Trihexyphenidyl Interactions with the Dopamine D$_1$-Selective Receptor Agonist SKF-82958 and the D$_2$-Selective Receptor Agonist N-0923 in 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-Induced Hemiparkinsonian Monkeys$^1$

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ABSTRACT
The effects of the antiparkinsonian agent trihexyphenidyl, a selective M$_1$ muscarinic cholinergic receptor antagonist, were studied in doses of 100, 320 and 1000 mg/kg i.m. alone. Trihexyphenidyl was then studied in combination with the selective dopamine receptor D$_1$ agonist SKF-82958 [(+)-6-chloro-7-8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-benzazepine hydrobromide] and the selective D$_2$ agonist N-0923 [(−)-2-(N-propyl-N-2-thienylethyl)amino-5-hydroxytetralin HCl] on rotational behavior in five 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned hemiparkinsonian monkeys. Given alone, trihexyphenidyl had no effect on ipsiversive and slightly enhanced contraversive circling. Contraversive circling produced by 74.8 and 234 mg/kg SKF-82958 i.m. was potentiated by increasing doses of trihexyphenidyl. On the other hand, contraversive circling produced by 10 and 32 mg/kg N-0923 i.m. was progressively reduced with increasing doses of trihexyphenidyl. The results obtained indicate differential actions on circling behavior between a selective M$_1$ muscarinic cholinergic receptor antagonist and selective D$_1$ and D$_2$ receptor agonists in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine monkey model of hemiparkinsonism.

Animal models of Parkinson’s disease are widely used to screen potential drugs or to better understand the complex interactions of multiple neurotransmitter systems in extrapyramidal motor function. The unilateral 6-OHDA rodent and the unilateral MPTP primate models both use circling behavior as behavioral endpoints. Ungerstedt (1974) reported that indirectly acting DA receptor agonists such as amphetamine cause unilateral lesioned rodents to circle to the side of the lesion (ipsiversive), and directly acting DA agonists cause rodents to circle opposite to the side of the lesion (contraversive). Pycock et al. (1978) studied the interaction of tertiary and quaternary cholinergic agonists and antagonists on circling behavior of unilateral 6-OHDA-lesioned mice with the DA agonists amphetamine and apomorphine. Given alone, the mAChR antagonists benztpine and scopolamine increased ipsiversive circling, whereas the mAChR agonists areoline, physostigmine and pilocarpine had no effect on circling behavior. Benztpine and scopolamine significantly potentiated ipsiversive circling induced by amphetamine but had no consistent effect on apomorphine-induced contraversive circling. All three mAChR agonists reduced circling induced by amphetamine and apomorphine. In addition, peripherally acting quaternary methscopolamine and neostigmine had no significant effects, whereas α-methyl-para-tyrosine and haloperidol abolished amphetamine- and scopolamine-induced ipsiversive circling.

Trihexyphenidyl is a selective M$_1$ mAChR antagonist (Giauchetti et al., 1986; Tien and Wallace, 1985). Recently, trihexyphenidyl has been shown to potentiate the effects of L-DOPA methyl ester in MPTP-induced hemiparkinsonian monkeys.$^2$ Inasmuch as this precursor of DA affects receptors D$_1$ through D$_5$, it was of interest to determine whether trihexyphenidyl had a more selective potentiation of D$_1$ vs. D$_2$ predominant postsynaptic receptor agonists. The hypothesis to be tested was that trihexyphenidyl would be synergistic (additive or potentiating) with both classes of DA agonists stud-

ABBREVIATIONS: 6-OHDA, 6-hydroxydopamine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; i.m., intramuscularly; DA, dopamine; mAChR, muscarinic cholinergic receptor; NMDA, N-methyl-d-aspartate; ACh, acetylcholine.

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2 E. F. Domino, unpublished observations.
The selective D2 agonist N-0923 (Belluzzi et al., 1986; Belluzzi et al., 1994; Domino et al., 1997; Domino and Sheng, 1993a, 1993b) was determined for 120 min after injection of the drug combinations. The data obtained indicated this was true only of the D1 predominant receptor agonist.

Materials and Methods

Animals. Five adult female Macaca nemestrina (pig-tailed Macaque) monkeys were studied. Details of their care, induction of MPTP-induced hemiparkinsonism and behavioral observations have been described in detail (Bankiewicz et al., 1986; Belluzzi et al., 1994; Domino et al., 1997; Domino and Sheng, 1993a, 1993b).

Drugs. All agents were reagent, medical or veterinary grade as available from commercial sources. Trihexyphenidyl hydrochloride was obtained from Sigma Chemical (St. Louis, MO). The selective D1 agonist SKF-82958 (Weinstock et al., 1980) initially was obtained from Dr. J. Weinstock (SmithKline Beecham Pharmaceuticals, King of Prussia, PA) and later from Research Biochemicals (Natick, MA). The selective D2 agonist N-0923 (Belluzzi et al., 1994) was obtained from Dr. D. McAfee (Discovery Therapeutics, Richmond, VA). Each drug solution was prepared fresh at 8:00 a.m. on the experimental day.

Drug administration. For convenience, all five monkeys received the same treatment on the same day. Control vehicle treatments were randomly interspersed throughout to ensure base-line stability over the duration of the study. All drugs were given intramuscularly in logarithmic doses. The same volume of vehicle [5% glucose in H2O (D5W) or 0.9% NaCl] was injected intramuscularly as a control. All five animals were usually treated once a week over a period of 6 months. Animals were run in the morning beginning at 8:30 a.m. with control vehicle for 30 min beginning at 8:30 a.m. at time 0, either control vehicle or trihexyphenidyl was given intramuscularly, and the animals were run for an additional 30 min. At 20 min, the DA agonist was given, and the animals were run for an additional 2.5-hr period. Before this study, the animals were drug free for 1 month.

Statistical analysis. The experiments were based on a repeated-measures design. The same group of monkeys was used in all experiments. The data were subjected to statistical analysis for active drug vs. vehicle or active drug alone vs. combination. Measurement of monkey circling behavior was based on a ratio scale of parametric data. The data were analyzed using separate one-way analysis of variance with repeated measures (InStat 2.0 for Macintosh, 1993) followed by Tukey’s multiple-comparison procedure when a significant F ratio was obtained. An α level (P value) of .05 was used as the level of significance for all of the statistical tests. Mean contraversive and ipsiversive circling to the side of the MPTP-induced brain lesion was determined for 120 min after injection of the drug combinations.

Measure of base-line stability. Base-line symptoms are known to vary with time after MPTP treatment. Therefore, each animal’s responses to vehicle control injections were made randomly before, during and after the study. The mean contraversive circling ± S.E.M. for a 2.5-hr period was 3.9 ± 6.9 and mean ipsiversive circling was 35.6 ± 14.0. There were no significant differences over the duration of the study. As expected, mean ipsiversive circling was greater than mean contraversive circling.

Effects of trihexyphenidyl. Trihexyphenidyl was given to all five lesioned monkeys in random increasing logarithmic doses of 100, 320 and 1000 μg/kg i.m. The animals were first given a dose of 5% D5W at −30 min. Trihexyphenidyl was given at time 0 and repeated three times on different weeks of the same month to determine reproducibility of the effect. After D5W alone, there was a tendency for more ipsiversive than contraversive circling, although shortly after the intramuscular injection, some animals showed transient contraversive circling. Trihexyphenidyl reduced ipsiversive circling in a dose-related manner and only slightly increased contraversive circling at a dose of 320 μg/kg i.m. (fig. 1). This effect lasted <1 hr. These effects were quite reproducible in the three different experiments.

Effects of trihexyphenidyl in combination with the D1 agonist SKF-82958. Moderate doses of SKF-82958 of 74.8 and 234 μg/kg i.m. were combined with increasing doses of trihexyphenidyl on different weeks using the injection schedule of D5W at −30 min, trihexyphenidyl at time 0 and SKF-82958 at +30 min. The dose-effect relationships are also shown in figure 1. Doses of 74.8 and 234 μg/kg SKF-82958 i.m., in combination with increasing doses of trihexyphenidyl, showed enhanced effects (F[3,72] = 11.008, P < .0001).

Effects of trihexyphenidyl in combination with the D2 agonist N-0923. Moderate doses of N-0923 of 10 and 32 μg/kg i.m. were combined with increasing doses of trihexyphenidyl using a similar schedule to that described above. The dose-effect relationships are shown in figure 2. Trihexyphenidyl in increasing doses significantly reduced the effects of N-0923 on contraversive circling behavior with no significant effect on ipsiversive circling. The reductions were

![Fig. 1. Trihexyphenidyl enhances the effects of the selective D1 receptor agonist SKF-82958 on circling behavior in MPTP-induced chronic hemiparkinsonian monkeys. The dose of trihexyphenidyl (in μg/kg i.m.) is shown on the x axis, and mean ± S.E.M. rotation behavior per 120 min for ipsiversive (ip) and contraversive (co) behavior is shown on the y axis. Trihexyphenidyl alone only slightly enhanced contraversive behavior in a dose of 320 μg/kg. In combination with SKF-82958 in various doses, it significantly enhanced contraversive circling as a potentiating effect. *P < .05 and ***P < .001.](image-url)
greater with 10 µg/kg [F(3,72) = 14.974, P < .0001] and less with 32 µg/kg [F(3,72) = 3.0848, P < .05] N-0923 i.m. These effects were so surprising that the study was replicated after 1 month with similar results. A large dose of 1000 µg/kg i.m. trihexyphenidyl was combined with varying doses of N-0923. Trihexyphenidyl reduced the effects of a small dose of N-0923 more than a larger one (fig. 3).

Discussion

The present study using MPTP-induced chronic hemiparkinsonian monkeys confirms previous reports in both rodents and monkeys that unilateral nigrostriatal DA-lesioned animals show predominant ipsiversive circling. Trihexyphenidyl, especially in large doses, reduced ipsiversive circling. As expected, both D₁- and D₂-selective agonists induce contraversive but not ipsiversive circling. Suitably DA-lesioned rodents and subhuman primates show similar gross behavioral motor effects. The differential effects of trihexyphenidyl with selective D₁ and D₂ agonists are reminiscent of the fact that in rats with a unilateral 6-OHDA lesion, NMDA receptor blockade by MK-801 (dizocilpine) potentiates D₁ but reduces D₂ actions (Engber et al., 1993; Morelli et al., 1992; Morelli and Di Chiara, 1990). Furthermore, Olney et al. (1987) showed that a number of antiparkinsonian drugs, including trihexyphenidyl, are NMDA antagonists. Could the results obtained in the present study be due to trihexyphenidyl acting as an NMDA antagonist? Probably not, because dizocilpine in MPTP-induced hemiparkinsonian monkeys reduces the effects of both selective D₁ and D₂ agonists contrary to its effects in unilateral 6-OHDA-lesioned rats (Domino and Sheng, 1993a). Another puzzling fact is that the effects of trihexyphenidyl in the present study with monkeys differ from those of both benzotropine and scopolamine alone and in combination with apomorphine, as described by Pycock et al. (1978) in unilaterally lesioned mice. Obviously, benzotropine alone and in combination should be studied in hemiparkinsonian monkeys. If an additional species difference exists, it is important from both a screening and neural mechanism point of view.

The interactions of dopaminergic and cholinergic neurotransmission in the striatum are still unclear. Even a cursory review of the literature indicates that in vitro, in vivo, normal...
and DA-deficient preparations from different species provide limited information on the complexities involved. Research by Bertorelli and Consolo (1990), Damsma et al. (1990), DeBoer and Abecrombie (1996), Lehman and Langer (1983), Scatton (1982), Stooft et al. (1979, 1992) and Stooft and Kubanin (1982) indicates that DA D2 receptors inhibit striatal ACh release. DA D1 receptors increase striatal ACh, as described by Consolo et al. (1987), Fage and Scatton (1986) and Login and Harrison (1996). The postsurgical interval (DeBoer et al., 1992), the degree of DA depletion, as well as the role of forebrain circuits influence D1 and D2 receptor modulation of striatal ACh release (Johnson and Bruno, 1995; Login et al., 1995; Robertson et al., 1992, 1993; Sato et al., 1994; Ueda et al., 1995). Koshikawa et al. (1996) found that stimulation of ACh or D1 or D2 receptors in nucleus accumbens altered striatal DA release, which correlated better with the latter than the former on contralateral circling behavior in rats. Damsma et al. (1991) reported that NMDA receptors are involved in D1 agonist striatal ACh release. In addition, Consolo et al. (1996) found that parafascicular thalamic nucleus projections onto NMDA receptors are critical in mediating D1 agonist release of striatal ACh. The complexities of striatal ACh release and circling behavior is further emphasized by the role of tachykinins acting via predominantly neurokinin NK2 receptors (Poncelet et al., 1996; Steinberg et al., 1995), serotonin via 5-hydroxytryptamine2 receptors (Ishida et al., 1996) and DA release via GABA heterocarrier activation (Fassio et al., 1996).

Perhaps the most important observation of the present research in hemiparkinsonian monkeys is that the overall evidence in rodents that DA D1-selective agonists increase and D2-selective agonists decrease striatal ACh can be used to form hypotheses for further research. Trihexyphenidyl potentiated the actions of the D1 agonist SKF-82958 and antagonized those of the D2 agonist N-0923 on contraversive circling behavior. Thus, antagonism of D1 agonist ACh release by an M1 postsynaptic antagonist potentiates but reduced release of ACh by a D2 agonist and postsynaptic M1 antagonism reduces circling behavior. Is this true of human hemiparkinsonian patients? Neurologists have not reported that trihexyphenidyl antagonizes the effects of levodopa or other directly acting DA agonists. In fact, our data in preparation for separate publication indicate that trihexyphenidyl potentiates the effects of L-DOPA methyl ester. Therefore, it follows that the effects of a generalized activation of all DA receptors in combination with trihexyphenidyl produce a predominant D1 effect! In human parkinsonian patients, the addition of trihexyphenidyl to levodopa therapy does not seem to further reduce bradykinesia and rigidity but does reduce the tremor. Obviously, further clinical research is warranted, especially in hemiparkinsonian patients, to determine whether the data from the present MPTP monkey model of hemiparkinsonism is useful. Certainly, more detailed human studies using various DA agonists are indicated. A cautionary note should be made with regard to trihexyphenidyl and other antimuscarinic drugs in combination with selective D2 agonists in the clinic; such a combination may be detrimental, and the clinician and patient should proceed cautiously.

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