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Dopamine D_1 receptors: efficacy of full (dihydrexidine) vs. partial (SKF38393) agonists in primates vs. rodents

Val J. Watts [a], Cindy P. Lawler [c,d], John H. Gilmore [b,c], Stan B. Southerland [c], David E. Nichols [e] and Richard B. Mailman [a,b,c,d]

Departments of [a] Pharmacology and [b] Psychiatry, [c] Brain and Development Research Center, [d] Curriculum in Toxicology, University of North Carolina School of Medicine, Chapel Hill, NC 27599-7250, USA, and [e] Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907, USA

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Although partial efficacy dopamine D_1 receptor agonists have little therapeutic benefit in parkinsonism, the first high potency, full efficacy dopamine D_1 receptor agonist dihydrexidine recently has been shown to have profound antiparkinsonian effects. One reason for the greater antiparkinsonian effects of dihydrexidine vs. SKF38393 might be that SKF38393, while a partial dopamine D_1 receptor agonist in rodent striatal preparations, has virtually no agonist activity in monkey striatum (Pifl et al., 1991, Eur. J. Pharmacol. 202, 273). To explore this hypothesis, we compared the dopamine D_1 receptor affinity and efficacy of dihydrexidine and SKF38393 in striatum from rat and monkey. In vitro binding studies using membranes from putamen of adult rhesus monkeys demonstrated that dihydrexidine competed for dopamine D_1 receptors (labeled with [^{3}H]SCH23390) with high potency (IC_{50} = 20 nM vs. ca. 10 nM in rat brain). SKF38393 was about 4-fold less potent than dihydrexidine in both monkey and rat brain. The in vitro functional activity of these drugs was assessed by their ability to stimulate adenylate cyclase activity in tissue homogenates. Dihydrexidine was of full efficacy (relative to dopamine) in stimulating cAMP synthesis in both monkey and rat. SKF38393 was only a partial efficacy agonist in both rat striatum and monkey putamen, but contrary to the original hypothesis, it had the same efficacy (ca. 40% relative to dihydrexidine) in membranes from both species. Interestingly, greater between-subject variation was found in the stimulation produced by SKF38393 in primate compared to rat brain, although the basis for this variation is unclear. The present data demonstrate for the first time that dihydrexidine is a full efficacy dopamine D_1 receptor agonist in primate brain. Moreover, these data indicate that the partial efficacy dopamine D_1 receptor agonist SKF38393 causes the same relative response (compared to dopamine) in rat and monkey dopamine D_1 receptors. Together, this information suggests that the antiparkinsonian effect of dihydrexidine vs. the relative inactivity of SKF38393 is not due to the fact that primate brains are simply unresponsive to SKF38393.

Dopamine D_1 receptors; Dihydrexidine; SCH23390; SKF38393; Adenylate cyclase; Parkinson's disease; (Rhesus monkey); (Rat)

1. Introduction

Traditionally, the division of dopamine receptors into two classes has been based on pharmacological and functional properties (Garau et al., 1978; Kebabian and Calne, 1979). The D_1 subclass preferentially recognizes 1-phenyl-tetrahydrobenzazepines (e.g., SCH-23390), while the D_2 subclass has selectivity for drugs of the benzamide and butyrophenone classes. Functionally, dopamine D_1 receptors stimulate adenylate cyclase, while dopamine D_2 receptors may have inhibitory effects on this enzyme. It has been shown that some populations of dopamine receptors may be linked to effector systems other than adenylate cyclase (e.g., Mailman et al., 1986; Undie and Friedman, 1992; Vallar and Meldolesi, 1989).

Molecular biological techniques have revealed that at least six molecular forms of dopamine receptors exist (see reviews by Civelli et al., 1991; O'Dowd, 1993). These include two molecular forms of dopamine D_1 receptors (D_{1A} and D_{1B}/D_5) and four forms of the D_2 subclass (D_{2A} and D_{2B}, D_3, and D_4). Although it is apparent that there is great diversity among dopamine receptors, there is limited understanding about their corresponding functional role(s) in the central nervous system. This is due in large measure to the lack of subtype-selective ligands. Until future studies clarify

Correspondence to: Richard B. Mailman, CB No. 7250, Brain and Development Research Center, University of North Carolina School of Medicine, Chapel Hill, NC 27599-7250, USA. Tel. (919) 966-3205, fax (919) 966-1844, email richard@css.unc.edu.

such issues, it seems useful to maintain a basic distinction between 'D_1-like' and 'D_2-like' receptors.

The role of dopamine D_1 and D_2 receptor subtypes in the treatment of idiopathic Parkinson's disease is an important, yet unresolved clinical issue. The therapeutic efficacy of levodopa was established more than 20 years ago (Cotzias et al., 1969), and is still the primary treatment for this disease. Historically, the beneficial effects of levodopa have been attributed to its conversion to dopamine and subsequent activation of dopamine D_2 receptors (e.g., Schachter et al., 1980). There is also evidence for some clinical improvement in Parkinson's patients who are administered D_2-selective agonists such as bromocriptine or pergolide, either alone or in combination with levodopa (Rinne, 1989). Unfortunately, disabling side effects and a loss of responsiveness in the later stages of the disease are major limitations of current treatment strategies (see reviews by Goetz, 1990; Koller and Hubble, 1990).

Given the limitations of treatment with either levodopa and/or D_2 agonists, the therapeutic potential of dopamine D_1 receptor agonists was considered. Yet a clinical trial with the D_1-selective agonist SKF38393 in subjects with idiopathic Parkinson's disease found no beneficial effects (Braun et al., 1987). Furthermore, numerous studies with SKF38393 in the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-HCl) primate model of Parkinson's disease failed to demonstrate anti-parkinsonian actions (Barone et al., 1987; Bedard and Boucher, 1989; Boyce et al., 1990; Close et al., 1985; Nomoto et al., 1988). Moreover, SKF38393 has been reported to decrease the efficacy of levodopa and the D_2 agonist quinpirole (Barone, 1987; Bedard and Boucher, 1989; Elliot et al., 1992; Nomoto et al., 1988) when coadministered with these compounds in MPTP-treated monkeys. Thus far, only one study has reported any beneficial effects of SKF38393 in MPTP-treated primates (Rouillard et al., 1990). This study found that the addition of SKF38393 to chronic bromocriptine treatment reduced the gradual loss of efficacy that occurs when bromocriptine is administered alone.

We had hypothesized that the lack of effectiveness of SKF38393 in treating parkinsonism could be attributed to the fact that this compound is only a partial efficacy agonist at dopamine D_1 receptors. For example, SKF38393 produces only about a half-maximal effect (compared to dopamine or dihydrexidine) in stimulating adenylate cyclase in various preparations including rat striatal membranes (e.g., Brewster et al., 1990), rat striatal slices (Cook et al., 1991), and the NS20Y neuroblastoma line (Lovenberg et al., 1991). Interestingly, Pifl et al. (1991) reported that in rhesus monkey membranes, SKF38393 only causes about a 10% maximal stimulation, versus about 50% in rodents. As would be expected from its partial efficacy, SKF38393 can actually *inhibit* dopamine-mediated stimulation of adenylate cyclase in both rodents and primates (Pifl et al., 1991). Thus, the lack of beneficial effects of SKF38393 in humans or MPTP-treated monkeys, as well as its functional antagonism of levodopa or D_2-selective agonists, could be explained by this relative lack of intrinsic activity in primates as opposed to rodents.

The recent availability of a novel class of dopamine ligands, the benzo[a]phenanthridines, has provided important new tools to study this hypothesis. One member of this class, dihydrexidine (trans-10,11-dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo[a]phenanthridine), has been shown to be the first high potency, full efficacy D_1-selective agonist in rodent brain (Lovenberg et al., 1989; Brewster et al., 1990; Mottola et al., 1992). We had suggested previously that a potent full efficacy dopamine D_1 receptor agonist may prove beneficial in the treatment of idiopathic Parkinson's disease (Lovenberg et al., 1989; Brewster et al., 1990). In support of this idea, and in contrast to the previous negative results obtained with drugs like SKF38393 in MPTP-treated monkeys, a low dose of the full efficacy dopamine D_1 receptor agonist dihydrexidine was shown to have dramatic effects in reducing parkinsonian signs (Taylor et al., 1991a). A more recent report with another full efficacy dopamine D_1 receptor agonist, A77636, has confirmed the beneficial effects of dopamine D_1 receptor stimulation in MPTP-treated marmosets (Kebabian et al., 1992).

In light of the potential clinical utility of dihydrexidine and other true full efficacy dopamine D_1 receptor agonists in parkinsonism, the present experiments were designed to compare the biochemical properties of dihydrexidine and SKF38393 in primate and rodent tissue. We hypothesized that dihydrexidine would be a potent, high affinity full agonist in primate brain, similar to its effects in rat brain. Based on the report of Pifl et al. (1991), we also hypothesized that the efficacy of SKF38393 would be much less in primate vs. rodent brain. The present study demonstrates that only the former of these hypotheses is correct.

2. Materials and methods

2.1. Compounds

Dihydrexidine (trans-10,11-dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo[a]phenanthridine) was synthesized as previously described (Brewster et al., 1990). SKF38393 and SCH23390 were obtained from Research Biochemicals (Natick, MA). Dopamine, cAMP, and isobutyl methylxanthine were purchased from Sigma Chemical Co. (St. Louis, MO). [α-^{32}P]ATP was supplied by New England Nuclear (Boston, MA).

[^{3}H]SCH23390 was prepared as described by Wyrick and Mailman (1985).

2.2. Subjects

Adult male Sprague-Dawley rats (200–250 g) were obtained from Charles River Breeding Laboratories (Raleigh, NC). Rats were killed by decapitation, the whole brains removed, chilled briefly in ice-cold saline, and sliced into 1.2 mm coronal sections with the aid of a dissecting block similar to that described by Heffner et al. (1980). Central striata were then dissected from two coronal sections containing the majority of this region. Tissue was frozen immediately on dry ice and stored at −70°C until the day of the assay. Brains of drug-naive adult male rhesus macaques (ex-breeders) were obtained from Buckshire Corporation (Perkasie, PA). Monkeys were anesthetized with ketamine and an overdose of sodium pentobarbital, and were perfused briefly with ice-cold saline via a cardiac catheter. Brains were removed from the skull rapidly, hemisected, and sliced into several 6–8 mm coronal slabs. These slabs were frozen rapidly on dry ice, and stored at −70°C. Putamen tissue was later dissected from these frozen coronal slabs. Dissected tissue chunks were kept at −70°C until the day of the assay.

2.3. Radioreceptor binding assays

Competition binding assays were performed on three separate days for rat tissue (pooled striata from two subjects in each assay; total n = 6 rats). Three separate experiments (putamen from one subject in each assay; total n = 3 monkeys) were conducted with monkey tissue. Frozen rat striata or monkey putamen were homogenized by seven manual strokes in a Wheaton Teflon-glass homogenizer in 8 ml ice-cold 50 mM HEPES buffer with 4.0 mM $MgCl_2$, (pH 7.4). Tissue was centrifuged at 27,000 × *g* for 10 min, the supernatant was discarded, and the pellet was homogenized (5 strokes) and resuspended in ice-cold buffer and centrifuged again. The final pellet was suspended at a concentration of approximately 2.0 mg wet weight/ml. The amount of tissue added to each assay tube was 1.0 mg, in a final assay volume of 1.0 ml. Dopamine D_1 receptors were labeled with [^{3}H]SCH23390 (0.30 nM). Total binding was defined as radioligand bound in the absence of any competing drug. Nonspecific binding was estimated by adding unlabeled SCH23390 (1 μM). Competition for [^{3}H]SCH23390-labeled sites was assessed using 10–12 concentrations of unlabeled competitor (dihydrexidine and SKF38393). As an internal control, a competition curve with six concentrations of unlabeled SCH23390 was included in each assay. Triplicate (rat) or duplicate (monkey) determinations were made for each drug concentration in each assay. Assay tubes were incubated at 37°C for 15 min. Binding was terminated by filtering with ice-cold buffer on a Skatron 12-well cell harvester (Skatron, Sterling, VA) using glass fiber filter mats (Skatron No. 7034). Filters were allowed to dry and 2.0 ml of Optiphase HI-SAF II scintillation fluid was added. After shaking for 30 min, radioactivity was determined on an LKB Wallac 1219 RackBeta liquid scintillation counter (Wallac, Gaithersburg, MD). Tissue protein levels were estimated using the BCA (bicinchoninic acid) protein assay reagent (Pierce, Rockford, IL).

Binding data from each assay were analyzed separately. Data were normalized by expressing the average dpm at each competitor concentration as a percentage of total binding. These data were then subjected to nonlinear regression analysis using the algorithm for sigmoid curves in the curvefitting program InPlot (Graphpad, San Francisco, CA). This program generated IC_{50} values and a Hill coefficient for each curve. Analysis of the residuals indicated an excellent fit for curves from the present experiments; r values were above 0.99 in all cases.

2.4. Dopamine-sensitive adenylate cyclase

Putamen samples from four monkeys were assayed separately. Two or three independent experiments were conducted with tissue from each of these subjects (n = 9 total experiments conducted on separate days). For most experiments, tissue from rat striatum (n = 1 rat per assay; total eight rats) was run simultaneously with the monkey samples to allow a controlled comparison of drug efficacy in rat vs. monkey. The automated HPLC (high pressure liquid chromatography) method of Schulz and Mailman (1984) was used to measure adenylate cyclase activity by separating cAMP from other labeled nucleotides. Briefly, striatal tissue from rat or monkey (ca. 50 mg) was homogenized with eight manual strokes in a Wheaton-Teflon glass homogenizer in 2.5 mM HEPES buffer (pH 7.5) containing 2 mM EGTA (50 ml/g tissue) Following the addition and mixing of 50 ml/g of 50 mM HEPES buffer (pH 7.5) containing 2 mM EGTA, a 20 μl aliquot of this tissue homogenate was added to a prepared reaction mixture (final volume of 100 μl) containing 0.5 mM ATP, 0.5 mM isobutyl methylxanthine, [^{32}P]ATP (0.5 μCi), 1 mM cAMP, 2 mM $MgCl_2$, 100 mM HEPES buffer, 2 μM GTP, 0–100 μM dopamine, dihydrexidine, or SKF38393, 10 mM phosphocreatine and 5 U creatine phosphokinase. Triplicate determinations were performed for each drug concentration. The reaction proceeded for 15 min at 30°C and was terminated by the addition of 100 μl of 3% sodium dodecyl sulfate (SDS). Proteins and much of the non-cyclic nucleotides were precipitated by addition of 300 μl each of 4.5% $ZnSO_4$ and 10% $Ba(OH)_2$. Samples were centrifuged

(10,000 × *g* for 8–9 min), and the supernatants injected on an HPLC system (Waters Z-module or RCM 8 × 10 module equipped with a C18, 10 μm cartridge). The mobile phase was 150 mM sodium acetate (pH 5.0) with 23% methanol. A UV detector (254 nm detection) was used to quantify the unlabeled cAMP added to the samples to serve as internal standard. The radioactivity in each fraction was determined by a flow-through radiation detector (Inus Systems, Tampa, FL) using Cerenkov counting. Sample recovery was based on UV measurement of total unlabeled cAMP peak areas quantified using PE Nelson (Cupertino, CA) Model 900 data collection modules and TurboChrom software. Tissue protein levels were estimated using the BCA protein assay reagent (Pierce, Rockford, IL).

Data obtained were expressed as pmol cAMP/mg protein/min. To accommodate any differences in the absolute levels of both basal and stimulated levels of cAMP between rat and monkey, each individual dose-response curve was normalized. Specifically, basal levels of cAMP synthesis were subtracted from the average values obtained at each drug concentration, and this value was then expressed as a percent of the stimulation produced by 100 μM dopamine. Data from multiple experiments conducted with tissue from the same monkey were then averaged to yield a single set of dose-response curves for each of four monkeys. Corresponding dose-response curves for rat tissue run simultaneously with each monkey were generated. The limited number of drug concentrations used and the variability of the data precluded the use of formal statistical methods for determination of EC_{50} values in these experiments.

3. Results

3.1. Radioreceptor binding assays

As can be seen in fig. 1 and table 1, dihydrexidine had high affinity for D_1 receptors in the monkey putamen (IC_{50} = 20 nM). The affinity of dihydrexidine for D_1 receptors was similar in rat and monkey brain. Furthermore, in both species, dihydrexidine affinity was 3–5-fold higher than that of the partial agonist SKF38393. The Hill coefficients for dihydrexidine and SKF38393 were less than unity in all cases.

3.2. Adenylate cyclase stimulation

Both basal and dopamine-stimulated levels of cAMP synthesis showed greater between-subject variation in monkeys compared to rats. The means and standard errors of basal levels (expressed as pmol/mg per min cAMP) were 70.2 ± 14.6 in monkey compared to 74.9 ± 9.3 in rat. The corresponding absolute values for stimulation produced by 100 μM dopamine were both more variable and somewhat lower in monkeys (mean ± S.E.M. = 146.3 ± 26.1 pmol/mg per min cAMP in monkey putamen vs. 201.9 ± 15.3 in rat striatum).

For ease of comparison across species, normalized dose-response curves (expressed as percent stimulation produced by 100 μM dopamine) for dihydrexidine and

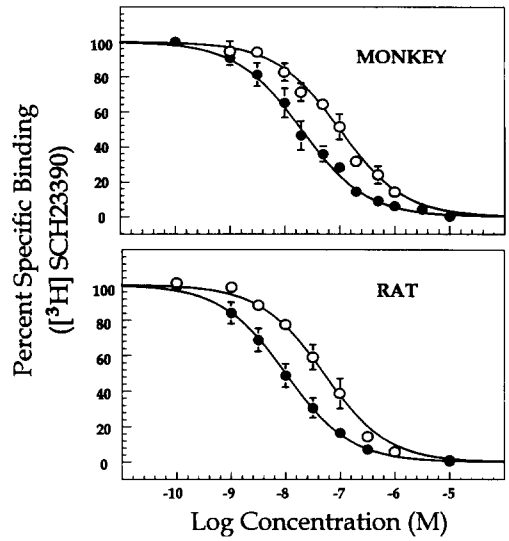

Fig. 1. D_1 receptor binding affinity of dihydrexidine (●) and SKF38393 (○) in rat and monkey striatum. Dihydrexidine bound with similar high affinity to dopamine D_1 receptors in monkey (top panel) and rat (bottom panel) striatum. The affinity of the prototypical D_1 partial agonist SKF38393 was 3–5-fold lower than dihydrexidine in both rat and monkey. Dopamine D_1 receptor binding potency was determined in homogenates of caudate/putamen (rat) or putamen (monkey), using 0.3 nM [^{3}H]SCH23390 as the radioligand. Data represent means and standard errors of competition curves generated in three separate experiments with tissue from monkeys (n = 3 monkeys) or rats (n = 6 rats). Parameter estimates (IC_{50} values and Hill coefficients) for these competition curves are presented in table 1. In some cases, the standard error bars fall within the size of the symbol and are not shown.

TABLE 1

Dopamine D_1 receptor binding affinity of dihydrexidine and SKF38393 in rat and monkey striatum.

Dopamine D_1 receptor binding affinity was determined in homogenates of caudate/putamen (rat) or putamen (monkey), using 0.3 nM [^{3}H]SCH23390 as the radioligand. Data represent means and standard errors of competition curves generated in three separate experiments with tissue from monkeys (n = 3 monkeys) or rats (n = 6 rats). Competition curves are shown in fig. 1. Parameter estimates were obtained from nonlinear regression analysis using an algorithm for sigmoid curves in the curvefitting program InPlot.

Drug	IC_{50} (nM)		n_H	
	Rat	Monkey	Rat	Monkey
Dihydrexidine	9.7± 3.2	20.2± 3.3	−0.70±0.02	−0.72±0.09
SKF38393	41.4±18.9	106.2±35.7	−0.79±0.05	−0.67±0.04
SCH23390	0.8± 0.1	1.3± 0.2	−1.2 ±0.1	−0.9 ±0.10

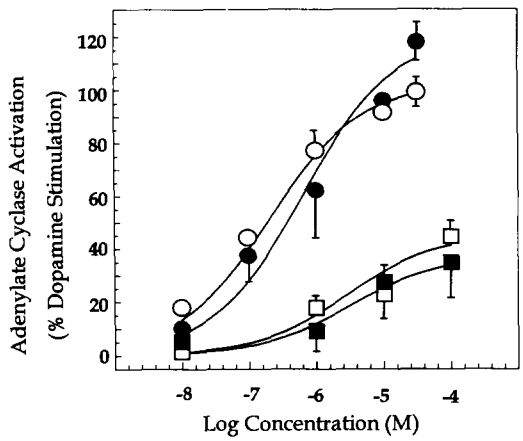

Fig. 2. Dopamine D_1 receptor-mediated adenylate cyclase stimulation by SKF38393 in rat (□) and monkey (■) and dihydrexidine in rat (○) and monkey (●) striatum. Dihydrexidine is a full efficacy agonist in rat and monkey striatum, while SKF38393 is a partial agonist in both species. The potency and efficacy of the two agonists do not appear to differ markedly between species. Adenylate cyclase activation was estimated by assaying the amount of cAMP accumulated in striatal homogenates (pmol/mg protein per min) in the presence or absence of dihydrexidine or SKF38393. Under these conditions, the maximal increase induced by dihydrexidine (30 μM) was at least 2-fold compared to baseline levels. The means and standard errors of basal levels (expressed as pmol/mg per min cAMP) were 70.2 ± 14.6 in monkey compared to 74.9 ± 9.3 in rat. The corresponding absolute values for stimulation produced by 100 μM dopamine were 146.3 ± 26.1 pmol/mg protein per min cAMP in monkey putamen vs. 201.9 ± 15.3 in rat striatum. Data for rat and monkey were normalized separately by subtracting basal levels and expressing the resulting value as a percent of the stimulation induced by 100 μM dopamine. The dose-response curves shown represent means and standard errors of data collected from four monkeys and eight rats in nine independent experiments. In some cases, the standard error bars fall within the size of the symbol and are not shown.

SKF38393 were generated for each monkey and for corresponding rat tissue run simultaneously. The averages and standard errors of these individual dose-response curves for rat vs. monkey are shown in fig. 2. Both dihydrexidine and SKF38393 produced dose-dependent increases in cAMP production in rat and monkey. In both species, dihydrexidine was a full efficacy agonist, inducing cAMP synthesis comparable to that of the endogenous transmitter dopamine.

In contrast, SKF38393 was a partial agonist in both cases, inducing less than 50% stimulation relative to dopamine. Although formal statistical methods were not applied to determine EC_{50} values for these data, visual inspection of the curves clearly indicates that dihydrexidine was a high potency agonist in both species (EC_{50} of 100–300 nM). While the low efficacy of SKF38393 makes it more difficult to estimate accurately the potency of this compound, it is clear that neither the potency nor efficacy of SKF38393 varied markedly between species. We did, however, detect increased between-subject variation in the response to SKF38393. Specifically, we failed to detect consistent dose-dependent stimulation by SKF38393 in tissue from one of four monkeys, while each of the remaining three monkeys (and all eight rats tested) demonstrated consistent stimulation at the higher doses of SKF38393. (Data from the non-responder were included in the average dose-response curves depicted in fig. 2.) Interestingly, dihydrexidine produced robust stimulation in all four monkeys; in each case, inducing stimulation equivalent to that of 100 μM dopamine.

4. Discussion

Dihydrexidine is one member of a novel structural class of dopaminergic ligands, the hexahydrobenzo [a]phenanthridines. The design of dihydrexidine and related analogs is part of our continuing efforts to develop novel ligands selective for the dopamine D_1 receptor subtype. One major impetus for our efforts is the potential relevance of such compounds to an understanding of the role of dopamine D_1 receptors in human neuropathology, including various movement disorders. We have suggested previously that a potent full efficacy dopamine D_1 receptor agonist such as dihydrexidine may prove beneficial in the treatment of idiopathic Parkinson's disease (Lovenberg et al., 1989; Brewster et al., 1990). The dramatic effects of dihydrexidine in the MPTP primate model have supported this prediction, as have more recent studies describing the anti-parkinsonian efficacy of the full dopamine D_1 receptor agonist A77636 (Kebabian et al., 1992). The importance of these findings is substantial inasmuch as the dopamine D_2 receptor had been considered the primary target of dopamine replacement therapy (e.g., by levodopa or D_2 agonists) in Parkinson's disease.

Our initial reports describing the pharmacological characteristics of dihydrexidine at dopamine D_1 receptors were based on experiments conducted in rodents (Brewster et al., 1990; Lovenberg et al., 1989; Darney et al., 1991; Mottola et al., 1992). The possible clinical application(s) of full dopamine D_1 receptor agonists such as dihydrexidine made it essential to extend our studies to include primate tissue. The present results suggest that the effects of dihydrexidine do not vary significantly between rodents vs. non-human primates. That is, dihydrexidine retains it affinity, potency, and efficacy at dopamine D_1 receptors in rhesus macaque brain. At present, it is not clear how these properties of dihydrexidine relate to the different molecular forms of 'D_1-like' receptors. Experiments in clonal cell lines are now underway to answer this question (Watts et al., 1992).

Our results indicate a similarity between the structural requirements for ligand binding and activation of

the dopamine D_1 receptor in rat and primate. This aspect of our data is in accord with several other findings. First, there is a high degree of amino acid sequence homology between the dopamine D_{1A} receptor recently cloned in rat, human, and monkey (Dearry et al., 1990; Monsma et al., 1990; Zhou et al., 1990; Machida et al., 1992); this molecular form is thought to be the major D_1 subtype present in the caudate and putamen. Second, the binding affinities reported for D_1 ligands in previous studies conducted with primates correlate well with those obtained in rat brain, although dopamine D_1 receptor density is 2- to 4-fold lower in primate caudate and putamen compared to rat (O'Boyle and Waddington, 1987; Faedda et al., 1989; Madras et al., 1988). Finally, studies in both human and nonhuman primates have demonstrated that dopamine D_1 receptor stimulation is linked to increases in the activity of the effector enzyme adenylate cyclase (e.g., Pifl et al., 1992); this relation has been well-documented in rats (e.g., Andersen and Jansen, 1990).

Despite the evidence for a strong correspondence between dopamine D_1 rat and primate receptors, a recent study by Pifl et al. (1991) reported a large difference in the efficacy of the partial dopamine D_1 receptor agonist SKF38393 in rat vs. monkey brain. In their studies, Pifl et al. (1991) noted SKF38393 produced ca. 40% stimulation (relative to dopamine) in rat brain membranes, but had 3-fold lower efficacy in membranes from rhesus monkeys. This finding of interspecies differences in efficacy of SKF38393 (Pifl et al., 1991) might possibly be explained by the presence of a smaller receptor reserve at dopamine D_1 receptors in primates compared to rats. This idea would be consistent both with the reduced number of dopamine D_1 receptors in primate vs. rat brain, and also with the difference in motor activation produced by this compound in dopamine denervation models in rats vs. primates (cf. Setler et al., 1978; Arnt, 1985; Rouillard and Bedard, 1988; Barone et al., 1987; Bedard and Boucher, 1989; Boyce et al., 1990; Close et al., 1985; Nomoto et al., 1988).

Although invoking a difference in spare receptors in rat vs. monkey striatum may have some heuristic value, the present results are at odds with this notion. We found no appreciable differences in the potency or the maximal response obtained with either dihydrexidine or SKF38393 between the two species. Given the fact that the affinity of these compounds for the dopamine D_1 receptor did not vary markedly with species, the presence of fewer spare receptors in primate brain should have decreased the maximal efficacy of the partial agonist SKF38393. Fewer spare receptors should also result in large rightward shifts in the potency of a higher efficacy agonist such as dihydrexidine (Ariëns et al., 1960). Thus, the present data do not support the idea that there is a greater receptor reserve in rodents versus primates.

We did find increased individual differences in the response to SKF38393 in monkey tissue compared to rat, yet such individual differences were not correlated with differences in either basal or dopamine-stimulated absolute levels of cAMP. Furthermore, in contrast to the variable response to SKF38393, the response to dihydrexidine was consistent and robust in all monkeys (and all rats tested). At present, the reason(s) for the discrepancy between the present study and that of Pifl et al. (1991) is unclear. It is of note, however, that neither their study nor the present one found appreciable differences between species in the potency of the full agonists dopamine (Pifl study) and dihydrexidine (present study). Thus, the discrepancy is restricted to SKF38393. It is possible that the intersubject variability we note is a wide-ranging phenomenon in primate brain, and therefore may be the reason for the discrepant results of the two studies. If so, then some unidentified subject factor(s) may be important in determining primate response to low intrinsic efficacy agonists such as SKF38393.

Notwithstanding the uncertainty concerning the degree of relative efficacy of SKF38393 in rat vs. monkey, it is clear that SKF38393 is a partial agonist in both species. In contrast, dihydrexidine binds with 3–4-fold greater affinity to dopamine D_1 receptors than does SKF38393, and was a full agonist in these studies, as in all earlier ones (Lovenberg et al., 1989, 1991; Brewster et al., 1990; Mottola et al., 1992). The large differences in biochemical efficacy of dihydrexidine and SKF38393 correlate with the dramatic antiparkinsonian actions of dihydrexidine, and the lack of effect of SKF38393, in the MPTP primate model (Taylor et al., 1991a; Nomoto et al., 1988; Close et al., 1985; Boyce et al., 1990). These findings, together with the recent report of marked beneficial effects of the isochroman derivative A77636 in the MPTP model (Kebabian et al., 1992) suggest that full D_1 efficacy is required for eliciting antiparkinsonian effects. One apparent contradiction comes from the recent report evaluating the effects of the dopamine D_1 receptor agonist SKF82958. This phenyltetrahydrobenzazepine is a full agonist in rat brain (O'Boyle et al., 1989), yet caused only weak motor activation in MPTP-treated primates (Rupniak et al., 1992). It should be noted, however, that the pharmacology of SKF82958 has some unusual properties. Mottola et al. (1992) have noted that the functional dose-response curve for stimulating adenylate cyclase (at least in rat striatal membranes) is very shallow, with full activation not occurring until concentrations of 100 μM were reached (vs. maximal effects occurring at 10-fold lower concentrations of dihydrexidine). Thus, although O'Boyle et al. (1989) have termed SKF82958 a full agonist, it may not actually be so in a

classical sense. In any event, the differences in antiparkinsonian efficacy of dopamine D_1 receptor agonists from three different structural classes may be an important mechanistic clue. Future studies which include direct biochemical and behavioral comparisons of dihydrexidine, A77636, and SKF82958 (and other novel dopamine D_1 receptor agonists that are developed) are essential, and should be instrumental in refining the requirements for clinical efficacy.

One aspect of the pharmacology of dihydrexidine should be noted. While this drug is very selective for dopamine receptors, it has only 10-fold selectivity for dopamine D_1 vs. D_2 receptors (Mottola et al., 1992). Thus, its affinity for dopamine D_2 receptors is similar to that of the prototypical D_2 agonist quinpirole (IC_{50} of ca. 100 nM vs. [^{3}H]spiperone). At present, we do not know whether dihydrexidine has selectivity for any of the molecular forms of 'D_2-like' receptors. Two obvious questions arise from these data. The first concerns the possible effect of the D_2 properties of dihydrexidine on the measurement of adenylate cyclase in the present study. In intact tissue preparations (striatal slice superfusion), we have, in fact, demonstrated an inhibitory component of dihydrexidine on cAMP efflux when high dihydrexidine concentrations are used (much like occurs for dopamine; Stoof and Kebabian, 1982). This effect appears to be mediated via dopamine D_2 receptors, suggesting that dihydrexidine behaves as a D_2 agonist (Mottola et al., 1992). In contrast, we have been unable to demonstrate the D_2 effects of dihydrexidine (or of any other D_2 agonists or antagonists) when adenylate cyclase stimulation is measured in homogenate preparations, as in the present experiment (Cook et al., 1991). Thus, the D_2 properties of dihydrexidine are unimportant in these biochemical studies. A second issue is whether the antiparkinsonian effects of dihydrexidine require its D_2 agonist properties. Preliminary studies conducted in MPTP-treated monkeys indicate that the therapeutic benefits of dihydrexidine (Taylor et al., 1991a) are derived from its actions at dopamine D_1 receptors, since they are not blocked when dihydrexidine is combined with the D_2 antagonist remoxipride (Taylor et al., 1991b). Thus, we believe that the D_2 properties of dihydrexidine neither confound interpretation of the present results, nor are of importance to the antiparkinsonian effects of the drug in MPTP monkeys.

In summary, dihydrexidine binds to dopamine D_1 receptors in primate brain with high affinity, and is a potent and full efficacy agonist. These results, in conjunction with our studies in MPTP-treated monkeys (Taylor et al., 1991a), suggest that dihydrexidine and similar compounds may have great promise as novel therapeutic agents for the treatment of idiopathic Parkinson's disease or other forms of parkinsonism, and underscore the importance of dopamine D_1 receptors in the maintenance of normal motor function. Furthermore, the present results demonstrate a similarity between the recognition characteristics and activational requirements of rat and primate dopamine D_1 receptors, enhancing the usefulness of dopamine D_1 receptor models based on data derived from rodent tissue.

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