHO/D_2 and CHO/D_3 receptors (Sokoloff et al., 1990), is used to determine the affinities of dopaminergic compounds for dopamine D_2 and D_3 receptors in rat brain.

2. Materials and methods

All experiments were carried out in accordance with the Declaration of Helsinki and with the NIH Guide for the Care and Use of Laboratory Animals.

2.1. Tissue preparation

Adult male Sprague-Dawley rats (200–300 g; Charles River Laboratories, Wilmington, DE, USA) were killed by decapitation; the brains rapidly removed, frozen in isopentane, and stored at −70° C until sectioning. Sagittal brain sections (20 μm, lateral 1.00–1.40 mm) were cut on a cryostat so that each section contained caudate/putamen and cerebellar lobule X. Sections were thaw-mounted onto chrome alum/gelatin-coated slides and stored at −70° C until use.

2.2. Receptor autoradiography with [^{125}I]iodosulpiride

Slide mounted sagittal brain sections (20 μM, lateral 1.0–1.4 mm) from male Sprague-Dawley rats were processed for [^{125}I]iodosulpiride autoradiography (Bouthenet et al., 1987). After being brought to room temperature, slide-mounted brain sections were incubated for 1 h at 23° C with ~0.3 nM [^{125}I]iodosulpiride, alone or in the presence of 5 concentrations of competing drug (10^{-9} to 10^{-5} M) in assay buffer (50 mM Tris-HCl, 5 mM KCl, 2 mM $MgCl_2$, and 2 mM $CaCl_2$, 120 mM NaCl, 0.1% ascorbate, pH 7.4 at 23° C). Duplicate sections from each animal were used for each data point. Nonspecific binding was defined using 1 μM spiperone. This concentration of spiperone was experimentally determined to inhibit binding to all receptors of interest in preliminary studies. After incu-

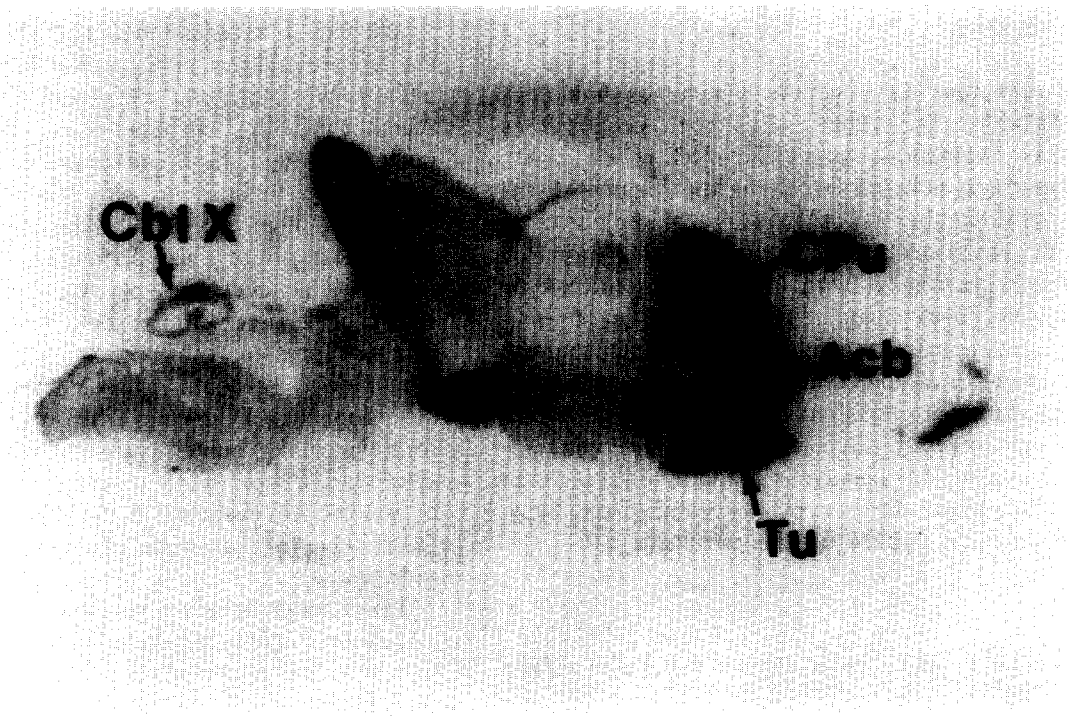

Fig. 1. Autoradiographic localization of [^{125}I]iodosulpiride-labeled receptors in rat brain. Sagittal sections (20 μM, lateral 1.2 mm) were incubated with ~0.3 nM [^{125}I]iodosulpiride at 23° C for 1 h. Nonspecific binding was defined with 1 μM spiperone. Acb – nucleus accumbens, ICj – islands of Calleja; Tu – olfactory tubercle; Cbl X – cerebellar lobule X.

bation, slides were dipped in ice-cold assay buffer, washed for 2 consecutive 5-min periods in ice-cold assay buffer, dipped in ice-cold deionized H_2O, and dried. Radiolabeled sections were then apposed to ^{3}H-Hyperfilm for a period of 24 h (for quantitation of binding in striatum) or 1 week (for quantitation of binding in cerebellar lobule X) with [^{125}I]methylmethacrylate autoradiographic standards (20 μm). ^{3}H-Hyperfilm was developed according to the manufacturer's instructions.

2.3. Quantification of autoradiograms and data analysis

Autoradiographic images were digitized and quantified using the Macintosh-based video densitometry program NIH 'Image' version 1.4. Best-fit curves of film optical density generated by the [^{125}I]methylmethacrylate standards resulted when a Rodbard plot was used to describe the relationship between optical density and radioactivity. Brain regions were identified according to the atlas of Paxinos and Watson (1986). The density of [^{125}I]iodosulpiride binding in specific brain regions was sampled and expressed as fmol/mg tissue equivalent. These data were used to generate competition curves from which the IC_{50} values were derived. Because (−)-sulpiride exhibited slightly differing (2-fold) affinities for striatal dopamine D_2 and cerebellar dopamine D_3 receptors in previous studies (Levant and DeSouza, 1993), K_i values were not determined.

2.4. Drugs

[^{125}I]Iodosulpiride (specific activity 1800–2000 Ci/mmol) was obtained from Amersham (Arlington, IL, USA). 7-OH-DPAT ((±)-2-dipropylamino-7-hydroxy-1,2,3,4-tetrahydronaphthalene), quinpirole, (−)-sulpiride, spiperone, haloperidol, and domperidone were obtained from Research Biochemicals (Natick, MA, USA). UH 232 (*cis*-(+)-(1*S*,2*R*)-5-methoxy-1-methyl-2-(di-*n*-propylamino)tetralin) and (+)AJ 76 (*cis*-(+)-(1*S*,2*R*)-5-methoxy-1-methyl-2-(*n*-propylamino)tetralin) were the generous donation of Dr. M. Piercey of the Upjohn Co. (Kalamazoo, MI, USA).

3. Results

Dense specific binding of [^{125}I]iodosulpiride was observed in the caudate/putamen, nucleus accumbens, olfactory tubercles, islands of Calleja and the molecular layer of cerebellar lobule X as previously described (Bouthenet et al., 1987) (Fig. 1). Caudate/putamen, a brain region associated primarily with dopamine D_2 receptor mRNA, was used as a prototypical D_2 tissue; cerebellar lobule X (D_3 mRNA associated) was used as a D_3 tissue for all further analyses. All drugs tested produced maximal inhibition of [^{125}I]iodosulpiride binding similar to that produced by spiperone (1 μM).

Data from autoradiographic competition studies are summarized in Table 1. Dopaminergic compounds inhibited [^{125}I]iodosulpiride binding in caudate/putamen with the following rank order of potencies: spiperone > domperidone ≥ haloperidol > (−)-sulpiride > UH 232 > 7-OH-DPAT > quinpirole > (+)AJ 76. In cerebellar lobule X, a somewhat different rank order was observed: spiperone ≥ 7-OH-DPAT ≥ (−)-sulpiride > haloperidol ≈ UH 232 ≥ quinpirole > domperidone > (+)AJ 76.

7-OH-DPAT had the greatest selectivity for cerebellar D_3 sites (24-fold), followed by quinpirole (6-fold). In contrast, haloperidol and domperidone were more potent at striatal dopamine D_2 receptors (4- and 18-fold, respectively). UH 232, (+)AJ 76, (−)-sulpiride, and spiperone exhibited relatively little D_2/D_3 selectivity.

Table 1
Pharmacological profiles of dopaminergic compounds in competition for [^{125}I]iodosulpiride binding in caudate/putamen and cerebellar lobule X

Brain region / Drug	IC_{50} (nM) Caudate/putamen mean ± S.E. (*n*)		IC_{50} (nM) Cerebellar lobule X mean ± S.E. (*n*)		IC_{50} (caudate)/ IC_{50} (cerebellum)	K_i (CHO/D_2)/ K_i (CHO/D_3)
7-OH-DPAT	99 ± 25	(4)	4.2 ± 0.8	(3)	24	78 [a]
Quinpirole	157 ± 46	(4)	28 ± 10	(4)	5.6	36 [b]
UH 232	56 ± 7.0	(4)	23 ± 1.5	(3)	2.4	4.4 [c]
(+)AJ 76	338 ± 97	(4)	143 ± 19	(4)	2.4	3.0 [c]
(−)-Sulpiride	11 ± 1.8	(4)	6.3 ± 0.6	(3)	1.8	0.36 [c]
Spiperone	2.1 ± 0.4	(8)	3.4 ± 0.5	(8)	0.62	0.11 [c]
Haloperidol	6.3 ± 1.0	(4)	23 ± 7.0	(3)	0.27	0.046 [c]
Domperidone	4.5 ± 1.2	(4)	83 ± 26	(4)	0.05	0.032 [c]

Comparison with dopamine D_2 and D_3receptors expressed in CHO cells. [a] Lévesque et al. (1992); [b] Sokoloff et al. (1992); [c] Sokoloff et al. (1990).

4. Discussion

Messenger RNA for the dopamine D_3, D_4, and D_5 receptor subtypes, and presumably the receptors themselves, are present in much lower abundance than dopamine D_1 and D_2 receptors (for review see: Sibley and Monsma, 1992). Because of the low abundance of the receptors and the lack of highly selective pharmacological tools, most investigations of these novel subtypes have employed receptor-expressing transfected cell lines. While this approach is of considerable merit, it has the inherent limitation that the transfected cell may not exhibit the same binding characteristics as would be observed in brain tissue. Of note, somewhat differing pharmacological profiles for dopamine D_2 and D_3 receptors have been reported by a number of laboratories using rat or human receptors expressed in a variety of expression systems (Boundy et al., 1993; Chio et al., 1994; Freedman et al., 1994; MacKenzie et al., 1992; Sokoloff et al., 1990; Sokoloff et al., 1992; Tang et al., 1994). This variation in results may also result from different in vitro assay protocols employed in various laboratories. Hence, it is desirable to also study these receptors in native brain tissue under assay conditions that are directly comparable to those used in other laboratories.

The aim of the present studies was to compare the pharmacological profile of dopamine D_2 and D_3 receptors in rat brain. The experimental approach used in these experiments utilizes a nonselective radioligand that labels both D_2 and D_3 sites. Discrete brain regions are used as prototypical dopamine D_2 and D_3 receptor-expressing tissues as an alternative to using transfected cell lines. Caudate/putamen, a brain region associated primarily with dopamine D_2 receptor mRNA, was used as a prototypical D_2 tissue; cerebellar lobule X, a D_3 mRNA-expressing region lacking D_2 RNA, was used as a D_3 tissue. It should be noted that because a small percentage of dopamine D_3 receptors are expressed in the caudate/putamen, these data may somewhat underestimate the relative D_3/D_2 selectivity of D_3-selective ligands.

The present findings confirm our previous finding that striatal and cerebellar dopamine sites exhibit differential pharmacological profiles that are roughly similar to those reported for CHO/D_2 and CHO/D_3 receptors, respectively (Levant and DeSouza, 1993) using a different radioligand and in vitro assay system. In addition to confirming our previous findings, the data indicate that the pharmacological profiles of striatal and cerebellar dopamine receptors labeled by [^{125}I]iodosulpiride are quite similar to those reported by Sokoloff et al. (1990, 1992 for CHO/D_2 and CHO/D_3 (Table 1). Accordingly, 7-OH-DPAT, one of the most D_3-selective compounds identified to date, was the most D_3-selective compound evaluated exhibiting 24-fold selectivity for cerebellar sites over striatal dopamine D_2 receptors. Quinpirole, which had also been proposed as a D_3-selective compound, was also more potent in displacing [^{125}I]iodosulpiride binding in cerebellum than in striatum. Conversely, compounds which have been observed to be D_2-selective in studies using transfected cell lines, such as domperidone, were more potent in competition for [^{125}I]iodosulpiride binding in striatum than in cerebellum.

The very low potency of spiperone in all brain regions in the present study is similar to what was observed in competition for [^{3}H]quinpirole binding in our previous autoradiographic study (Levant et al., 1992a). The apparent low affinity of spiperone in our autoradiographic studies must be addressed in further studies. The present studies, however, were designed to determine relative affinities rather than the absolute affinity of each ligand for striatal and cerebellar receptors. The relative affinities observed in this study are in close agreement with the literature.

A secondary goal of these studies was to compare the relative affinities of dopaminergic compounds for dopamine D_2 and D_3 sites under different in vitro assay conditions. Our previous study used [^{3}H]quinpirole to label dopamine D_2 and D_3 receptors. Binding of this agonist ligand is inhibited by Na^+ (Levant et al., 1992b). Accordingly, Na^+ was not included in that assay system. [^{125}I]Iodosulpiride, the ligand used in the initial characterization of CHO/D_2 and CHO/D_3 receptors (Sokoloff et al., 1990), is a benzamide antagonist. Like other benzamides, binding of this ligand is potentiated by Na^+ (Niznik et al., 1985). Hence, the [^{125}I]iodosulpiride assay system incorporates this ion. Accordingly, (−)-sulpiride was considerably more potent at both [^{125}I]iodosulpiride-labeled striatal and cerebellar receptor than observed in our previous study using [^{3}H]quinpirole (Levant and DeSouza, 1993). It should be noted, however, that (−)-sulpiride exhibited little D_2/D_3 selectivity in either study.

While the overall rank order of selectivity between striatal and cerebellar receptors was similar between this and our previous study, the relative potencies of all compounds examined were shifted towards the dopamine D_3 receptor in this study compared to our previous study using [^{3}H]quinpirole. For example, 7-OH-DPAT was 24-fold more potent in competition for [^{125}I]iodosulpiride-labeled D_3 sites than D_2, but only 5-fold more potent when receptors are labeled with [^{3}H]quinpirole (Levant and DeSouza, 1993). Likewise, spiperone exhibited 80-fold selectivity for [^{3}H]quinpirole-labeled striatal dopamine D_2 receptors, but was only 1.6-fold more potent in the present study. These data clearly demonstrate differences in pharmacological profile of striatal and cerebellar dopamine receptors obtained under different in vitro assay conditions.

In summary, the present data demonstrate that

[^{125}I]iodosulpiride-labeled receptors in striatum and cerebellar lobule X exhibit pharmacological profiles quite similar to that reported for CHO/D_2 and CHO/D_3 receptors, respectively. These data also indicate that the selectivity of some dopaminergic drugs may vary under different assay conditions. Clearly, in vitro assay conditions must be considered in the demonstration of receptor selectivity of novel compounds with the aim of identifying potential therapeutic agents.

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