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Effects of Chronic Oral Administration of L-Deprenyl in the Dog

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MILGRAM, N. W., G. O. IVY, M. P. MURPHY, E. HEAD, P. H. WU, W. W. RUEHL, P. H. YU, D. DURDEN, B. A. DAVIS AND A. A. BOULTON. *Effects of chronic oral administration of* L-*deprenyl in the dog.* PHARMACOL BIOCHEM BEHAV **51**(2/3) 421–428, 1995.—Dogs were administered capsules containing L-deprenyl daily over 3 weeks at dose levels of 0, 0.1, 0.5, and 1.0 mg/kg. Spontaneous behavior was measured using a canine open field test, and was not significantly affected by L-deprenyl. Plasma levels of amphetamine showed a clear dose-dependent elevation 2 h following treatment, but were markedly lower after 24 h, and were undetectable 5 days following the last treatment. Plasma levels of phenylethylamine were increased, but were highly variable. Animals sacrificed 1 day following the last treatment showed a dose-dependent inhibition of monoamine oxidase B in the brain, liver, and kidney, whereas monoamine oxidase A was unaffected in these tissues. L-Deprenyl also caused an increase in phenylethylamine in the striatum and hypothalamus, but not in the neocortex. Brain levels of DA, DOPAC, 3-MT, HVA, 5-HT, and 5-HIAA were unaffected. The pharmacological profile for the dog is distinct from that of other species in that long-term treatment did not produce any significant inhibition of MAO-A activity. The absence of an effect on biogenic amines or metabolites suggests that the metabolism of dopamine is mediated at least in part through pathways other than MAO-B in the normal adult dog.

L-Deprenyl Dog Monoamine oxidase A Monoamine oxidase B Phenylethylamine Amphetamine Monoamines Monoamine metabolites

L-DEPRENYL is a selective, irreversible inhibitor of monoamine oxidase B (MAO-B) that is widely used in the symptomatic treatment of Parkinson's disease (PD) (4). Recent research has also indicated a possible neuroprotective application. Treatment during the early stages of Parkinson's disease delays the development of PD symptoms (36,37). L-Deprenyl has also been found to both increase the survival of PD patients (3) and to prolong the life of aged laboratory rats (18, 19,22). These findings suggest an applicability for L-deprenyl beyond the treatment of PD. This possibility is under investigation in several animal studies with laboratory rats. However, the utility of the rat as a model system for human applications is problematic: there are marked species differences in both the ratio of MAO-B to MAO-A and in the role of each in catecholaminergic deamination. In both the human and monkey, brain dopamine (DA) is either a substrate of MAO-B or of both isoforms (12,25), and basal levels of DA are increased by chronic L-deprenyl treatment (5,32). On the other hand, MAO-B does not play a direct role in brain dopamine metabolism in the rat brain, and DA levels are less affected by L-deprenyl than they are in humans (16,26).

The primary purpose of the present experiment was to provide an overview of the pharmacological effects of long-term oral administration of L-deprenyl in the dog. There is evidence that MAO-B is the predominant form of monoamine oxidase (MAO) in the canine brain (24,34), but there is also evidence to the contrary (15), and there have not been any studies of the role of MAO-B in catecholamine metabolism in this species. Thus, it was of importance to determine the pharmacological profile of L-deprenyl in the dog. Following treatment with L-deprenyl, determinations were made of brain levels of both forms of MAO, serotonin (5-HT), DA, and metabolites

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of DA and 5-HT. In addition, we also evaluated brain and plasma levels of phenylethylamine (PE) and amphetamine. Amphetamine is a metabolite of L-deprenyl (30,41), and levels in both brain and urine are increased following L-deprenyl treatment (28,31). Phenylethylamine levels have also been found to increase following L-deprenyl treatment, most likely due to PE being a substrate of MAO-B (27–29). We also monitored spontaneous behavioral responses using a canine open field test that we have previously described (13). L-Deprenyl has been found to increase activity in the rat (11), and we have reported that there is a dose-dependent effect on locomotor activity in the dog following a single treatment (13). It is not known how chronic administration of this drug affects locomotor activity in this species.

METHOD

Subjects

Twenty-two dogs, including 17 beagles and 5 dogs of mixed breeds, were used in this study. Twenty-one of the animals were assigned to one of four treatment groups: a control group (placebo, $N = 3$), a low-dose group (0.1 mg/kg, $N = 6$), a medium-dose group (0.5 mg/kg, $N = 6$), and a high-dose group (1.0 mg/kg, $N = 6$). An additional control animal (untreated) provided brain tissue, but was not used for the blood assays. The additional animal was included to increase the size of the control group, with the assumption that the effects of placebo treatment on brain chemistry were similar to that of no treatment. The group assignment was with a procedure that counterbalanced age, sex, and breed. Administration was oral using capsules filled with either L-deprenyl or sucrose (for the placebo control). The animals were treated daily [as in clinical studies in the human (3,4,17,36)], approximately 4 h before feeding, over a 3-week period. All personnel associated with data collection and dosing of the dogs were blinded with respect to the experimental treatment.

Thirteen beagles were used in an analysis of the effects of L-deprenyl on brain tissue. This included four control animals and three animals at each of the three dose levels. Twenty-four hours following the last treatment, the animals were sacrificed with an overdose of sodium pentobarbital, their brains immediately removed, and the striatum, hypothalamus, frontal cortex, and hippocampus dissected out. Additionally, samples of liver and kidney were taken. All tissue samples were immediately frozen in liquid nitrogen and stored at −70°C until assayed.

Test Procedures

Analysis of plasma amphetamine and PE. Blood samples were taken for subsequent analysis of plasma levels of amphetamine and PE on five occasions: 1) just prior to the initial dosing; 2) 2 h following the seventh day of treatment; 3) 2 h after the 14th treatment; 4) 24 h following the last dose (3 weeks of treatment); and 5) 5 days following the last dose. We chose a 2-h sample delay to coincide with the peak amphetamine levels after L-deprenyl dosing (33). The final 5-day test was from the nine dogs that were not sacrificed. Blood samples were drawn (10 ml) and centrifuged in the morning, prior to feeding. The plasma was removed and frozen at −70°C. Sample analysis of the monoamines was completed within 1 week of tissue harvesting. All other assays were completed within 2 weeks.

PE and amphetamine were quantified using the method of Durden, Davis, and Boulton (9). The *N*-acetyl-*N*-pentafluorobenzoyl derivatives were detected by negative chemical ionization gas chromatography-mass spectrometry (NCI-GC-MS) and quantified by high resolution selective ion monitoring (HRSIM) of the negative molecular ions (9). The ions monitored were m/z 357.0810 and 361.1061 for PE and deutero-PE, respectively, and m/z 371.0966 and 376.1279 for amphetamine and deutero-amphetamine. Levels of detection for phenylethylamine and amphetamine were 8–10 pg/ml and 30 pg/ml, respectively.

Determination of brain amines and metabolites. Brain samples from striatum, hypothalamus, and cortex were analyzed for dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-MT, 3-methoxy-tyramine, 5-hydroxytryptamine (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) by high performance liquid chromatography (HPLC) with electrochemical detection (43).

Determination of MAO-A and MAO-B activities. A radioenzymatic procedure modified from that described by Wu and Dyck (40) as described previously (23) was used in analysis of MAO-A and B activities (from samples of hippocampus, striatum, liver, and kidney). Briefly, the tissue was homogenized in phosphate buffer, centrifuged at 1000 × *g* and the supernatants collected. MAO-A activity was determined using [^{14}C]5-HT as substrate, and MAO-B using [^{14}C]2-PE. Clorgyline (5×10^{-7} M) and L-deprenyl (10^{-6} M) were used to determine nonspecific activity in the appropriate assays. Reactions were carried out at 37°C for 30 min and terminated by the addition of 2 N citric acid. The radioactive metabolites were extracted into 1 : 1 toluene : ethylacetate (v/v) following the termination of the reaction. MAO-A activity is defined as the enzyme activity that is not inhibited by 10^{-6} M L-deprenyl, but is inhibited by 5×10^{-7} M clorgyline. MAO-B activity is defined as the enzyme activity that is not inhibited by 5×10^{-7} M clorgyline, but is inhibited by 10^{-6} M L-deprenyl. Protein determination was by the method of Lowry (21).

Measurement of PE in brain tissue. PE in dog brain regions was analyzed by mass spectrometry using the NCI-GC-MS described above, and modified for tissue type (8).

Open field activity. Spontaneous behaviors were measured as described by Head and Milgram (13). Briefly, the animals were placed in a standard test room, 3.66 × 3.66 m, for 10 min. An observer in an adjoining room used a dedicated computer program to record all locomotion, exploratory sniffing, urination, and inactivity. Each animal was tested twice: 1 day before the start of treatment and again 2 h following the seventh treatment. The analysis was based on difference scores obtained by subtracting the baseline from the seventh day test score.

Statistical Analysis

Dose–response data were analyzed using SAS statistical procedures. Dose response data for the spontaneous behavior measure was analyzed using a repeated measures one-way analysis of variance (ANOVA). A repeated-measures ANOVA was used in the analysis of plasma PE and amphetamine. Analysis of brain MAO-A, MAO-B, and phenylethylamine was by multivariate analysis of variance (MANOVA). Multiple comparisons were conducted using Dunnett's test.

RESULTS

Plasma Levels of Amphetamine and Phenylethylamine

There was no detectable baseline amphetamine in the plasma. However, there was a significant dose-dependent ef-

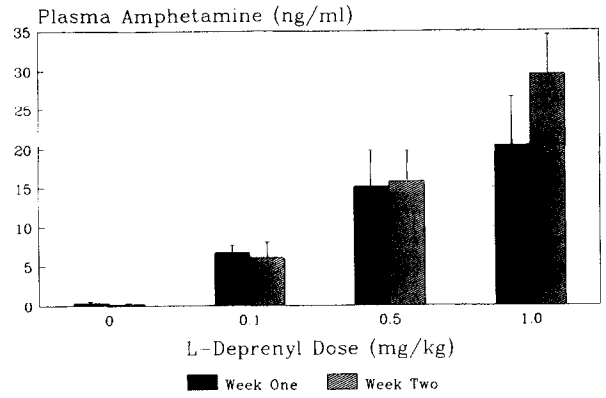

FIG. 1. Plasma levels of amphetamine following oral administration of L-deprenyl. The figure shows two replications during the period of chronic treatment, after 1 and 2 weeks of treatment. Four dose levels were studied and samples were taken 2 h following treatment. Bars show SE.

fect of L-deprenyl. The data from the 1- and 2-week tests are shown in Fig. 1.

There was a significant main effect of dose on plasma amphetamine concentration, $F(3, 17) = 9.45$, $p < 0.0007$. Levels of amphetamine were not statistically different between weeks 1 and 2, suggesting that the plasma levels of this metabolite had reached a steady-state after only a single week of treatment (no significant time or time × dose interaction). The apparent increase in plasma amphetamine within the 1.0-mg/kg group at 2 weeks was not significant (paired t-test). As shown in Fig. 2, amphetamine concentration was reduced when the samples were taken 24 h following the last treatment, but there was still a significant overall elevation, $F(1, 17) = 22.22$, $p < 0.0005$. When measured 5 days following treatment, amphetamine levels were undetectable.

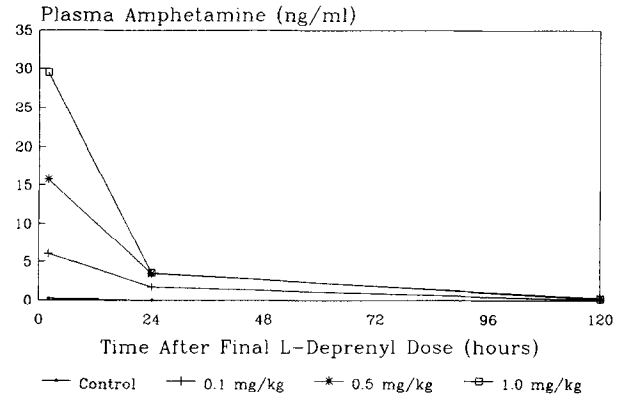

FIG. 2. Time-dependent decrease in plasma levels of amphetamine following termination of treatment with L-deprenyl. The results are plotted for threee different dose levels and placebo controls. Amphetamine was measured at 2 h, 24 h, and 5 days following treatment. The data plotted for the 2-h test are from the 2-week administration shown in Fig. 1. The 24-h data were based on samples collected from animals 24 h following their final treatment (3 weeks). The data from 5 days following treatment termination were collected from the nine animals that were not sacrificed (three per dose of L-deprenyl). Due to extensive overlapping of points at the 24-h and 5-day time points, error bars have been omitted for clarity (the error bars for the 2-h group may be seen in Fig. 1).

Levels of plasma PE following treatment with L-deprenyl are shown in Table 1. Plasma PE was increased on both the 1- and 2-week tests, and decreased 24 h following the last treatment. There was a statistically significant difference between control and treated animals at the 2-week test ($p < 0.05$). Even though the dose-response relationship was not statistically significant, there was a clear trend towards increasing PE concentrations with increasing dose of L-deprenyl. Much of the variability in the data was attributable to one dog in the 0.5-mg/kg group, that had levels three times greater than any other animal. The data from this dog were similar on both tests, and dropped to baseline levels after treatment was discontinued.

Brain MAO-A, MAO-B, Monoamines, Monoaminergic Metabolites, and PE

The results of the analysis of dopamine, serotonin, and metabolites are summarized in Table 2. There were no significant effects of L-deprenyl.

In the control animals, mean MAO-B specific activity from the striatum and hippocampus did not differ significantly ($t = 2.29$, $p < 0.11$). As shown in Fig. 3(A,B), L-deprenyl produced a dose-dependent inhibition of MAO-B activity in these brain structures. The results of the ANOVA were highly significant for both structures [$F(3, 9) = 13.84$, $p < 0.001$ for the striatum, and $F(3, 9) = 17.98$, $p < 0.0005$ for the hippocampus]. A similar dose-dependent inhibition was observed in both the liver, $F(3, 9) = 16.6$, $p < 0.0005$, and kidney, $F(3, 9) = 36.2$, $p < 0.0001$ [Fig. 3(C,D), respectively].

The baseline level of MAO-A activity in the hippocampus was significantly greater than the striatum ($t = 3.35$, $p < 0.05$). As is clear from Fig. 3, L-deprenyl had no detectable effects on MAO-A activity in any of the tissue types examined in this study. Activity levels of MAO-B in the liver and kidney were very high in comparison with measured levels of MAO-A.

Differences in PE levels between frontal cortex, hypothalamus, and striatum were statistically significant, $F(2, 18) = 11.29$, $p < 0.0007$ (Fig. 4). The results of the repeated-measures analysis revealed a significant overall effect of dose, with a clear upward trend in all three regions, $F(3, 9) = 5.17$, $p < 0.025$. Dunnett's test for comparison with a control showed a significant effect at 1.0 mg/kg in both the hypothalamus and striatum, but not in the frontal cortex. No other comparisons were significant.

TABLE 1

EFFECT OF L-DEPRENYL ON PLASMA CONCENTRATIONS OF PHENYLETHYLAMINE

Dose (mg/kg)	N	Baseline	1 Week Test	2 Week Test*	1 Day Post
0	3	48.3 ± 4.5	40.3 ± 5.7	40.3 ± 15.7	72.3 ± 30.5
0.1	6	25.2 ± 2.2	53.3 ± 13.2	50.3 ± 10.2	54.3 ± 10.7
0.5	6	33.8 ± 8.8	114.8 ± 22.3	105.0 ± 40.9	62.0 ± 7.8
1.0	6	33.0 ± 3.3	85.8 ± 16.2	74.8 ± 5.9	61.0 ± 10.5

Values are mean ± SE in pg/ml.

*Difference between control and treated animals significant, $p < 0.05$.

TABLE 2

EFFECT OF L-DEPRENYL ON BRAIN MONOAMINES AND METABOLITES

Dose (mg/kg)	N	DA	DOPAC	HVA	3-MT	5-HT	5-HIAA
				Striatum			
0	4	7526 ± 1080	1643 ± 397	12159 ± 404	420 ± 12	880 ± 190	424 ± 62
0.1	3	7148 ± 2682	1290 ± 465	12544 ± 1703	491 ± 162	856 ± 210	356 ± 59
0.5	3	8692 ± 415	1389 ± 166	14330 ± 1901	490 ± 98	1454 ± 475	552 ± 134
1.0	3	8251 ± 1480	2030 ± 374	11504 ± 243	439 ± 67	1080 ± 79	470 ± 29
				Cortex			
0	4	17 ± 2	6 ± 2	263 ± 48	0	180 ± 35	84 ± 16
0.1	3	27 ± 10	10 ± 3	145 ± 41	0	151 ± 30	77 ± 14
0.5	3	23 ± 5	8 ± 2	361 ± 151	5 ± 2	210 ± 28	82 ± 12
1.0	3	16 ± 2	4 ± 1	292 ± 195	0	215 ± 131	95 ± 40

Values are means ± SE in ng/g of tissue; there were no significant effects.

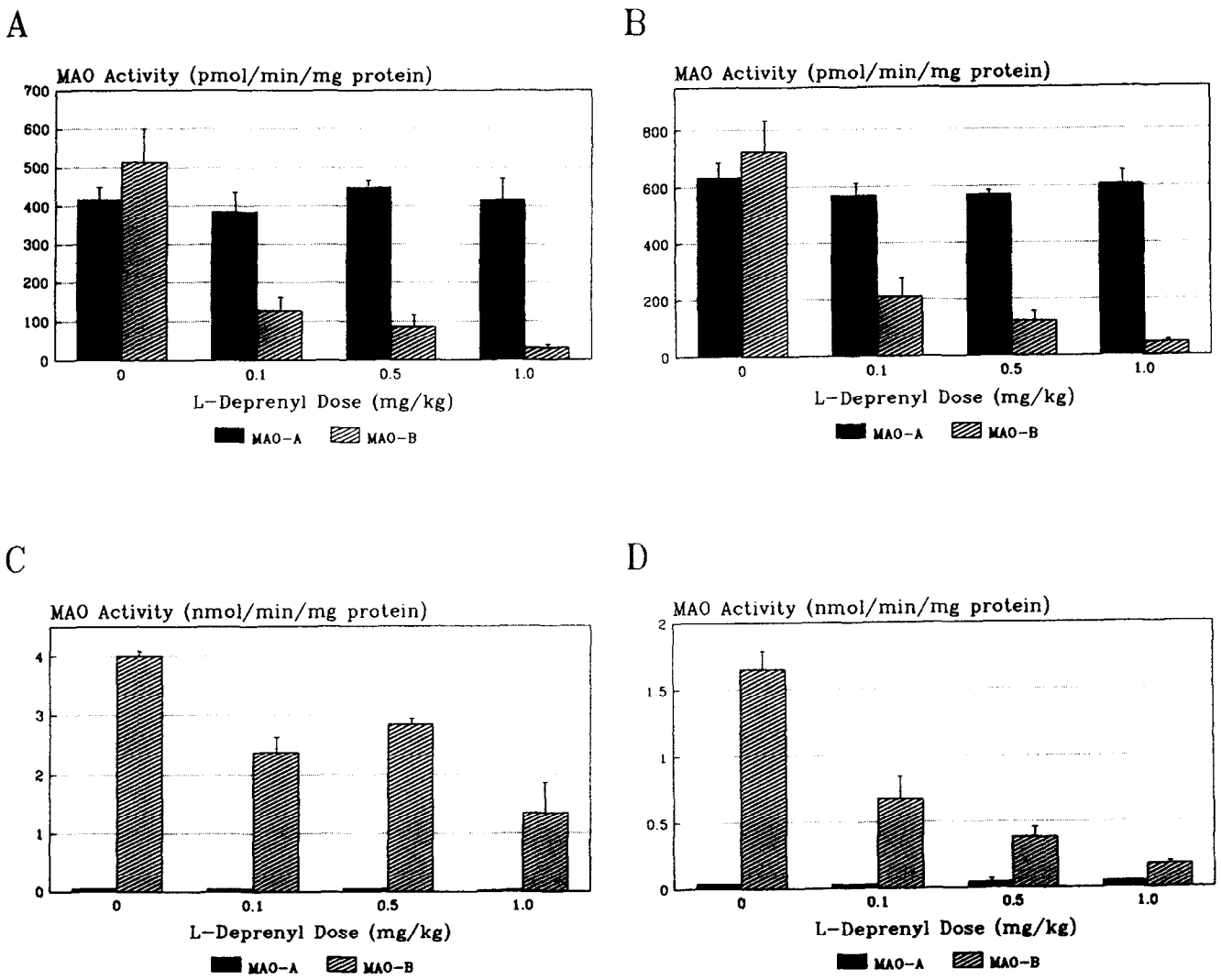

FIG. 3. Effect of L-deprenyl on MAO-A and B activity in striatum (A), hippocampus (B), liver (C), and kidney (D) at four different doses. Enzyme activity in striatum and hippocampus is given in pmol/min/mg protein, whereas activity in liver and kidney is given in nmol/min/mg protein.

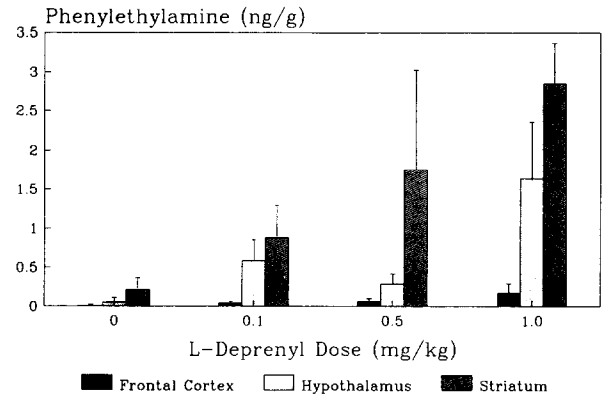

FIG. 4. Effect of increasing dose of L-deprenyl on levels of phenylethylamine in three different brain regions.

Open Field Results

There were no significant effects of L-deprenyl on open field activity. Figure 5 shows the results from the four measures that had previously been found to be sensitive to L-deprenyl: total movement, exploratory sniffing, inactivity, and sniffing. There was a small, nonsignificant increase in total movement, $F(3, 20) = 2.62$, $p < 0.09$. Sniffing decreased in all groups, but to a lesser extent in the animals administered L-deprenyl. Also, examination of the movement patterns revealed no group differences.

DISCUSSION

The present report is the first systematic study of the effects of chronic oral administration of L-deprenyl in the dog. At the dose levels used in this study (0.1, 0.5, 1.0 mg/kg), L-deprenyl caused a reliable increase in plasma levels of amphetamine and phenylethylamine, and in brain levels of phenylethylamine. L-Deprenyl did not affect locomotor activity or other mea-

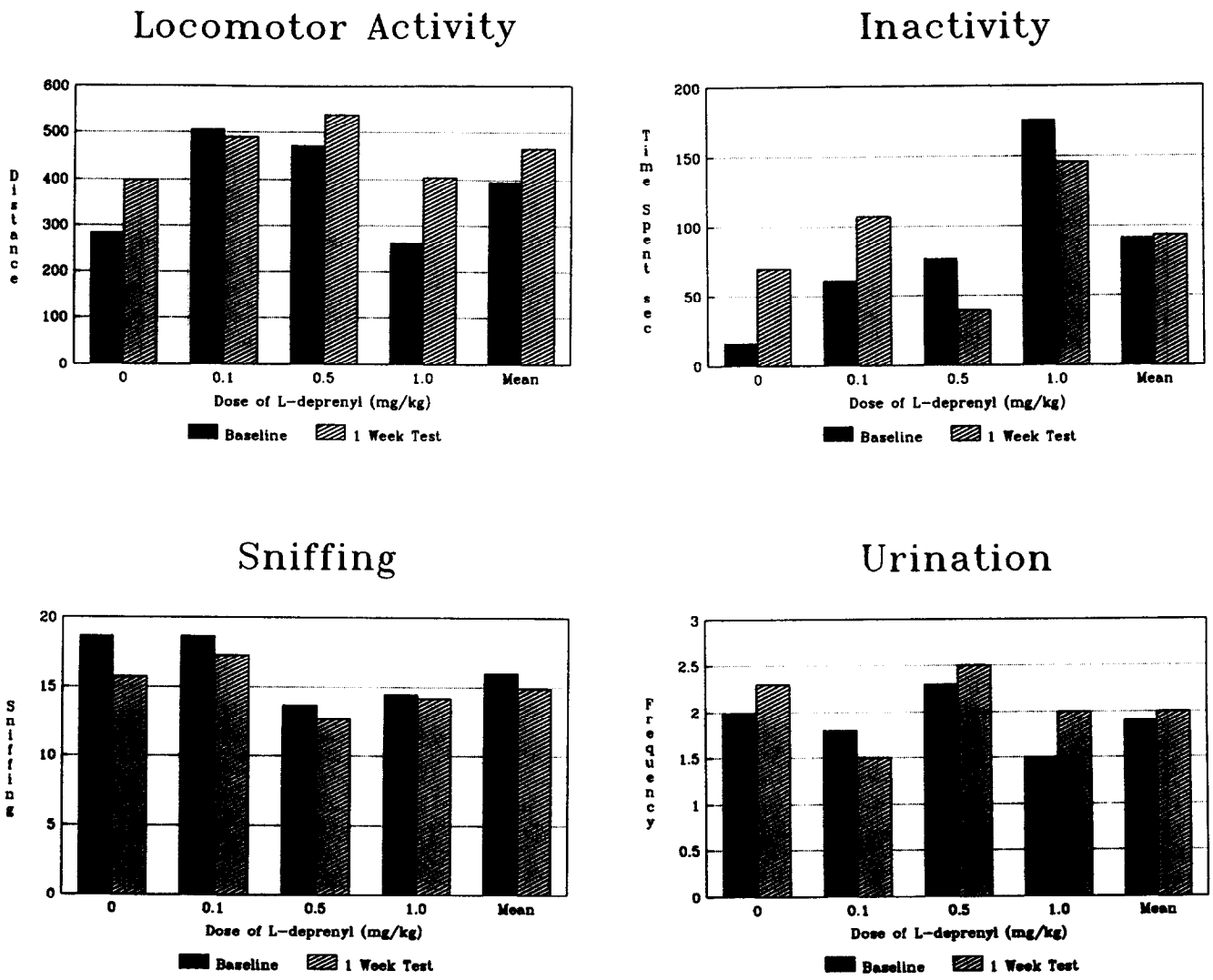

FIG. 5. Effect of L-deprenyl on spontaneous behaviors. The baseline results are compared with the scores obtained following 1-week of treatment at four different dose levels for four measures of spontaneous behavior. Locomotor activity is in arbitrary distance units with one unit approximately equal to 61 cm. Inactivity is represented as time. Both sniffing and urination are scored in terms of frequency of occurrence. None of the differences approached statistical significance. Error bars have been omitted for clarity.

sures of spontaneous behavior, activity of brain MAO-A, and brain levels of DA, DOPAC, 5-HT, and metabolites. As expected, MAO-B activity was significantly inhibited.

Analysis of plasma amphetamine levels after treatment with L-deprenyl revealed a dose-dependent relationship 2 h after treatment, and detectable levels of amphetamine after 24 h, but not after 5 days. The plasma levels of amphetamine recorded in animals chronically treated with L-deprenyl are consistent with those recently reported by Salonen (33) and with earlier evidence of increased levels of amphetamine in the brain and urine of mice (28). The possibility has previously been raised that some of the therapeutic effects of L-deprenyl are attributable to the presence of active metabolites such as amphetamine and methamphetamine (10). The amphetamine metabolites of L-deprenyl are of the L-form and no racemic transformation has been detected (14,41), and this has also been determined in the dog (33). In the dog, the levels of amphetamine produced by daily administration of L-deprenyl at doses less than or equal to 1.0 mg/kg are unlikely to have significant behavioral effects. The highest levels of amphetamine that we recorded were between 20 and 40 ng/ml. These levels are less than one-tenth of the levels reported by Bareggi et al. (2), who observed hyperthermia and stereotypy when *d*-amphetamine was given at 2 mg/kg orally, producing approximately 500–600 ng/ml of serum L-amphetamine.

In a previous study, we reported distinctive changes in behavior following single oral doses of L-deprenyl at 3 mg/kg or higher (13). The pattern was characterized by repetitive locomotion, decreased exploratory sniffing, and decreased urinary marking. In the present study, chronic treatment led to a small, nonsignificant increase in total locomotion at a dose of 1.0 mg/kg, and the activity pattern observed following chronic administration of this dose was markedly different from that observed following acute treatment with higher doses (i.e., 3 mg/kg). There was no repetitive behavior, and the animals receiving L-deprenyl showed similar levels of exploratory behavior compared to controls.

We also found a trend towards a dose-dependent increase in plasma levels of phenylethylamine after L-deprenyl, but this was not statistically significant. This effect was also considerably weaker than the elevation observed in plasma amphetamine. In this study, therefore, the measurement of plasma PE is a poorer dose-dependent correlate of the efficacy of oral L-deprenyl than amphetamine.

Assays of brain PE, another substrate of MAO-B (27–29), also revealed that L-deprenyl caused a dose-dependent increase. The magnitude of the increase was somewhat greater than has been reported previously in studies with other species. Paterson et al. (26) did not find a significant change in striatal PE following injection of rats with 0.5 mg/kg of L-deprenyl. In contrast, we found that an oral dose of 0.1 mg/kg caused an increase of almost 400% in the striatum and 1000% in the hypothalamus when the tissue was taken 24 h after the last treatment with L-deprenyl (Fig. 4). These species differences are probably not attributable to differences in the inhibition of MAO-B. Oral administration (as was used for the dog) produces less inhibition of brain MAO-B than the same dose given by injection (23,35) in the rat. Species differences in basal levels of PE is another factor that should be considered, because the basal levels in the dog caudate are about 25% of those of the rat. Possibly most importantly, in the present study the dogs were treated chronically whereas previous reports have been based on changes following a single treatment (24). However, we cannot rule out the possibility that the procedure for harvesting the tissue of rats (decapitation) can result in different MAO and metabolite measures than those obtained by drug overdose, as was done in the dog.

We also found that the effect of L-deprenyl on brain PE varied among brain regions. There were dose-dependent increases in the striatum and hypothalamus, but not in the cortex; however, an upward trend (nonsignificant) in cortical PE was evident. It has been suggested that brain PE is synthesized inside dopaminergic terminals, where it diffuses out and is rapidly metabolized (1). If so, we would not expect there to be a large absolute change in PE from structures with low basal DA levels. In fact, we found that cortical levels of DA were minimal when compared with those of the striatum (17 vs. 7526 ng/g). Knoll (20) has argued that the action of L-deprenyl is selective to the nigrostriatal dopaminergic system. Although the present findings do not directly support this suggestion, differences between brain structures in the release and metabolism of PE provide a possible mechanism for a structure-specific action of L-deprenyl.

With respect to MAO-A and MAO-B, we found a dose-dependent inhibition of brain MAO-B, but no significant change in MAO-A at any dose. The dose-dependent inhibition of brain MAO-B by L-deprenyl was expected, but the absence of any inhibition of MAO-A was not, because our animals were treated daily over a 3-week period. With other species, a single treatment with L-deprenyl at these doses (23) has little effect on MAO-A, but repeated treatment will cause MAO-A inhibition (23,35,39,42).

In this study, assays were also done of DA, 5-HT, and of their metabolites. There were no significant effects of L-deprenyl, and there were no clear trends. These results are different from those obtained from the primate and guinea pig, both of which show increased levels of DA following L-deprenyl (5,16). In the rat, DA levels are not increased following single treatment (16), but following repeated administration there is an increase in brain DA and a decrease in DOPAC (7,38). It appears that for both the dog and the rat, dopamine metabolism is mediated by MAO-A rather than MAO-B. The species differ in their response to repeated treatment, which results in MAO-A inhibition in the rat, but not in the dog. It is important to emphasize, however, that we used oral administration with a maximum dose of 1.0 mg/kg. We cannot rule out the possibility that a higher dose or a different route of administration could affect measurements of DA. Although it is possible that the sodium pentobarbital influenced the biochemical results, this is unlikely because barbiturates have little effect on DA metabolism (6).

The absence of any effect of L-deprenyl on brain DA and metabolites does not rule out L-deprenyl affecting dopaminergic transmission. As discussed previously, L-deprenyl caused a substantial increase in brain levels of PE, and both Paterson et al. (26,27) and Balster and Schuster (1) have shown that PE can facilitate catecholaminergic transmission.

To conclude, the pharmacological profile of the effects of repeated L-deprenyl administration in the normal dog is unique in the selective inhibition of MAO-B, with the absence of any effect on dopamine levels. Because brain MAO-A levels are not affected even after chronic administration of L-deprenyl, the dog could provide a useful model for in vivo studies of MAO-B.

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