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Hexahydrobenzo[a]phenanthridines: novel dopamine D_3 receptor ligands

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We report that certain substituted hexahydrobenzo[a]phenanthridines are novel high affinity ligands selective for the dopamine D_3 receptor. These data demonstrate that substitutions on the heterocyclic nitrogen and the pendant phenyl ring of this nucleus cause a marked increase in both affinity and selectivity for dopamine D_3 vs. D_2 receptors. Thus, these compounds represent important new tools to study the pharmacology of dopamine D_3 receptors, and may also provide an opportunity for the synthesis of new radioligands for dopamine D_3 receptors.

Dopamine D_3 receptors; Dopamine D_2 receptors; Dihydrexidine

The dopamine D_3 receptor (Sokoloff et al., 1990) is a member of the dopamine 'D_2-like' receptor family. Compared to the previously cloned dopamine D_2 receptors (D_{2S} and D_{2L}), the dopamine D_3 receptor appears to be much less abundant, and has a more restricted anatomical distribution. The majority of dopamine D_3 receptors and mRNA is found in limbic areas, including the olfactory tubercle, nucleus accumbens, islands of cajella and the hypothalamus (Sokoloff et al., 1990; Levesque et al., 1992; Boundy et al., 1993). A major problem in understanding dopamine D_3 receptor pharmacology has been the limited number of selective ligands. The development of novel structural classes of D_3 selective ligands is essential for studying the pharmacophoric requirements of ligand-dopamine D_3 receptor interactions, and ultimately activation of dopamine D_3 receptors. In the present communication, we report a significant breakthrough in the development and initial characterization of a high affinity, D_3 selective class of ligands.

A major focus in our effort to understand the pharmacophoric requirements of dopamine receptors has involved drugs of the hexahydrobenzo[a]phenanthridine series. The prototype of this series was dihydrexidine (DHX), the first potent full efficacy dopamine D_1 receptor agonist (Brewster et al., 1990). Although DHX has 10-fold selectivity for dopamine D_1 vs. D_2-like receptors in rat striatum, it still has significant potency for dopamine D_2-like receptors. Moreover, several analogs (including N-n-propyl-DHX, 4-methyl-DHX, and 4-methyl-N-n-propyl-DHX) have even higher affinity for dopamine D_2-like receptors.

For the present studies, we compared the radioligand binding affinity of several hexahydrobenzo[a]phenanthridines for two molecular forms of dopamine receptors (D_3 and D_{2L}) that had been stably transfected in C-6 glioma cells. Saturation analyses of the two transfected cell lines using [^{3}H]spiperone yielded similar maximal receptor expression levels (B_{max} ca. 800–1400 fmol/mg) and K_D values (0.07 nM (D_{2L}) vs. 0.14 nM (D_3)). These were the radioligand concentrations used to label receptors for the competition binding assays. Test compounds, along with one or both of the two prototypical D_3 selective ligands, quinpirole and 7-OH-DPAT, were compared within the same assay using membranes prepared from the two expression systems.

As reported previously, quinpirole and 7-OH-DPAT displayed ca. 100-fold selectivity for the D_3 versus the D_{2L} receptor (Levesque et al., 1992). The four hexahy-

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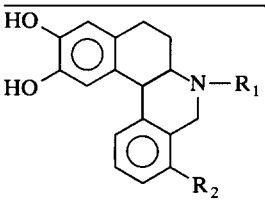
TABLE 1

Apparent affinities ($K_{0.5}$) and D_3:D_{2L} selectivity ratios of a series of benzo[a]phenanthridines and two prototypical dopamine D_3 receptor ligands (quinpirole and 7-OH-DPAT) for clonal rat dopamine D_3 and D_{2L} receptors expressed in C-6 glioma cells.

Membranes from each cell line were prepared identically. Ten to 15 μg protein were incubated with each test compound and [^{3}H]spiperone (0.07 nM; D_{2L}; 0.14 nM, D_3) for 50 min at 37°C in 50 mM Tris-HCl (pH 7.4), with 0.9% NaCl, 0.025% ascorbic acid, and 0.001% bovine serum albumin in the absence of GTP. (+)-Butaclamol (2 μM) was used to define nonspecific binding. Apparent affinities ($K_{0.5}$) were estimated by the Cheng-Prusoff equation after nonlinear regression analyses (sigmoid algorithm; InPlot; Graphpad, San Francisco). $K_{0.5}$ values are expressed as the mean (nM) ± standard error of the mean, with the number of experiments for each compound given below in parentheses. The Hill slope (n) is expressed as the mean ± standard error of the mean.



	C-6 D_3		C-6 D_{2L}		Selectivity ratio
Drug	$K_{0.5}$, nM	n	$K_{0.5}$, nM	n	D_3:D_{2L}
DHX (R_1=H, R_2=H)	170 ±23 (3)	1.03±0.12	1490±270 (3)	0.90±0.07	8.8
4-Me DHX (R_1=H, R_2=CH_3)	85 ±28 (3)	1.10±0.10	674±160 (3)	0.74±0.09	7.9
N-n-Pr DHX (R_1=C_3H_7, R_2=H)	8.70± 1.3 (4)	0.85±0.06	434±100 (4)	0.96±0.09	50
4-Me-N-n-Pr DHX (R_1=C_3H_7, R_2=CH_3)	2.08± 0.45 (5)	0.55±0.07	229±32 (5)	0.74±0.06	110
Quinpirole	26.8± 2.6 (7)	0.92±0.09	4210±870 (7)	0.68±0.08	157
7-OH-DPAT	3.71± 0.22 (5)	0.83±0.05	426±123 (5)	0.76±0.08	115

drobenzo[a]phenanthridines examined here showed D_3 selectivity ranging from 8- to 110-fold. Both the 4-methyl and N-n-propyl substitutions result in increases in dopamine D_3 receptor affinity and selectivity; when substituted in combination, the result is a ligand with affinity in the nanomolar range and greater D_3 selectivity. In particular, 4-methyl-N-n-propyldihydrexidine (4MP-DHX) exhibited selectivity similar to that of quinpirole and 7-OH-DPAT (> 100-fold), and higher affinity ($K_{0.5}$ = 2.08 nM) than any of the ligands examined (table 1). Furthermore, comparison of the Hill slopes for 4MP-DHX and 7-OH-DPAT revealed a significantly lower coefficient for 4MP-DHX (table 1). This finding suggests that, compared to 7-OH-DPAT, 4MP-DHX has much greater affinity for the high affinity site of the dopamine D_3 receptor.

Presently, the signal transduction mechanism(s) of dopamine D_3 receptors are unknown. Thus the functional implications of such selective dopamine D_3 receptor ligands are unclear. Of interest are our earlier reports that DHX, and its analog N-n-propyl-DHX, seem to bind to both pre- and postsynaptic dopamine D_2-like receptors, yet have agonist functional activity that is confined to postsynaptic receptors (Mottola et al., 1991). That is, both compounds act as agonists to inhibit adenylate cyclase activity, inhibit prolactin release, and increase K^+ channel conductance. Conversely, many presynaptic events (e.g., striatal dopamine synthesis or release) are unaffected by receptor actions of these drugs. This unusual pharmacology may be explained, in part, by the D_3 actions of these compounds.

In summary, 4MP-DHX is a new selective, high affinity dopamine D_3 receptor ligand that can be a powerful tool to study this receptor. Modeling of the pharmacophore for this receptor should facilitate the development of other members of this class with desired pharmacological properties. Moreover, the radiolabeling of 4MP-DHX may provide a dopamine D_3 receptor radioligand with useful characteristics compared to available dopamine D_3 receptor radioligands (Levant et al., 1991; Levesque et al., 1992; Kung et al., 1993).

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