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Rapid communication

Dihydrexidine, a full dopamine D_1 agonist, reduces MPTP-induced parkinsonism in monkeys

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The antiparkinsonian effects of dopamine agonists have largely been attributed to activation of D_2 dopamine receptors. Reports in human and non-human primates using SKF38393, the prototypical D_1 agonist, have failed to demonstrate significant antiparkinsonian effects (Barone et al., 1986), suggesting that D_1 receptors were not involved. Although SKF38393 is a potent D_1 agonist, it causes only half as much stimulation of cAMP synthesis as dopamine (a biochemical function distinguishing D_1 from D_2 receptors). Recently, dihydrexidine (trans-10,11-dihydroxy-5,6,6a,7,8,12bhexahydrobenzo-[a]phenanthridine) has been reported to be as fully efficacious as dopamine, bioavailable in brain, and to have a 10-fold selectivity for the D_1 over D_2 receptor (Lovenberg et al., 1989; Brewster et al., 1990). Since there are reasons to hypothesize that appropriate occupation of D_1 receptors may be an essential component of efficacious antiparkinsonian therapy (Brewster et al., 1990), we tested dihydrexidine in non-human primates pretreated with the dopaminergic neurotoxicant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-HCl (MPTP), which results in a parkinsonian syndrome in humans and non-human primates.

Five adult male monkeys (*Cercopithecus aethiops sabaeus*) were injected intramuscularly with MPTP (Research Biochemicals Inc., Natick, MA) over a period of five days (cumulative dose of 2.0 mg/kg) two months before this study. For evaluation of drug efficacy, behavioral observations were made 'blind' at 10 min intervals before and after injections of dihydrexidine or saline. The monkeys were observed for 5 min using a time-sampled technique and observer ratings (see Taylor et al., in press). The status of the monkeys was assessed using a *Parkinsonian summary score*, derived from factor analyses, that represented the sums of tremor, freezing, difficulty eating, food response, delayed initiation and poverty of movement, response to threat, appearance, and inability to stand (or 'face-down'). Since there is evidence that spontaneous eye blink rates are decreased in Parkinson's disease, and after MPTP treatment, they were then counted for 2 min (see Lawrence and Redmond, 1991). Doses of dihydrexidine (0.3, 0.45 or 0.6 mg/kg) were given acutely to four subjects; due to limited drug availability. Multiple doses were given to the fifth subject (0.93 mg/kg cumulative). Dihydrexidine or saline was administered intramuscularly in a balanced pseudo-random sequence over two days. Analyses of variance was used to determine significant differences.

MPTP treatment resulted in unequivocal signs of parkinsonism – tremor, poverty of movement, difficulty in initiating movement, bradykinesia, motor freezing, and a decrease in eye blink rate. Dihydrexidine resulted in a dramatic reduction in parkinsonian signs, as well as an increase in blink rats (see fig. 1) within minutes of injection. Significant changes over time were observed (parkinsonian summary score $F(6,24) = 38.41$ and blink rate $F(6,24) = 5.64$, $P < 0.001$). Conversely, signs of parkinsonism and decreased blink rates were unchanged following the injection of saline. Blink rates were increased to normal levels (Lawrence and Redmond, 1991) and were also inversely correlated with parkinsonian scores (Spearmen's correlation coefficient, $R^2 = 0.73$, $P < 0.01$). The effects of dihydrexi-

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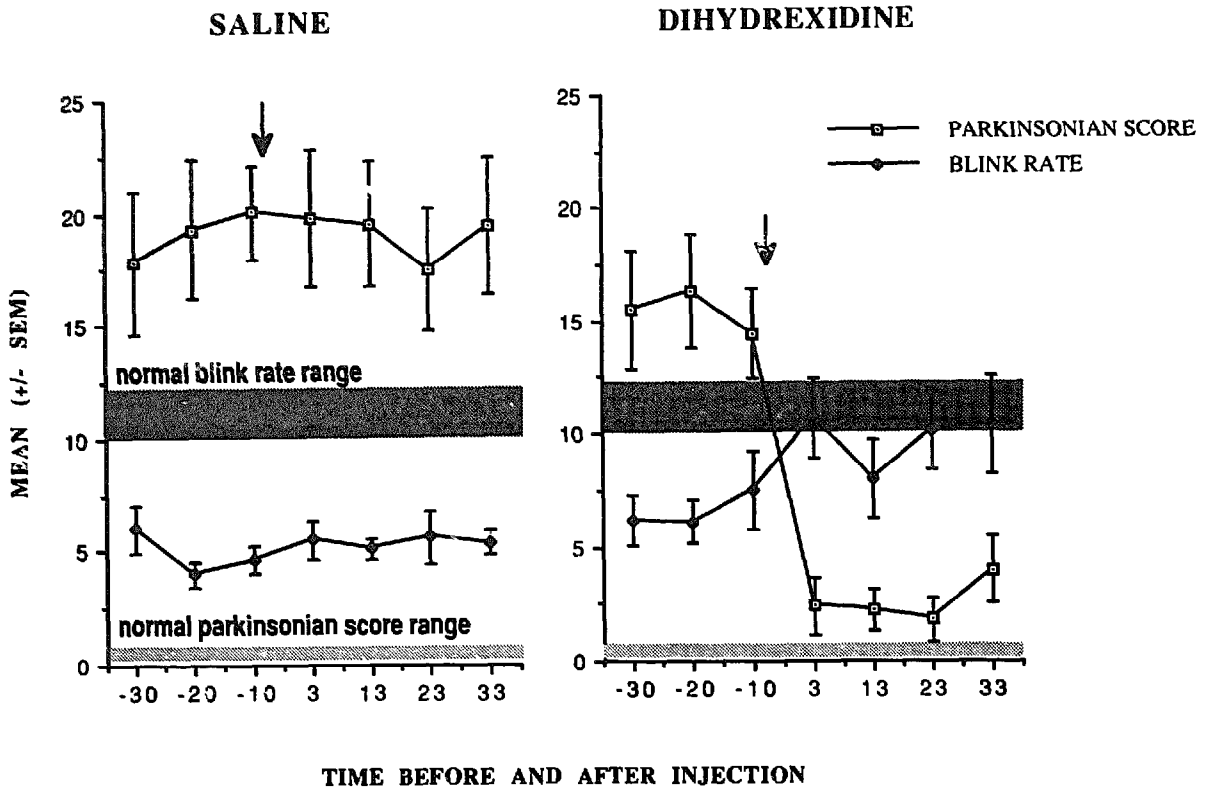

Fig. 1. Effects of saline and dihydrexidine administration (arrows) in five MPTP-treated monkeys (with the time of injection designated as 0 min, observations were made at −30, −20, −10, +3, +13, +23, +33). Measures of parkinsonism (parkinsonian summary score) were decreased only after administration of dihydrexidine (drug/saline $F(1,3) = 39.84$, $P < 0.01$; time $F(6,18) = 25.16$, $P < 0.01$; drug/saline by time interaction $F(6,18) = 20.15$, $P < 0.01$). Conversely, eye blink rates were increased only after administration of dihydrexidine (drug/saline $F(1,3) = 8.27$, $P < 0.05$; time, $F(6,18) = 5.59$, $P < 0.001$; drug/saline by time interaction $F(6,18) = 2.59$, $P < 0.05$). The range of parkinsonian scores observed in normal subjects is shown by a light stippled band and the range of normal blink rates is shown by a dark stippled band; the mean blink rate of healthy adult male vervets is approximately 10-12 blinks per min compared with 7-8 blinks per min in MPTP-treated subjects.

dine lasted for approximately 45 min. Although one subject showed minimal drug effects, a cumulative dose of 0.93 mg/kg resulted in similar effects. Since most subjects appeared to be normalized after injection of dihydrexidine, the 'blind' was essentially broken; however, four observers rated random segments of videotape and were 100% correct in identifying the treatment condition.

These data show that dihydrexidine dramatically attenuated parkinsonian signs, including tremor, motor freezing, abnormal posture, rigidity, and bradykinesia, while increasing eye blink rates in MPTP-treated monkeys. Thus, contrary to accepted views, these results suggest a critical role for D_1 receptor occupancy in attenuation of parkinsonian signs.

While the D_1 component of dihydrexidine is clearly essential for its action, its D_2 potency (one tenth of the D_1) may also play a role (Brewster et al., 1990). Behavioral studies in rodents have shown that D_1 effects predominate at low doses, whereas D_2 effects may be seen as the doses increase (Darney et al., in press). Experiments in primates have shown that dihydrexidine-induced increases in blink rates could be blocked selectively by D_1 and not D_2 antagonists, indicating an independent regulation in primates (Elsworth et al., submitted). It is unclear if both effects are needed for the efficacy we have found. It is unknown if dihydrexidine has selectivity for any of the multiple subtypes of dopamine receptor that have been recently cloned. Moreover, we are aware that there are many differences between idiopathic Parkinson's disease and the MPTP model. Nonetheless, these data do demonstrate that striking improvements in parkinsonism can result from drugs with activity at D_1-like receptors.

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