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Long-Term Treatment of Male F344 Rats with Deprenyl: Assessment of Effects on Longevity, Behavior, and Brain Function

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BICKFORD, P. C., C. E. ADAMS, S. J. BOYSON, P. CURELLA, G. A. GERHARDT, C. HERON, G. O. IVY, A. M. L. Y. LIN, M. P. MURPHY, K. POTH, D. R. WALLACE, D. A. YOUNG, N. R. ZAHNISER AND G. M. ROSE. *Long-term treatment of male F344 rats with deprenyl: Assessment of effects on longevity, behavior, and brain function.* NEUROBIOL AGING **18**(3) 309–318, 1997.—L-Deprenyl (selegiline) was chronically administered to male Fischer 344 rats via their drinking water beginning at 54 weeks of age (estimated daily dose: 0.5 mg/kg/day). Beginning at 84 weeks of age, the rats were behaviorally evaluated using a sensorimotor battery, a motor-learning task, and the Morris water maze. At 118 weeks of age, cerebellar noradrenergic function was evaluated in the surviving rats using in vivo electrochemistry. The rats were then sacrificed to measure brain monoamine oxidase activity and perform quantitative autoradiography to evaluate the effect of chronic deprenyl treatment on β-adrenergic receptors in the cerebellum, α_2-adrenergic receptors several brain regions, and D_1 and D_2 dopamine receptors in the striatum. Deprenyl treatment reduced brain monoamine oxidase B activity by 85%, but had no effect on brain monoamine oxidase A. A clear effect of chronic deprenyl treatment upon longevity was not observed. Several measures of CNS function were altered in the deprenyl-treated animals: 1) spatial learning in the Morris water maze was improved; 2) electrochemical signals recorded following local application of NE were reduced, and the responsiveness to the reuptake blocker nomifensine was enhanced, in the cerebellum; 3) β-adrenergic receptor binding affinity was increased in the cerebellum; 4) α_2-adrenergic receptor density was increased in the inferior colliculus; and 5) striatal D_1 dopamine receptor density was reduced but binding affinity was enhanced. In contrast, chronic deprenyl treatment did not cause changes in: 1) sensorimotor function, as evaluated by balance beam, inclined screen, or wire hang tasks; 2) motor learning; 3) α_2-adrenergic receptor density in any region examined except for the inferior colliculus, or binding affinity in any region examined; or 4) striatal D_2 dopamine receptor number or affinity. Thus, long-term oral administration of deprenyl extended the functional life span of rats with respect to cognitive, but not motor, performance. © 1997 Elsevier Science Inc.

Selegiline Aging Longevity Hippocampus Water maze Cerebellum Motor learning Dopamine receptors Noradrenergic receptors Electrochemistry

EXTENDING functional life span is an important goal of gerontological research. To date, progress in this area has been made primarily through the development of treatments for age-related diseases. An alternative approach is to investigate manipulations for prolonging the life of healthy individuals. While this problem has received considerable experimental attention, very few positive results have emerged [e.g., (32, 34)]. At present, essentially life-long caloric restriction is the only intervention that is generally recognized to enhance longevity in mammals (1, 33, 53).

L-Deprenyl (selegiline) is a selective and irreversible inhibitor of monoamine oxidase B (MAO-B) that has been used as an adjunct to the pharmacotherapy of Parkinson's disease. A decade ago it was reported that patients receiving deprenyl and *l*-dopa lived longer than patients receiving *l*-dopa alone (10). Subsequent studies in experimental animals have suggested that chronic treatment with deprenyl alone can enhance longevity [see Table 5; (24,25,29,30,35,47,55); but see (5,21,43)]. In previous studies in rats, deprenyl has routinely been administered by subcutaneous

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injection, several times per week, starting late in the expected life span of the animal. The purpose of this study was to evaluate the effect of chronic deprenyl treatment, administered orally, to male Fischer 344 rats starting at middle age.

Treatments that enhance longevity need to have beneficial effects above and beyond life extension. Ideally, these agents should also delay age-related declines in behavioral function. Thus, in the present study the effect of long-term deprenyl treatment on sensorimotor capacity and on two different types of learning tasks were studied prior to evaluating several measures of central nervous system function. Because of the known interaction of deprenyl with catecholamine systems, its effect upon noradrenergically mediated cerebellar motor learning (6,7) was made a particular focus of this work. In addition to a behavioral assessment of deprenyl's ability to affect the age-related decline in motor learning, in vivo electrochemistry and β-adrenergic receptor binding were used to evaluate noradrenergic function in the cerebellum.

METHOD

Animals and Treatment

Forty male Fischer 344 (F344) rats, 52 weeks (12 months) of age, were purchased from the NIA contract colonies maintained by Harlan Laboratories. The animals were housed in pairs in polyacrylate cages, which were placed in a laminar flow hood. A normal light cycle (12 h on/12 h off) was maintained, and food (Purina Lab Rodent Diet 5001) and tap water were available ad lib. Cage bedding (Sani-Chips, Murphy Forest Products, Montville, NJ) was renewed every 3 days.

After a 2-week acclimation period, the animals were divided randomly into two groups. Twenty rats had deprenyl (a gift from Somerset Pharmaceuticals, Tampa, FL) added to their drinking water (8 μg/ml), while the other 20 rats received no drug treatment. The concentration of deprenyl was calculated to provide an estimated dose of 0.5 mg/kg/day. Deprenyl solutions were made in tap water every 3 days and were stored in the dark at 4°C. Behavioral testing (described below) took place between 1000 and 1600 h.

Behavioral Testing

Sensorimotor Evaluation. At 84 weeks of age the rats were tested using a battery of tasks to evaluate sensorimotor skills [adapted from Wallace et al. (51)]. These tasks included walking on a balance beam, climbing an inclined screen, and hanging by the forepaws from a horizontally suspended wire. The balance beam apparatus was a 60 cm long, longitudinally grooved, wooden dowel rod (both 2.5 and 5 cm diameters were used) that was suspended between two safety platforms located 60 cm above a padded surface. The rat was placed in the center of the rod and the latency to fall was recorded. A rat that either remained on the rod for 2 min or reached a safety platform was assigned a score of 120 s. Each rat was given three trials (10-s intertrial interval); the best score of the three trials was used for subsequent analysis. Another task utilized a wire screen that was inclined at an angle of 60°. The rat was placed on the screen facing downward and allowed to stay for a maximum of 15 min. A single trial was given, and the latency to slip off the screen was recorded. In the final test, the rats were suspended by their forepaws from a 12-gauge copper wire (diameter: 2.03 mm), which was strung between two poles at a height of 60 cm above a padded surface. For a single trial, a rat was allowed to hang on to the wire for up to 120 s; otherwise, latency to fall was recorded. The data were analyzed using the Mann–Whitney *U*-test.

Motor Learning. Motor learning was assessed on 86-week-old rats using methods described previously (8). The apparatus for this task consisted of a straight runway (length = 127 cm, height = 25 cm, and width = 6 cm), at either end of which was a goal box (25 × 25 cm) in which a water spout was located. The floor of the runway consisted of an arrangement of 38 horizontally oriented aluminum rods. The rods (diameter = 4 mm, 2 to 4 cm long from the inner wall, and a minimum interrod distance of 2.5 cm) could be arranged in either a regular (REG) or irregular (IRR) configuration (see Fig. 3.) The goal of the rat was to traverse the runway to receive a water reward (0.3 ml) in the goal box. Water delivery was accompanied by a tone that served as a conditioned reinforcer. A number of photobeam detectors were positioned along the runway to allow the measurement of running time.

The rats were deprived of water for 12 h before training was begun. Initially, the rats were acclimated to the runway by covering the rods with a piece of Plexiglas. Shaping continued for 1 week, or until the animals consistently ran back and forth in the runway to drink at the goal boxes. The rats were given 3 min ad lib water access after each daily session, but were maintained on water restriction such that they averaged 90% (never less than 85%) of their original body weight during the entire 5-week training period. After shaping, training proceeded by gradually removing sections of the Plexiglas that covered the rods. Data collection began when the rat had performed two successful traverses of the entire length of the uncovered rods in less than 1 min. Average daily performance was calculated by determining the running time for 20 successive trials. After 5 days of training with the rods in the REG pattern, the rats were rested for a 2-week period. The animals were then trained in the IRR pattern until their performance was asymptotic. Comparisons between groups were made by simultaneous modeling of the data as has been previously described (8).

Spatial Learning. At 104 weeks of age, place learning in the Morris water maze was tested according to the protocol described in detail in Engstrom et al. (18). Briefly, the rats were required to use information provided by extra-apparatus cues to learn the location of a hidden escape platform located in a circular tank, 1.5 meters in diameter and 0.3 meters high, which was filled with water made opaque by adding 250 ml of white Createx, a nontoxic latex paint. Water temperature was maintained at 24–25°C. The tank was located in a room containing numerous sensory cues (e.g., a poster on one wall, a stand containing the cages of rats, an incandescent light) that were maintained in constant locations during the period of behavioral testing.

Prior to the first training trial, each rat was placed on the platform and allowed to remain there for 30 s. Then, with the platform in a consistent location, all animals were given four trials/day with an intertrial interval of approximately 10 min. A trial consisted of being placed into the water in each one of the four starting quadrants, as described above. The rat was required to swim for 60 s or until it located the platform and climbed onto it. If the platform was not located within 60 s, the rat was hand guided to it. In either case, the animal was allowed to remain on the platform for 15 s before being removed and returned to its home cage.

Training consisted of 20 trials given over 5 days. The behavioral measure recorded was the time to locate the platform (maximum 60 s). The data were analyzed by MANOVA, using trials as the repeated measure, with Tukey–Kramer post hoc comparisons.

In Vivo Electrochemistry

At 118 weeks of age, the surviving rats (four control, six deprenyl-treated) were anesthetized with urethane (1.5 g/kg) and