Ca^{2+} channels, and adenylyl cyclase. Thus, G proteins, in addition to acting as signal transducers, can link different effectors into membrane networks; (iii) in the heart β-adrenergic agonists produce opposite changes in I_{Ca} and I_{Na}, but the effects on I_{Na} require that the membrane potential is depolarized. These effects will be exaggerated in the ischemic myocardium because ischemia causes membrane depolarization through extracellular K^+ accumulation and is accompanied by an increased catecholamine concentration (*16*). Thus, our results may explain the data linking high levels of catecholamine to a greater risk of severe arrhythmias associated with myocardial infarction.

REFERENCES AND NOTES

1. M. Windisch and H. Tritthart, *J. Mol. Cell. Cardiol.* **14**, 431 (1982); I. Hisatome, T. Kiyosme, S. Imanishi, M. Arita, *ibid.* **17**, 657 (1985).
2. W. A. Catterall, *Science* **242**, 50 (1988); T. L. Seelig and J. J. Kendig, *Brain Res.* **245**, 144 (1982).
3. A. L. Hodgkin and A. F. Huxley, *J. Physiol. (London)* **117**, 500 (1952). The inactivated state is a closed state favored by depolarization from which the channels cannot be activated. It differs in this way from the resting, closed state. In this context, inactivation and unavailability of channels are used interchangeably.
4. A. M. Brown and A. Yatani, *The Heart and Cardiovascular System* (Raven Press, New York, 1986), vol. 1, p. 627.
5. E. Fenwick, A. Marty, E. Neher, *J. Physiol. (London)* **331**, 599 (1982); J. M. Fernandez, A. P. Fox, S. Krasne, *ibid.* **356**, 565 (1984); D. L. Kunze *et al.*, *J. Gen. Physiol.* **86**, 691 (1985).
6. N. M. Scherer *et al.*, *Arch. Biochem. Biophys.* **251**, 431 (1987); S. W. Halvorsen and N. M. Nathanson, *Biochemistry* **23**, 5813 (1984).
7. H. Reuter, *Nature* **301**, 569 (1983); M. Kameyama *et al.*, *Pfluegers Arch.* **405**, 285 (1985).
8. A. Brown *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **53**, 365 (1988).
9. R. Cerione *et al.*, *Biochemistry* **26**, 1485 (1987).
10. A. Otero *et al.*, *Science* **242**, 443 (1988).
11. L. Birnbaumer *et al.*, *Kidney Int.* **32**, S14 (1987); A. G. Gilman, *Annu. Rev. Biochem.* **56**, 615 (1987).
12. A. M. J. VanDongen *et al.*, *Science* **242**, 1433 (1988).
13. B. I. Khodorov, *Prog. Biophys. Mol. Biol.* **374**, 49 (1981).
14. B. Hille, *J. Gen. Physiol.* **69**, 494 (1977).
15. V. L. Salgado, J. Z. Yeh, T. Narahashi, *Biophys. J.* **47**, 567 (1985); W. A. Caterall, *J. Gen. Physiol.* **74**, 375 (1979); P. M. Vassilev, T. Scher, W. A. Catterall, *Science* **241**, 1658 (1988).
16. A. Wollenberger and L. Shaab, *Nature* **207**, 88 (1965); H. J. Hirche *et al.*, *J. Mol. Cell. Cardiol.* **12**, 579 (1980).
17. Primary cardiac cell cultures were prepared from hearts of 1- to 3-day-old neonatal rats [G. E. Mark and F. F. Strasser, *Exp. Cell Res.* **44**, 217 (1966)] and used as described in Kunze *et al.* (*5*).
18. We used the patch-clamp technique [O. P. Hamill, A. Marty, E. Neher, B. Sakmann, F. J. Sigworth, *Pfluegers Arch.* **391**, 85 (1981)] to record whole-cell and single-channel Na^+ currents that had the gating, conductance, and pharmacological properties that have been reported for fast cardiac Na^+ channels (*4*). For whole-cell measurements we selected small spherical cells (10 μm in diameter) with membranes that behaved as a simple resistance-capacitance (*R-C*) circuit with a time constant <100 μs [D. L. Kunze *et al.*, in (*5*)]. Test potentials were always on the positive limb of the current-voltage (*I-V*) curve and the currents had none of the features of inadequate space clamp. Patch pipettes had tip resistances of 1.5 to 5.0 megohms and the input resistance of the cells was about 1.0 gigaohm. Capacitive transient cancellation and series resistance adjustments were made to provide optimum settling and attenuation of the capacitive current transient.

Currents were digitized and recorded at 44 kHz on a pulse-code modulated videocassette recorder for off-line analysis. Before digitization, currents were filtered at 5 kHz (−3 dB) with a 4-pole Bessel filter. The data were then transferred to a MicroVax II computer for further analyses. No corrections for leak currents were made for whole-cell recordings. The single-channel records were filtered before analysis with a Gaussian finite-impulse response filter.

The experimental chamber (200 to 500 μl) was placed on an inverted microscope stage. When necessary, external solutions were superfused at 2 ml per minute by gravity. To suppress outward currents, the pipettes were filled with a Cs^+-rich solution of 118 m*M* CsOH, 118 m*M* aspartic acid, 6.4 m*M* $MgCl_2$, 5 m*M* EGTA, 4.2 m*M* ATP, 2.7 m*M* $CaCl_2$, 5 m*M* Hepes, *p*H 7.3 with CsOH (290 mOsm adjusted with cesium aspartate). The calculated free concentrations of Mg^{2+} and Ca^{2+} were 2 m*M* and 0.1 μ*M*, respectively. After breaking the patch, the pipette solution was allowed to equilibrate with the cell interior for a few minutes. The external solution contained 137 m*M* NaCl, 5.4 m*M* KCl, 1 m*M* $MgCl_2$, 2 m*M* $CaCl_2$, 5 m*M* $CoCl_2$, 10 m*M* glucose, 10 m*M* Hepes, *p*H 7.4, adjusted with NaOH (290 mOsm). Ca^{2+} currents were suppressed by addition of Co^{2+} in the presence of Mg^{2+}. All experiments were performed at 20° to 22°C.
19. The G proteins used in this study were purified from human erythrocyte membranes to greater than 95% purity (as assessed by Coomassie blue staining) by fractionation techniques that yield G_s essentially free of G_k and G_k essentially free of G_s [A. Yatani *et al.*, *J. Biol. Chem.* **263**, 9887 (1988)].

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The Effect of Deprenyl (Selegiline) on the Natural History of Parkinson's Disease

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The effects of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), a neurotoxin that produces the symptoms of Parkinson's disease, can be fully prevented in experimental animals by inhibiting monoamine oxidase B. On the basis of this observation, a double-blind, placebo-controlled study in patients with early Parkinson's disease was initiated to determine whether deprenyl (a selective monoamine oxidase B inhibitor) would delay the need for L-dopa therapy by slowing the progression of the disease. Fifty-four patients were randomly assigned to deprenyl (10 mg/day) or placebo treatment groups and followed until L-dopa therapy was indicated or until the patient had been in the study for 3 years. Analysis of Kaplan-Meier survival curves for each group showed that deprenyl delayed the need for L-dopa therapy; the average time until L-dopa was needed was 312.1 days for patients in the placebo group and 548.9 days for patients in the deprenyl group. Disease progression, as monitored by five different assessment scales, was slowed (by 40 to 83% per year) in the deprenyl group compared to placebo. Therefore, early deprenyl therapy delays the requirement for antiparkinsonian medication, possibly by slowing progression of the disease.

PARKINSON'S DISEASE IS INVARIABLY progressive. Although drugs for symptomatic treatment are available, none slow the progress of the disease. L-Dopa remains the backbone of modern treatment, but complications, toxicity, and decreased effectiveness tend to appear with long-term use of all antiparkinsonian drugs or with progression of the disease (*1*). For these reasons, there is a critical need for a new treatment aimed at retarding disease progression.

In 1983, two seemingly unrelated events set the stage for the therapeutic trial reported here. First, Birkmayer and colleagues (*2*) reported that parkinsonian patients receiving both L-dopa and the selective monoamine oxidase (MAO) inhibitor, deprenyl, appeared to live longer than patients receiving L-dopa alone. They surmised that deprenyl might be preventing death of neurons in the substantia nigra, the central neuropathological feature in Parkinson's disease, thus slowing disease progression. Their report provoked little response, perhaps because it was retrospective in nature. That same year, we published an account of four young drug abusers who developed pure parkinsonism after using a new "synthetic heroin"; the offending agent was tentatively identified as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (*3*). MPTP was quickly shown to be selectively toxic to the substantia nigra in both rhesus (*4*) and squirrel monkeys (*5*), thus providing a striking pathological analogy to Parkinson's disease. After the discovery that MAO

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B (*6*) mediated the conversion of MPTP to 1-methyl-4-phenylpyridinium ion (MPP^+) (*7*), its putative toxic metabolite, a number of groups, including our own, were able to block the biological effects of MPTP with MAO inhibitors, including deprenyl (*7, 8*).

Armed with this experimental and empirical data, we initiated a double-blind, placebo-controlled study of patients with early, untreated Parkinson's disease (*9*) to test the hypothesis that chronic administration of deprenyl would delay the need for initiation of L-dopa therapy by slowing the rate of progression of Parkinson's disease in humans.

Only patients with Parkinson's disease for less than 5 years, and an examination indicative of typical Parkinson's disease (including at least two of the three cardinal features: rigidity, bradykinesia, and rest tremor) were accepted (*10*). Participation was limited to individuals between 30 and 80 years of age to minimize the number of patients with atypical disease, or, in the elderly, the higher risk of developing other medical problems.

Patient recruitment was initiated with a letter to local neurologists and media announcements. As a result, over 200 patients were screened by telephone, 57 of whom qualified for a baseline evaluation. This evaluation included a history, physical examination, and the Mini–Mental State Examination (MMSE) (*11*), as well as five assessment scales to measure the severity of parkinsonism. These were the (i) Unified Parkinson's Disease Rating Scale (UPDRS) Motor Examination (*12*) [scoring method: each of 13 different motor features were rated on a 5-point scale (0, normal; 4, severely affected); these ratings were then summed for a total score]; (ii) Hoehn and Yahr Staging [scoring method: see (*13*)], (iii) The Webster Step-Second Test (*14*) (scoring method: time in seconds to stand and walk a prescribed course and sit again); (iv) UPDRS Activities of Daily Living (ADL) [scoring method: 13 different ADLs were reviewed with the patient and the degree of dysfunction for each rated on a 5-point scale (0, normal; 4, severely disabled); these ratings were then summed for a total score]; (v) The Schwab and England ADL (*15*) (scoring method: percentage of normal function was estimated by the patient to the nearest 5%). Depression was also assessed [scoring method: the UPDRS subscale for depression, which is scored from 0 (none) to 4 (severe)]. Laboratory tests included a complete blood count, urine analysis, a standard blood chemistry panel including liver enzymes (Sequential Method Analysis no. 18), and an electrocardiogram. Baseline examinations took place between March 1986 and July 1987; 54 patients met entry criteria and were enrolled (Table 1).

Enrolled patients were randomized to either the placebo group (PG) or the deprenyl group (DG) by using a "biased coin" randomization method (*16*). Deprenyl (5 mg) and identical (except for the deprenyl) placebo tablets were provided by Somerset Pharmaceuticals. The dose of 5 mg twice daily, which has been used in most previous clinical trials, was selected for this study because it achieves nearly complete inhibition of MAO B in the brain (*17*). The patients, and all clinic personnel who came in contact with them, were blinded as to treatment status. Human Subjects Review Committee (Institute for Medical Research, San Jose, California) and Food and Drug Administration (IND no. 26,066) approvals were obtained. Follow-up evaluations were begun 1 month after initiation of the study drug (wash-in evaluation), and continued routinely at 5-month intervals or until patients reached end point (L-dopa therapy) (*18*); patients were seen earlier if they or their personal physician believed L-dopa therapy was needed before the next 5-month ap-

Table 1. Baseline characteristics of study patients. Values with plus or minus are means ± SEM.

Treatment	*n*	Age (years)	No. of		Sex M/F (*n*)	Age at onset of symptoms (years)	Age at diagnosis (years)	Duration of symptoms before entry* (months)
			Young <55 years	Old ≥65 years				
					All enrolled patients			
Placebo	27	61.1 ± 1.52	5	8	18/9	59.2 ± 1.54	60.1 ± 1.50	25.0 ± 2.71
Deprenyl	27	60.9 ± 1.85	6	7	19/8	59.3 ± 2.37	59.7 ± 1.90	29.0 ± 2.69
					Patients reaching end point			
Placebo	22	61.2 ± 7.70	3	6	14/8	58.8 ± 7.90	59.8 ± 7.60	25.0 ± 14.70
Deprenyl	22	60.3 ± 10.00	5	5	17/5	57.6 ± 10.10	59.0 ± 10.10	28.5 ± 12.80

*For placebo treatment, the range was 8 months to 5 years. For deprenyl treatment, the range was 10 months to 5 years.

Table 2. Absence of wash-in or wash-out effect. Average ratings (± SEM) for each assessment scale in the placebo and deprenyl groups for the wash-in (baseline compared to examination at 1 month) and wash-out (end-point examination compared to examination 1 month after stopping the study drug) studies. Only the change in the Webster Step-Second Test in placebo group at wash-out was statistically significant. Thus, there was no statistically significant symptomatic effect from deprenyl. See text for abbreviations.

Assessment	Treatment	Wash-in study			Wash-out study		
		n	Baseline	1 month after baseline	*n**	End point	1 month after end point
UPDRS motor findings (points)	Placebo	27	21.41 ± 2.18	22.48 ± 2.41	19	29.20 ± 1.80	27.70 ± 2.15
	Deprenyl	27	21.93 ± 1.47	20.50 ± 1.56	19	28.26 ± 1.67	27.90 ± 2.09
Hoehn & Yahr scale (stage)	Placebo	27	1.46 ± 0.13	1.55 ± 0.13	19	1.93 ± 0.15	1.76 ± 0.16
	Deprenyl	27	1.59 ± 0.10	1.69 ± 0.11	19	1.88 ± 0.13	1.81 ± 0.14
Webster Step-Second Test (step-s)	Placebo	27	61.11 ± 3.61	62.88 ± 3.55	19	67.28 ± 5.30	83.38 ± 12.65†
	Deprenyl	27	57.81 ± 3.53	58.25 ± 3.32	19	65.65 ± 4.93	65.83 ± 4.99
UPDRS ADL symptoms (points)	Placebo	27	8.00 ± 0.90	8.11 ± 0.76	19	12.25 ± 0.84	11.72 ± 0.93
	Deprenyl	27	7.74 ± 0.52	7.51 ± 0.51	19	10.29 ± 0.73	10.74 ± 0.79
S & E by patient (percent)	Placebo	27	91.30 ± 1.17	89.07 ± 1.55	19	79.75 ± 1.89	78.10 ± 3.83
	Deprenyl	27	90.19 ± 0.72	89.25 ± 1.07	19	81.50 ± 1.69	76.40 ± 3.73

*The number of patients in the wash-out study is lower due to drop-outs (five per group) and failure to return for wash-out assessment (three per group). †$P < 0.05$.

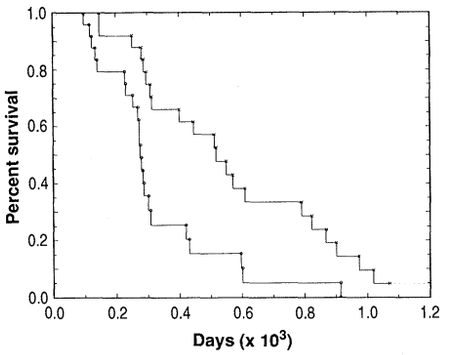

Fig. 1. Kaplan-Meier survival curves for placebo (○) (n = 25) and deprenyl (×) (n = 26) group. The curves are significantly different by the log rank test ($P < 0.002$).

pointment. After end point, the study drug was discontinued and a final follow-up examination (wash-out) carried out 1 month later (*19*). All five assessment measures and a depression rating were obtained at each follow-up visit. Patients reached end point after continuing on the study drug alone for 3 years or when it was determined that they needed L-dopa based on (i) threatened loss or significant change in employability or the ability to handle domestic responsibilities, or (ii) failing postural reflexes, placing the subject at risk for falling.

To analyze survivorship, a Kaplan-Meier curve, with all but the three excluded patients (n = 51), was calculated for each group by using time to end point, and the two curves were compared with the log rank test (*20*). To determine the rate of disease progression, we fitted a separate line for each assessment score versus time by least squares for each patient who reached end point (n = 44) with all of the data points available (*21*). These slopes were averaged for each assessment measure in DG and PG. Each assessment measure was then compared separately between DG and PG by a two-tailed Wilcoxon test. The wash-in (n = 54) and wash-out (n = 38) studies (Table 2) were assessed for statistical significance by using the Wilcoxon signed rank test.

One-half of the patients were assigned to each arm of the study. Baseline characteristics were comparable in the two groups (Table 1). All 54 returned for the 1 month wash-in examination. Three patients were excluded from further data analysis because they were unable to remain off antiparkinsonian medications beyond 1 month (two in PG, one in DG). Seven patients dropped out for the following reasons: lost to follow-up (two in DG); insomnia (one in DG); euphoria-intoxicated sensation (one in PG); transient elevation in liver enzymes (one in PG); dysphagia (one in DG); and incidental surgery (one in PG). The remaining 44 patients reached end point (22 in each group). Thirty-eight of these underwent wash-out examinations 1 month later (19 in each group).

Analysis of Kaplan-Meier survival curves for each group showed that deprenyl clearly delayed the time to end point ($P < 0.002$) (Fig. 1). The average time to end point was 312.1 days (44.47 ± SEM) for patients in the PG and 548.9 days (61.01 ± SEM) for patients in the DG. One patient in the DG reached end point by staying on the study drug alone for 3 years. Disease status was remarkably similar at end point in both groups (Table 2), indicating that (i) the decision to administer L-dopa had been consistently applied between groups and was therefore not biased in favor of one group or the other, and (ii) patients in the DG were taking nearly twice as long to reach this stage of disability as the PG. Scores did not change appreciably after the study drug was stopped (wash-out) in either group (Table 2), indicating that the delay to end point in the DG was not due to a transient therapeutic effect on parkinsonian signs or symptoms. Further, there was no evidence of improvement of parkinsonian symptoms or signs at wash-in (Table 2), nor was there any difference in the depression scores at wash-in or wash-out in either PG or DG [in agreement with a recently published study (*22*)], indicating that an antidepressant effect did not interfere with the decision to administer L-dopa. The rate of progression was slowed by 40 to 83% per year as measured by the five assessment scales (Table 3); only the Webster Step-Second Test failed to reach significance (P = 0.07).

At the end of the study (March 1989), the examiner and patients were asked to guess which drug patients had received. The examiner guessed the experimental treatment in 34 of 54 subjects (62%); patients were correct 61% of the time (42 responded). Ideally this exercise should have been done at end point for each patient before L-dopa was started, because deprenyl may enhance the effects of L-dopa, thereby giving the examiner and patients a clue as to the study drug. In any case, we do not believe that this small success in guessing the correct drug significantly affected our results.

The two potentially serious adverse events occurred in the PG; one patient experienced a transient elevation of liver enzymes and dropped out (see above), and the other developed a mild but persistent elevation in creatine phosphokinase. Subjective complaints were almost all transient and included insomnia [4 in PG, 14 in DG (including one dropout)], headache (10 in PG, 7 in DG), dizziness (6 in PG, 8 in DG), nausea (5 in each group), and euphoria [1 in each group, including a PG dropout (see above)].

At least three conclusions can be drawn from this study. First, deprenyl appears to be a remarkably safe drug in patients with early, untreated Parkinson's disease. We failed to observe any serious side effects, and the minor complications that did occur were almost evenly divided between the PG and DG. Second, early deprenyl therapy significantly delays the need for treatment with L-dopa. Finally, deprenyl appears to slow the rate of progression of Parkinson's disease as monitored by five different clinical measures. The latter two observations support the hypothesis that deprenyl slows the progression of Parkinson's disease. However, this conclusion must be tempered by two important caveats. First, to actually prove this hypothesis, it would be necessary to show that death of nigral neurons is being prevented, and as yet there is no way to determine this in living humans. Second, the study reported here is a relatively small one on which to base an entire new therapeutic

Table 3. Rate of disease progression for Parkinson's disease patients in placebo and deprenyl groups. Values represent change in score of assessment scales per year (± SEM). Decrease in rate, percentage decrease in rate of progression for the deprenyl group compared to placebo group. See text for abbreviations.

Assessment (30–35)	Placebo (n = 22)	Deprenyl (n = 22)	P-value*	Decrease in rate (%)
UPDRS motor examination (points/year)	13.40 ± 1.82	6.75 ± 1.05	0.002	50
Hoehn & Yahr scale (stage/year)	0.73 ± 0.15	0.263 ± 0.10	0.05	64
Webster Step-Second Test (step-s/year)	21.68 ± 7.75	3.67 ± 2.57	0.07	83
UPDRS ADL symptoms (points/year)	4.45 ± 1.01	2.69 ± 0.58	0.02	40
S & E by patient (%/year)	−9.20 ± 4.23	−5.25 ± 1.23	0.02	43

*Two-tailed Wilcoxon test.

strategy for Parkinson's disease. Therefore, it should be regarded as preliminary and in need of confirmation. Fortunately, a large multi-institutional study, DATATOP (*23*), which was designed to determine whether or not deprenyl alone or in combination with vitamin E can slow the course of Parkinson's disease, is now nearing completion. As the DATATOP study has enrolled 800 subjects, it should provide definitive evidence for (or against) the conclusions reached here. These issues are not minor, as setting a precedent for one age-related human neurodegenerative disease is likely to have consequences for others, such as Alzheimer's disease and amyotrophic lateral sclerosis.

There are several possible mechanisms by which deprenyl might achieve the effects reported here (*9*), including prevention of the formation of a pyridinium species after exposure to an exogenous or endogeous tetrahydropyridinium (*24*). Deprenyl could also be moderating oxidative stress by preventing oxidation of dopamine by MAO B (*25*). It will be important to explore this question, because determining the mechanism of this effect could provide a clue to the cause of Parkinson's disease. Knoll and colleagues (*26*) have dramatically increased the life-span of rats with chronic deprenyl administration, raising the possibility that more than dopaminergic neurons are being protected.

REFERENCES AND NOTES

1. J. Jankovic and C. D. Marsden, in *Parkinson's Disease and Movement Disorders*, J. Jankovic and E. Tolosa, Eds. (Urban and Schwarzenberg, Baltimore-Munich, 1988), pp. 95–119.
2. W. Birkmayer *et al.*, *Mod. Probl. Pharmacopsychiatr.* **19**, 170 (1983).
3. J. W. Langston *et al.*, *Science* **219**, 979 (1983).
4. R. S. Burns *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4546 (1983).
5. J. W. Langston *et al.*, *Brain Res.* **292**, 390 (1984).
6. K. Chiba, A. J. Trevor, N. Castagnoli, Jr., *Biochem. Biophys. Res. Commun.* **120**, 574 (1984).
7. J. W. Langston *et al.*, *Neurosci. Lett.* **48**, 87 (1984); S. P. Markey *et al.*, *Nature* **311**, 464 (1984).
8. J. W. Langston, I. Irwin, E. B. Langston, L. S. Forno, *Science* **225**, 1480 (1984); R. E. Heikkila, L. Manzino, R. C. Duvoisin, F. S. Cabbat, *Nature* **311**, 467 (1984); G. Cohen *et al.*, *Eur. J. Pharmacol.* **106**, 209 (1985).
9. J. W. Tetrud and J. W. Langston, *J. Neural. Transm. [Suppl.]* **25**, 69 (1987).
10. Exclusionary criteria included: (i) atypical features or the suspicion of any other neurologic disorder; (ii) Hoehn and Yahr stage greater than 2.5 (*13*) (we assumed an effect on progression would be more obvious in patients with milder disease, since a larger number of dopaminergic neurons would still be present to benefit from protective therapy); (iii) a major medical or psychiatric disorder; (iv) a modified Mini-Mental Status Examination (MMSE) score less than 24 (*11*), (v) previous use of any drugs known to induce parkinsonism; and (vi) chronic treatment with any medications, including all antiparkinsonian drugs, that might interact with deprenyl or complicate patient evaluation. Twenty-three patients [10 in DG (6, carbidopa/L-dopa; 1, amantadine; 1, trihexyphenidyl; 1, amitriptyline; 1, propranolol) and 13 in PG (7, carbidopa/L-dopa; 4, amantadine; 1, trihexyphenidyl; 1, propranolol)] had been on treatment for less than 1 year and were allowed to enroll after remaining off medication for at least 1 month prior to entry into the study. None of the patients received other medications that would affect the central nervous system during the study.
11. M. F. Folstein, S. E. Folstein, P. R. McHugh, *J. Psychiatr. Res.* **12**, 189 (1975).
12. S. Fahn and R. L. Elton, Members of the UPDRS Development Committee, in *Recent Developments in Parkinson's Disease*, S. Fahn, C. D. Marsden, D. B. Calne, M. Goldstein, Eds. (MacMillan Healthcare information, Florham Park, NJ, 1987) vol 2, pp. 153–163; M. B. Stern, in *The Comprehensive Management of Parkinson's Disease*, M. B. Stern and H. I. Hurtig, Eds. (PMA, New York, 1988), pp. 3–50; the 13 motor examination items actually yield 27 data points because each extremity is scored separately in many of the items.
13. M. Hoehn and M. D. Yahr, *Neurology* **17**, 427 (1967); For this study, the Hoehn and Yahr scale was slightly modified: stage 1, unilateral disease; stage 1.5, unilateral disease with neck rigidity; stage 2, bilateral disease without impaired balance; stage 2.5, bilateral disease with impaired balance after being pushed, but able to regain balance without assistance.
14. D. D. Webster, *Mod. Treat.* **5**, 257 (1968).
15. R. S. Schwab and A. C. England, in *Third Symposium on Parkinson's Disease*, F. J. Gillingham and M. C. Donaldson, Eds. (Livingstone, Edinburgh, 1969), pp. 152–157.
16. R. G. Miller, B. Efron, B. W. Brown, L. E. Moses, *Biostatistics Case Book* (Wiley, New York, 1980); B. W. Brown and J. Halpern, *Stat. Med.* **5**, 211 (1986).
17. P. Reiderer and M. B. H. Youdim, *J. Neurochem.* **46**, 1359 (1986).
18. The mean interval between follow-up examinations was 153 days in DG (range 109 to 204), and 149 days in PG (range 107 to 175).
19. J. Knoll, *Acta Neurol. Scand. Suppl.* **95**, 57 (1983); J. D. Elsworth *et al.*, *Psychopharmacology* **57**, 33 (1978). Since deprenyl and its metabolites are cleared within 24 to 48 hours after stopping the drug, the only pharmacologic effect that might be long-lasting is its action on MAO B, as it binds irreversibly to this enzyme. However, replacement synthesis results in a gradual return of MAO B activity within 2 to 3 weeks. Therefore, the washout period of 1 month should have been fully adequate to detect a transient dopaminomimetic effect, had there been one.
20. R. Miller, *Survival Analysis* (Wiley, New York, 1981).
21. Two patients had less than four data points (three data points in one, and two in the other; both were in PG).
22. J. J. Mann *et al.*, *Arch. Gen. Psychiatry* **46**, 45 (1989).
23. R. Lewin, *Science* **230**, 527 (1985); I. Shoulson, in *Handbook of Experimental Pharmacology: Drugs for Treatment of Parkinson's Disease*, D. B. Calne, Ed. (Springer-Verlag, New York, in press).
24. J. W. Langston, in *Parkinson's Disease and Movement Disorders*, J. Jankovic and E. Tolosa, Eds. (Urban and Schwarzenberg, Baltimore-Munich, 1988), pp. 75–85.
25. G. Cohen, *J. Neural. Trans. Suppl.* **19**, 89 (1983); ——— and M. B. Spina, *Ann. Neurol.*, in press.
26. J. Knoll, *Mech. Ageing Dev.* **46**, 237 (1988).

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Triggering of Allostery in an Enzyme by a Point Mutation: Ornithine Transcarbamoylase

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The origin of allostery is an unanswered question in the evolution of complex regulatory proteins. Anabolic ornithine transcarbamoylase, a trimer of identical subunits, is not an allosteric enzyme per se. However, when the active-site residue arginine-106 of the *Escherichia coli* enzyme is replaced with a glycine through site-directed mutagenesis, the resultant mutant enzyme manifests substrate cooperativity that is absent in the wild-type enzyme. Both homotropic and heterotropic interactions occur in the mutant enzyme. The initial velocity saturation curves of the substrates, carbamoyl phosphate and L-ornithine, conform to the Hill equation. The observed cooperativity depends on substrate but not enzyme concentration. The finding underscores the possibility that a single mutation of the enzyme in the cell could turn transcarbamoylation into a regulatory junction in the biosynthesis of L-arginine and urea.

COOPERATIVITY OF PROTEINS IN ligand binding has been known for more than 80 years since Bohr's observation of heme-heme interactions in hemoglobin (*1*). All allosteric proteins are either oligomeric or contain multiple interacting domains within one polypeptide chain, and models (*2, 3*) have been advanced to explain the binding properties of allosteric ligands. However, most oligomeric enzymes are not cooperative, and reasons such as promotion of stability have been offered for their existence. Recently, several oligo-

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