EFFECTS OF L-DEPRENYL ON LIFE EXTENSION AND SEXUAL BEHAVIOUR IN AGED FISCHER 344 RATS

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SUMMARY

The effect of l-deprenyl on sexual behavior and longevity was examined in aged male Fischer 344 rats. The rats (23-25 mo. old) were administered saline or l-deprenyl (0.25 mg/kg) subcutaneously on every other day. Sexual behavior was studied prior to the start of treatment and at bimonthly intervals thereafter. Over 90% of the rats were sexually active before treatment and remained so for the duration of treatment - 6 months. We interpret this result as due to the animals being too old at the start of treatment. However, significant increases in both mean (approximately 16%) and maximum (approximately 17%) survival were seen in the l-deprenyl treated rats. Blood chemistry results suggest that the increased survival may be due to improved renal function. Analysis of body weights ruled out the possibility that deprenyl-induced dietary restriction was responsible for the increased survival, since the rats on l-deprenyl showed a slower rate of body weight decrease than did controls.

Key Words: *deprenyl, life extension, sexual behavior, rats, Parkinson's disease.*

INTRODUCTION

Although originally developed as an antidepressant agent, l-deprenyl (selegeline hydrochloride) is now primarily used as an adjunct to l-dopa in the treatment of Parkinson's disease. It has been found to enhance and prolong the beneficial action of l-dopa[1] and to reduce the side effects which develop following long-term usage of l-dopa[2]. Furthermore, it significantly prolongs the survival of Parkinson's patients[3]. Recent work suggests an increased clinical utility, by indicating that l-deprenyl also slows the development of severe motor symptoms in Parkinson's patients[3]. Recent work suggests an increased clinical utility, by indicating that l-deprenyl also slows the development of severe motor symptoms in Parkinson's patients if administered at early stages[4,5].

It has been suggested that deprenyl may act by protecting the brain against toxic by-products of MAO-medicated metabolism, thus delaying the normal age-related deterioration of the nigro-striatal dopamine system and so prolonging the life of the animal[6,7,8]. In support of this Knoll (1988)[8] and Knoll et al (1989)[9] reported that 24 month old Logan-Wistar rats injected subcutaneously with deprenyl three times a week survived significantly longer than did the control rats. The deprenyl treated rats also showed a significant increase in sexual activity as compared to controls. The purpose of the present study was to attempt to replicate these findings in the Fischer 344 (F344) rat and to gather evidence on the possible mechanism(s) of action of deprenyl at the organismal level.

MATERIALS AND METHODS

Sexual behavior

A total of 70 male F344 rats were involved in tests of sexual behavior; 35 received saline and 35 received l-deprenyl (0.25 mg/kg) via subcutaneous injection every other day beginning at 23 months of age.

The rats were tested for sexual activity on three separate occasions at two week intervals, prior to the start of the experiment. This pretest procedure was intended to both provide baseline data, and give the

animals some sexual experience. Following the start of deprenyl treatment, the rats were tested every two weeks over a period of 18 weeks. At the time that testing was discontinued, all of the rats had become sexually inactive.

The female F344 rats were purchased ovariectomized at 3 months of age. Prior to testing, the females were brought into estrous by a subcutaneous injection with estradiol benzoate (8-10mg) dissolved in peanut oil 24 to 48 hours before testing and of progesterone (500mg) 4-6 hours prior to test. Before each test, the receptivity of the females was established using as a criterion the occurrence of lordosis in response to a genital probe.

The sexual behaviour tests lasted for 60 minutes in a cylindrical Plexiglas cage, illuminated with a red light, during the day part of the animal's cycle. The male was placed in the testing chamber for approximately 5 minutes and the female was then introduced. Each session was both videotaped and scored on line using a computer program which used key punches to record the time of occurrence of 5 separate behaviors: genital sniffing, attempted mounts, mounts, intromissions and ejaculations.

Survival studies

Male F344 rats received subcutaneous injection of saline (n=66) or l-deprenyl (n=66; 0.25 mg/kg) every other day beginning at 23-25 month of age. The rats survived until morbidity (in which case euthanasia was performed) or natural death. Body weights of all animals were received at each treatment time. Autopsy was done on each rat at time of death or shortly thereafter.

Serum biochemistry

Serum biochemistry data was collected from 22 control and 23 deprenyl treated rats after 3 months of treatment and was compared to measures in these animals taken before the start of treatment. Serum analysis was done by Vita-Tech, Canada, on the following measures: A/G ratio, albumin, bilirubin, BUN, creatinine, glucose, total protein, SGOT and SGPT.

RESULTS

Sexual behavior

The data from the sexual behavior testing are summarised in Tables 1 and 2. None of the animals reached ejaculation during any of the baseline sessions, and only 8 achieved intromission. Consequently, none of the animals were sexually sluggish, and the remaining animals were sexually inactive. Over the subsequent testing, only one animal on a single occasion achieved an ejaculation (a deprenyl treated animal on the first test after the start of treatment).

Experience dependent changes. Both genital sniffing and attempted mounting showed experience dependent changes. In the case of genital sniffing, the frequency score was highest on the first baseline test. Session two was significantly smaller than the score in session 1 (t =3.45, P=0.001). The difference from the last baseline session to the first post-drug session was also statistically significant. On the other hand, the latency measure showed a significant decrease from the first to second baseline test.

The attempted mount score showed an initial experience dependent increase, which continued from the last baseline test to the two week test. There was also a significant increase in frequency of attempted mounts (t =3.46, P=0.001), attempted mount latency (t =3.9, P=0.003) and latency to genital sniff (t =2.5, P=0.016).

Age dependent changes. Following baseline testing, there were progressive decreases in all indices of sexual activity, with all of the rats becoming sexually inactive after 10 weeks, when the animals reached 26 months of age (Table 2).

Effects of l-deprenyl. There were no significant differences between the rats maintained on l-deprenyl and the control rats on any measure at any time point. The only suggestion of any facilatory effect of l-deprenyl was an increase in attempted mounts at the two week test. However, an increase was also seen in the controls and, in any case, both groups showed a decrease in the four week test.

Table 1
Effect of l-deprenyl on measures of sexual responsiveness as a function of age

Test	Group	N	Genital Sniffing		Attempted Mounts	
			Frequency (Mean)	Latency (Minutes)	Frequency (Mean)	Latency (Minutes)
Base 1	Control	34	20.88±1.48	1.76±0.31	0.56±0.19	39.56±4.22
	Deprenyl	30	17.47±1.83	3.87±0.75	0.37±0.18	39.90±6.93
Base 2	Control	29	14.62±1.72	1.39±0.51	0.66±0.24	18.69±5.76
	Deprenyl	33	13.27±1.37	2.04±0.50	0.81±0.33	21.14±7.05
Base 3	Control	34	14.32±1.85	2.15±0.55	1.23±0.43	16.54±4.77
	Deprenyl	33	15.36±1.49	1.09±0.34	1.18±0.34	11.04±2.95
2 Weeks	Control	32	11.41±1.15	1.64±0.55	1.71±0.68	11.05±3.86
	Deprenyl	31	10.19±1.07	1.31±0.45	1.58±0.36	12.06±3.47
4 Weeks	Control	31	11.51±1.79	6.29±2.09	0.58±0.21	15.11±3.09
	Deprenyl	30	10.07±1.14	3.26±0.88	0.30±0.15	17.21±7.54
6 Weeks	Control	29	6.76±1.09	3.36±1.11	0.63±0.28	14.64±8.83
	Deprenyl	29	5.79±0.91	4.29±1.70	0.55±0.29	20.56±7.85
8 Weeks	Control	28	5.89±1.11	5.52±2.03	0.39±0.17	11.05±7.00
	Deprenyl	29	7.31±0.94	4.79±1.48	0.41±0.14	15.36±3.79
10 Weeks	Control	27	7.78±1.14	4.90±2.51	0.56±0.23	17.25±5.16
	Deprenyl	27	10.26±1.40	1.64±0.71	0.70±0.27	17.74±9.36
12 Weeks	Control	22	6.41±1.10	1.48±0.61	0.91±0.20	10.69±5.59
	Deprenyl	23	8.13±1.68	3.38±0.55	0.52±0.18	14.96± 7.44
14 Weeks	Control	19	6.11±0.78	1.91±0.78	0.16±0.12	15.30±12.16
	Deprenyl	22	5.59±0.89	4.72±1.96	0.36±0.14	11.83±8.94
16 Weeks	Control	18	5.39±1.17	1.09±0.23	0.22±0.10	9.24±5.47
	Deprenyl	19	4.47±0.84	7.27±3.57	0.16±0.12	5.15±1.22
18 Weeks	Control	13	3.08±0.54	8.53±5.19	-	-
	Deprenyl	14	3.14±0.74	2.38±1.26	0.07±0.07	2.09±0

Table 2
Frequency of occurrence of sexual activities as a function of age and deprenyl treatment. Scores represent number of animals showing behavior at each time point

Session	Group	N	Attempted Mount	Mount	Intromission	Ejaculation
Baseline 1	Control	34	10	4	1	0
	Deprenyl	30	7	3	1	0
Baseline 2	Control	29	9	3	2	0
	Deprenyl	33	9	5	1	0
Baseline 3	Control	34	12	3	3	0
	Deprenyl	33	14	4	2	0
2 Weeks	Control	32	16	4	1	0
	Deprenyl	31	21	4	1	1
4 Weeks	Control	31	10	1	0	0
	Deprenyl	30	5	0	0	0
6 Weeks	Control	29	6	1	0	0
	Deprenyl	29	7	1	0	0
8 Weeks	Control	28	6	0	0	0
	Deprenyl	29	8	0	0	0
10 Weeks	Control	27	7	1	0	0
	Deprenyl	27	7	1	0	0
12 Weeks	Control	22	5	0	0	0
	Deprenyl	23	8	0	0	0
14 Weeks	Control	19	2	0	0	0
	Deprenyl	22	6	0	0	0
16 Weeks	Control	18	4	0	0	0
	Deprenyl	19	2	0	0	0
18 Weeks	Control	13	0	0	0	0
	Deprenyl	14	1	0	0	0

Survival

Each rat was assigned a score equal to its survival in days following start of treatment, and the student's t-test was used to determine group differences. First, there was no significant difference (P=0.457) between rats which began treatment at 23 months and participated in sexual testing and the rats which began treatment at 25 months but did not undergo sexual testing. Data from all animals was thus pooled.

The mean survival for the deprenyl group (133.7 ± 8.3 days) was significantly longer than that of the control group (114.7 ± 7.7) by approximately 16% (P=0.048). Further, the maximum survival (defined as the mean of the longest surviving 10% of the animals) of the deprenyl group (248.4 ± 11.7 days; n =7) was also significantly longer than that of the control group (212.1 ± 8.9; n =7) by approximately 17% (P=0.015, one tailed test). The details of this study have been reported previously[10].

Serum biochemistry, autopsy and body weight

The only serum measure that was significantly affected by treatment with l-deprenyl was that of blood-urea nitrogen (BUN). This measure was significantly lower in the deprenyl group after three months of treatment, indicating maintenance of renal function during aging. Detailed serum chemistry results have been reported previously[10].

Autopsy results indicated numerous tumors, cataracts and liver and kidney problems, but there were no significant differences between the deprenyl and control groups.

Analysis of body weights indicated that the deprenyl treated rats maintained body weights significantly better into old age than did the control rats. Details have been reported in Milgram et al. (1990)[10].

DISCUSSION AND CONCLUSIONS

Our results indicate that while survival can be significantly increased by 16-17% in 23-25 month male F344 rats by treatment with deprenyl, sexual activity is not increased.

These results warrant discussion since Knoll and colleagues[7,8,9] have previously reported an increase lifespan of 210% (our calculations) after start of deprenyl treatment in aged (24 months) Logan-Wistar rats, as well as significant increase in sexual behavior in aged deprenyl treated rats.

These discrepancies may well be due to strain differences. Knoll began treatment at 24 months in Logan -Wistar rats. His control rats survived to about 35 months; the rats had thus attained about 68% of their mean lifespan at start of treatment. We began treatment at 23-25 months in F344 rats that have a mean lifespan of 22-24 months; our rats had thus already attained 100% of their mean life expectancy. At start of treatment, 90% of our rats exhibited no sexual behavior and the other 10% exhibited very little. It is likely that the rats were simply too old to undergo a major behavior change by this time. Also, at start of treatment, a number of our rats already had visible tumors and renal problems that are characteristic of this strain. It is unlikely that deprenyl treatment could reverse these already present pathologies.

One important difference between our work and that of Knoll and colleagues[8,9] was the baseline level of sexual activity of the animals at the start of the experiment. In their experiment, although no animals were sexually active (reached ejaculation) during baseline testing, 44 of 132 animals (33%) were sexually sluggish (showed intromission and mounting) and 46 animals (35%) were sexually inactive. We also had no animals which achieved ejaculation, but only 8 (6%) of our animals were sexually sluggish, while 120 (90%) of our animals were sexually inactive at the start of the experiment. These differences raise the possibility that the mechanisms controlling sexual activity were in a more advanced stage of our experiment than they were in the rats used by Knoll's group.

Since the Fischer rat is capable of sexual activity at 23 months, the contrast between our findings and those of Knoll and colleagues suggest that the primary effect of l-deprenyl is on the learning component. According to this hypothesis, deprenyl appears to facilitate the acquisition of sexual activity in inexperienced rats. A similar conclusion is suggested by the results of Dallo et al., (1986)[12] who found that deprenyl was effective in inducing sexual activity in young rats that had been sexually inactive.

Finally, we can't rule out the possiblity that strain or species differences are of critical importance. Species differences were also suggested for data reported by Chambers and Phoenix (1983)[11] who were also unable to demonstrate changes in sexual behavior following long term treatment with deprenyl of both aged and young rhesus monkeys.

The fact that the deprenyl treated animals in our study survived longer that the controls indicated that deprenyl may have had a beneficial effect on one or more of the systems that would contribute to aging and death of the animals. The maintenance of lower serum BUN levels in the deprenyl group may indicate that deprenyl provides protection of renal function and that this may be one way of prolonging life in these rats. The maintenance of higher body weights by the deprenyl-treated, as compared to the control, rats into old age may likewise indicate that deprenyl protects metabolic function - or at least helps to maintain apetite - into old age. These latter results are also important in that they indicate that deprenyl is probably not working via a self-imposed dietary restriction mechanism. However, in future studies, it will be important to measure caloric intake in control and deprenyl treated rats and to determine if deprenyl may be effecting various metabolic parameters in a way similar to the effects of dietary restriction.

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