Effects of Pramipexole on Contraversive Rotation and Functional Motor Impairments in 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-Induced Chronic Hemiparkinsonian Monkeys

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ABSTRACT
Rotational and functional motor behavioral changes were studied in five MPTP lesioned chronic hemiparkinsonian Macaca nemestrina monkeys after i.m. pramipexole, a predominant D2 subfamily agonist. Pramipexole induced contraversive rotations in a dose-dependent manner with an optimal dose of 56 mg/kg for 2 to 4 hr after injection. Three different rating scales were used to determine drug-induced functional improvement. They included a monkey parkinsonism rating scale, volitional responses to fruit presentations, and number of hand movements that appeared volitional. A dose of 56 μg/kg of pramipexole produced functional improvements on hand disability, and on a parkinsonian rating scale for monkeys in a dose-dependent manner from 32 to 100 mg/kg. These doses produced an increase in significant hand movements in the affected (contralateral) as well as in the normal (ipsilateral) hand to the side of the brain lesion compared with 5% dextrose in water vehicle control. With a dose of 100 μg/kg, the therapeutic effects of pramipexole on hand movements were less than with 56 μg/kg, due to side effects such as scratching.

The Parkinson Study Group (1997) reported on the efficacy and safety of pramipexole (Mirapex) in the treatment of early Parkinson's disease. Its clinical pharmacology and pharmacokinetics were reported in phase I trials in humans before its use in parkinsonian patients (Peters et al. 1996a, 1996b; Wright et al., 1996a, 1996b). Pramipexole has high binding specificity as a full agonist for the D2 subfamily of dopamine receptors. It is somewhat more selective for D3 than for D2 or D4 receptors. In vitro it has negligible alpha-2 and 5-HT3 antagonistic activities (Mierau and Schingnitz, 1992; Hashimoto and Kuriyama, 1993; Georgie et al., 1995; Hoffmann et al., 1995; Piercey et al., 1995). Mierau and Schingnitz (1992) also showed that it was effective in treating MPTP-lesioned monkeys.

Although clinical emphasis has been placed on the therapeutic use of pramipexole as a monotherapy in early human parkinsonism (Shannon et al., 1997), and only as an adjunctive treatment with levodopa in late Parkinson's disease (Lieberman et al., 1997), it was of interest to determine its effects in chronic hemiparkinsonian monkeys who were lesioned with MPTP 6 to 8 years previously. The animals studied were unique in that they maintained their clinical signs of hemiparkinsonism over an extended period of time. A series of motor functional tests, including a clinical monkey parkinsonian scale (that had drug side effects incorporated in it), volitional responses to food presentations, obvious volitional ipsilateral and contralateral hand movements, and contraversive rotation to the side of the brain dopaminergic lesion, were used to determine the experimental therapeutic usefulness of pramipexole in this very rare chronic animal model. This manuscript describes the results obtained.

Methods
Some of the methods used in this study have been described previously by Domino and Sheng (1993a, 1993b), Domino et al. (1997, 1998) and Domino and Ni (1998). Animals and MPTP-induced hemiparkinsonism. Five adult female Macaca nemestrina (pig-tailed macaque) monkeys, ranging in...
weight from 5.8 to 9.2 kg at the time of this study, were initially obtained from the Charles River Co., Port Washington, NY.

The monkeys were placed in a sound-quiet, air-conditioned, certified animal room (31.5 x 11.3 x 8.0 feet) on a light/dark cycle with lights on from 7:00 a.m. to 7:00 p.m. Each animal was given an enriched environment consisting of ease of visualization of their colleagues, Kong toys, TV entertainment, etc. Food and water were given in the mornings except when a drug was tested, in which case the animals were fed in the afternoon. Monkey chow and water were available ad libitum until consumed. At the time of the present experiments, the animals had symptoms of hemiparkinsonism for 6 to 8 years that did not show any gross change over this period of time. During the intervening years, the animals were intermittently given various dopamine D1-like and D2-like agonists i.m., as well as levodopa/carbidopa orally in their fruit.

MPTP-induced hemiparkinsonism was produced by a modified method of Bankiewicz et al. (1986). MPTP was slowly infused unilaterally into one common carotid artery in a total dose of ~0.6 mg/kg. This was done 6 to 8 years earlier. During the intervening period of years, the lesioned animals were studied with a large variety of antiparkinsonian agents. The animals were completely drug free for at least 1 month before the present study.

Medication. Pramipexole (S)-2-amino-4,5,6,7-tetrahydro-6-propylamino-benzothiazole dihydrochloride monohydrate, SN 919 Cl2Y was obtained from Boehringer Ingelheim KG (Ingelheim, Germany). D5W was purchased from Abbott Laboratories (North Chicago, IL).

Drug administration. Pramipexole was given i.m. in logarithmic doses to all five monkeys. The drug was given on the same day each week, usually Thursday A.M. If the drug was given more than once in the same dose, mean data were used for analysis. The same volume of vehicle (D5W) was injected i.m. as the control.

Rating of parkinsonism in monkeys. A modified Parkinson's disease rating scale (PD scale) was used to quantify the overall status of the MPTP lesioned monkeys before and 10, 60, 120, 180 and 240 min after drug administration. This scale includes any drug side effects. Use of such a scale allows comparability of data from different centers using similar lesion paradigms. Each monkey was videotaped in its own cage and rated from the videotape recordings by a trained researcher blind to the drug dose. The scale consisted of ratings of one point each for parkinsonian features (tremor, posture, gait, bradykinesia, balance, gross motor skills, defense reaction, and freezing), three drug-related side effects (dyskinesia, psychological disturbance, and vomiting) and overall level of activity. Higher scores indicate a greater degree of disability.

Volitional responses to fruit presentation. Volitional responses to presentation of pieces of fruit were scored in overnight fasted monkeys. Two pieces of food were offered sequentially; the second piece was presented when the first piece had been taken by one hand and brought to the animal's mouth. In normal monkeys, the second piece of food was always taken with the other hand. In hemiparkinsonian monkeys, however, the first piece was held in the mouth while the same hand that was used initially was again used to obtain the second piece of food; the rigid and tremorous limb contralateral to the MPTP infusion side usually remained unused. The ability to use the "parkinsonian arm" to obtain a second piece of food was used as an index of improvement in volitional motor function. Monkeys were scored by a trained researcher according to the following rating scale: 0, normal use; 1, can grasp small objects, independent use; 2, can grasp only big objects without assistance of intact arm, tremor present; 3, can grasp large objects but only with assistance of the intact limb; 4, arm use only when intact arm used; 5, unable to use affected limb. A rating of less than 3 indicates effective arm use.

Scoring each significant hand and arm movement. The entire experiment was videotaped without the presence of the investigators. All hand and arm movements counted the same, 1 point each. The total score for 5 min was the sum of the points for each hand's movements as described: 1) Grasps the cage with the hand. 2) Supports the body with the hand. 3) Grooms with the hand. 4) Grasps and holds food with the hand. 5) When the monkey circles, it will use the hand to assist itself. Sometimes the monkey will use the hand several times during a circling movement. 6) The hand will be moved in what appears to be voluntary motion even in air. The following hand movements were very difficult to score: 1) Grooming incessantly with the same hand. 2) Holding food to eat and moving the hand incessantly. 3) Grabbing and shaking the cage incessantly. 4) Playing with and touching an object incessantly.

All hand movements were scored in real time from the videotapes. When the observed hand was transiently behind the monkey and could not be seen, hand movements were not scored. If the last four movements continued for more than 2 sec, they were scored as one movement. The sum of hand movements per each 5-min period began at ~30 min with the drug vehicle injection, and at 0 min when the actual drug was administered. Hand movements were scored for a minimum of 120 min.

Observations of circling behavior. Each monkey was placed in a standard primate cage modified with a clear plexiglas front for viewing and recording free moving behavior. The upper cages were illuminated with 34 watt fluorescent ceiling lights. The lower cages were illuminated with 20-W fluorescent lights. Gross animal behaviors were observed via three separate video color cameras. The behaviors of two monkeys in separate cages were recorded with one camera. Thus, a total of three video cameras with zoom autofocus were used. All three cameras were connected electrically to their own videocassette recorders. Time and date of each camera were synchronized. The animal was video recorded in its own home cage without human presence for 30 min after vehicle injection, and for an additional 2 hr or more, as necessary, after drug or vehicle injection. The videotapes were scored by persons blind to the purpose of the study for ipsiversive (ipsi) and contraversive (contra) circling to the side of the brain lesion. The number and direction of complete 360-degree turns during each consecutive 5-min period were counted and recorded. The total number of complete contraversive turns in 2 hr after drug administration was computed. This time period was used because all of the drugs studied were relatively short acting. The coefficient of reliability r for two interraters was >0.9.

Statistical analysis. Correlation coefficients, Dunnett multiple comparison tests, and Student's t tests were run using the Statview program (Abacus Concepts, Berkeley, CA) and on a Macintosh SE30 computer (Apple Computer, Cupertino, CA). One-way ANOVAs with repeated measures were run using Instat 2.0 followed by the Tukey multiple comparison procedure when a significant F ratio was obtained. An alpha level of .05 was used for all statistical tests.

Results

Effects of pramipexole using a rating scale of monkey parkinsonism. The mean effects of increasing doses of pramipexole on a rating scale of parkinsonism in monkeys are shown in figure 1. The data before and 10, 60, 120, 180 and 240 min after i.m. injection are illustrated in bar graph form. Five percent dextrose in water vehicle control (dose 0) had no effect. A dose of 10 µg/kg i.m. pramipexole had a tendency to reduce parkinsonian signs, but these were very small effects. Doses of 32 and 100 µg/kg of pramipexole improved the clinical signs of parkinsonism to a small degree. The duration of effects of 100 µg/kg i.m. pramipexole on this rating scale was at least 240 min. One-way ANOVA with repeated measures was done for all five animals for the doses of pramipexole of 0, 10, 32 and 100 µg/kg. Before pramipexole [F(3,12) = .52, P = .67] there was no significant change. After pramipexole at 10 min [F(3,12) = 5.14, P = .016], 60 min [F(3,12) = 8.32, P = .0029] and 120 min [F(3,12) = 13.01, P =
there were statistically significant reductions of parkinsonian signs. Thus, the vehicle alone produced no significant change but there was a progressive improvement that was highly significant with doses of 32 to 100 \( \mu \text{g/kg} \).

**Effects of pramipexole on volitional responses to fruit.** Pramipexole did not significantly improve volitional responses of the affected hand to fruit presentations. The animals were scored before and 10, 60 and 120 min after D5W vehicle control, as well as after 10, 32 and 100 \( \mu \text{g/kg} \) i.m. pramipexole given at weekly intervals (data not shown). The rating scores were all \( \sim 5.1 \) and were not statistically significantly different.

**Effects of pramipexole on significant hand movements.** As illustrated in figure 2, pramipexole produced significant movements of both the normal (ipsilateral) and the affected (contralateral) hand. With a dose of 56 \( \mu \text{g/kg} \) of pramipexole, there was a maximal increase in hand movements. Smaller doses of pramipexole were less effective. A dose of 100 \( \mu \text{g/kg} \) i.m. pramipexole induced scratching behavior.

**Relation of significant hand movements and circling behavior.** The dose-effect relationships between mean significant hand movements of both the normal and affected hand and the relationship to circling behavior over a 120-min period are illustrated in figure 3. This figure illustrates the mean data of control vehicle D5W and three doses of pramipexole. The data after 10 \( \mu \text{g/kg} \) did not differ significantly from the control vehicle and, therefore, were not included. Note that the 100 \( \mu \text{g/kg} \) i.m. dose had a faster onset and longer duration of action than 56 \( \mu \text{g/kg} \), but the latter dose produced a greater peak effect. Both normal (ipsilateral) and affected (contralateral) hand movements increased. The time course of these effects was similar to the effects on contraversive circling to the side of the MPTP-induced dopaminergic lesion.
The present research indicates that pramipexole produces mild to modest improvement in parkinsonian symptoms and functional hand movements in MPTP unilaterally lesioned chronic monkeys. Pramipexole produced a significant dose-effect relationship with an optimal dose of 56 μg/kg i.m. The clinical parkinsonism rating scale and the number of significant hand responses improved after increasing doses of pramipexole. Although pramipexole increased affected hand movements, the volitional response to food test was not sensitive enough to determine any effects.

Pramipexole increased movements of the normal hand similar to another antiparkinsonian drug, talipexole (Domino et al., 1997, 1998). When normalized, this effect was less dramatic than with the affected hand. These effects were related to the degree and duration of pramipexole-induced contraversive circling. The duration of action of i.m. pramipexole was >2 hr in these tests. Moreover, on the rating scale of parkinsonism in these monkeys the duration of action of pramipexole was 4 hr or more. This indicates that pramipexole would be therapeutically optimal in human parkinsonian patients if given several times per day, as is recommended in its package insert (three times/day). The major side effect of pramipexole was scratching of the skin (possibly hallucinations and formication) when given in large doses. Clinically, pramipexole should be given slowly to achieve an optimal dose and maintained at that level, also as described in its package insert.

One of the major issues to be addressed is whether the in vitro selectivity of pramipexole for D3 receptors is reflected in vivo as a better therapeutic agent in this MPTP monkey model of parkinsonism. The best drug comparisons in these animals are our related studies with talipexole, N-0923, SKF-82958, A-86929 and L-DOPA methyl ester (see Asin et al., 1997; Domino and Ni, 1998; Domino and Sheng, 1993a, 1993b; Domino et al., 1997, 1998). Selective D1 and D2 dopamine agonists and L-DOPA methyl ester produced similar improvements in this monkey model. Except for differences in effective doses and side effects, all of these agents are equally effective! Although these drugs differ in their biochemical mechanisms of action, the fact that they are all equally therapeutically effective offers hope that the clinical symptoms of parkinsonism can be relieved with agents affecting different molecular targets within the dopamine receptor family. In contrast, Konitsiotis et al. (1997) showed that PD 128,709a, a D3 preferring agonist, may give a better overall therapeutic profile in a similar monkey model, although it did not provide a better therapeutic index than the nonselective dopamine agonist apomorphine in levodopa-primed animals. Only further research will provide answers to this crucial issue.

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