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Behavioral Activating Effects of Adrafinil in Aged Canines

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SIWAK, C. T., P. GRUET, F. WOEHRLÉ, M. SCHNEIDER, B. A. MUGGENBURG, H. L. MURPHEY, H. CALLAHAN AND N. W. MILGRAM. *Behavioral activating effects of adrafinil in aged canines.* PHARMACOL BIOCHEM BEHAV **66**(2) 293–300, 2000.—Adrafinil, a vigilance enhancing pharmaceutical, was administered to aged dogs for 14 consecutive days at doses of 10, 20, 30, or 40 mg/kg using a crossover design. The effects on spontaneous behavior in a 10-min canine open-field test were systematically recorded every fourth day, starting with day 1 of treatment. The open field tests were given 2 or 10 h following oral administration of capsules containing either adrafinil or lactose, the placebo control. Adrafinil caused an increase in locomotor activity at the three highest doses at both the 2- and 10-h intervals and during both the first (days 1 and 5) and second treatment week (days 9 and 13). Adrafinil also caused a transient increase in directed sniffing. At the highest dose level, adrafinil caused a decrease in urination frequency. The increased locomotion was generally unaccompanied by stereotypical behavior in the test session. There was some variability; a subpopulation of animals showed either no effect, or decreased locomotion. The individual differences were correlated with changes in serum levels of adrafinil 10 h following treatment. © 2000 Elsevier Science Inc.

Adrafinil Modafinil Locomotor activity Spontaneous open-field behavior Canine Aging

(DIPHENYLMETHYL)SULFINYL-2 ACETOHYDROXAMIC ACID (adrafinil) is a recently developed pharmaceutical that is effective in increasing alertness and enhancing vigilance in the elderly (1,14). Treatment of animal subjects with adrafinil causes increased behavioral activity, without inducing amphetamine-like stereotypy. This result has been reported in mice (24), monkeys (19), and rats (4). Adrafinil also affects other behaviors, but not necessarily by producing facilitation. Delini-Stula and Hunn (4) reported that adrafinil produced a selective suppression of apomorphine-induced yawning in the rat. Similar behavioral effects are also caused by treatment with modafinil, the partly metabolized amide form [(diphenylmethyl)sulfinyl-2 acetamide] (13). Direct administration of modafinil causes increased behavioral activity in mice, rats (29,32), and monkeys (13). There is an issue, however, about whether the increased activity is due to increased movement or increased awake time (7). Two other metabolites have been identified—an acid form (CRL40467), and a sulfone (CRL41056) form—but no evidence exists linking them to the behavioral effects induced by adrafinil (2).

The mechanism of action of adrafinil is generally linked to an agonistic effect on the noradrenergic system in the central nervous system, specifically to postsynaptic alpha-1 receptors. Alpha-1 antagonists reduce or block the locomotor enhancing effects of adrafinil, while yohimbine, an alpha-2 antagonist, is ineffective unless administered at very high doses (24,25). Other mechanisms, however, have also been suggested. Recent work indicates a possible inhibitory action on GABA release, increased glutamate release or an increase in cerebral metabolism (8,31,33).

The behavioral activation produced by both adrafinil and modafinil has been compared to that produced by amphetamine, and it appears to involve a different mechanism. In addition to increased activity, amphetamine also causes behavioral stereotypy, tachycardia, hypertension, tolerance, dependence, anorexia, and anxiety. These effects are not observed following treatment with adrafinil or modafinil (26,30).

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The present investigation was concerned with the behavioral activating effects of adrafinil in aged canines. Our interest in aged dogs stems from the possibility that dogs can provide a useful model for studying human aging (3). The specific focus of this study was on spontaneous behavior. We have previously found that aspects of spontaneous behavior vary as a function of age (11,12). Age-dependent changes in behavior have also been noted in rodents (15,27), monkeys (9), cats (16), and rabbits (6). As previously mentioned, adrafinil has been reported to affect spontaneous behavior in animal subjects, but no published studies have been done on canines or on aged animals. Spontaneous behavior of the dogs was studied using a modified open field procedure, which we previously developed to assess effects of drugs on activity of canines (12). The animals were administered capsules containing adrafinil on a daily basis for 14 consecutive days. Measurements of spontaneous behavior were recorded on four different occasions during this period, and on four corresponding occasions during a 14-day placebo control phase.

METHOD

This placebo-controlled, fully blinded study was performed in accordance with Good Laboratory Practices (GLP) and NIH guidelines for the care and use of research animals. The study was approved by the Institutional Animal Care and Use Committee at the study site, Lovelace Respiratory Research Institute.

Subjects

The experiment was conducted with 32 beagle dogs (16 male and 16 female), from 9 to 16 years of age, at the Lovelace Respiratory Research Institute colony in Albuquerque, New Mexico. The dogs' weights ranged from 7.0 to 16.3 kg. The animals were housed either individually or in pairs in outdoor kennel runs with water available ad lib from a wall spout. The subjects were fed Teklad Certified 25% Lab Dog Diet once a day in the morning. Twenty-four of the animals had been born into the colony. Eight of the dogs were from a similar type of laboratory colony, and had been at the institute for at least 5 years before the start of the study. None of the dogs had participated in any other study within the year prior to this study.

Behavioral Test Procedures

Open-field activity was tested in an empty room, 274.32 × 252.73 cm, with two open doorways that were blocked off during testing. To simplify tracking of the animals' behavior pattern, the floor of the room was marked into 25 rectangles 49.02 × 53.34 cm with black electrical tape. Prior to each test, the floor was cleaned with a disinfectant solution to prevent odor cues from other dogs having disruptive effects.

The test sessions were 10 min in duration. The dog was placed just inside one doorway of the room, and was observed by two experimenters. One recorded the animal's behavior with a video camera. The other used dedicated computer software (12) to monitor locomotor activity, directed sniffing, urination, inactivity, grooming, jumping, rearing, and vocalization. For locomotor activity, grooming, and inactivity, the program provided a measure of total distance or time. A frequency of occurrence measure was used in characterizing the other behaviors.

Experimental Design

Prior to the start of the treatment phase, every animal was given two activity tests, which were used to calculate a mean measure of baseline locomotion. Each dog was then assigned to one of four dose levels using a counterbalancing procedure that took into consideration both sex and baseline locomotor activity. The dose levels used were 10, 20, 30, and 40 mg/kg, and a total of eight animals were placed in each group.

Vétoquinol Spécialités Pharmaceutiques Vétérinaires B.P. 189-70204 Lure Cedex, France, provided weighed capsules, containing either adrafinil or lactose, the excipient substance. All capsules were identical to assure that the experimenter remained blinded as to the content of the capsules. The capsules were placed in balls of Hill's® Prescription Diet® p/d® for oral administration and were administered once daily.

The study was divided into treatment and control phases of 14 days each. Half of the animals from each dose group were first tested with the placebo control substance, while the other half were first tested after administration of adrafinil. The open field test was given on days 1, 5, 9, and 13 of each phase. On days 1 and 9 the test was 2 h following treatment, while on days 5 and 13 the test was performed 10 h following treatment.

After completion of the initial test phase, the animals were given an 8-day washout period and were then retested on the second test phase. Thus, half of the animals were tested first with adrafinil and second with the placebo control. The treatment order was reversed for the other half of the animals. The experimenter was blinded to the treatment order.

Plasma Levels

Immediately following each behavioral assessment, 7 ml of blood was taken from the jugular vein of the dog, placed in a heparinized tube, and centrifuged. Two aliquots of plasma were stored at −20°C for analysis. The concentrations of adrafinil and its two metabolites, modafinil and CRL40467, were determined by means of validated high-performance liquid chromatography (HPLC) ultraviolet methods [see (2)].

Adrafinil, modafinil, and CRL40467 were extracted from the dog plasma using a two-step extraction procedure. Modafinil was extracted first using sodium hydroxide (final pH 13–14) and dichloromethane. A solution containing two internal standards of pharmaceutical purity, CRL51157 and CRL40940, was also added. The resulting solution was centrifuged, and the organic layer was evaporated to dryness. This was then solubilized in a mobile phase, vortex mixed, sonicated, and centrifuged. The solution was injected into the HPLC.

The remaining aqueous phase was acidified to pH 3 with hydrochloric acid and centrifuged. The supernatant was loaded onto a solid phase cartridge (Sep-Pack C18, Waters) previously primed with methanol and water. After sample loading, the cartridge was eluted with methanol. The methanol was evaporated and the dry residue solubilized in a mobile phase. The solution was vortex mixed, sonicated, and centrifuged. This was injected into the HPLC.

Data Analysis

All statistical analyses were performed using the Statistica software package. Each measure of spontaneous behavior was evaluated using a five-factor analysis of variance (ANOVA) procedure with three repeated measures; treatment, drug-test interval, and length of time on drug. Test order and dose served as independent variables. The level of significance used was 0.05.

We also obtained computer-generated printouts of the animals' activity patterns to provide a qualitative index of the response to adrafinil.

The levels of adrafinil and each of the two metabolites were also examined using a three-factor ANOVA with drug-test interval and length of time on drug as repeated measures and dose as the independent variable. We also looked at the correlations between serum levels and the change in activity levels over baseline.

RESULTS

Effect of Adrafinil on Spontaneous Behavior

Adrafinil appeared to affect several behaviors, but the present analysis focused only on the three with the highest frequency of occurrence; locomotion, sniffing, and urination (Fig. 1). Adrafinil produced a marked increase in locomotion and a more transient increase in sniffing. Urination, however, was decreased. These effects were a function of dose, time of test, and duration of test.

Locomotion

The increase in locomotion was revealed by a highly significant main effect of treatment, $F(3, 24) = 24.41957$, $p = 0.000048$, and a statistically significant interaction between dose and treatment, $F(3, 24) = 3.25$, $p = 0.0392$. As shown in Fig. 2, these results are due to a dose-dependent increase in locomotion at all but the lowest dose (10 mg/kg). The increased activity was also apparent in the activity pattern printouts, which revealed increased activity without any obvious changes in activity pattern (Fig. 3).

The ANOVA also revealed a main effect of drug-test interval, $F(1, 24) = 7.7818$, $p = 0.0102$, and a significant interaction between drug-test interval and duration of time on treatment, $F(1, 24) = 9.1201$, $p = 0.0059$. Increased activity occurred at both 2 and 10 h posttreatment. Furthermore, activity was greater at 2 h than at 10 h, but for the second 2-h test only. This increased activity in the second 2-h test block accounted for the significant interaction.

Sniffing

Treatment with adrafinil caused an increase in sniffing, but the effect was smaller and more transient. Increased sniffing was only observed at the 2-h test interval, and only for the first test session. This was also indicated by a significant three way interaction between treatment, length of time on drug, and drug-test interval, $F(1, 24) = 9.16$, $p = 0.0058$.

Urination

Urination frequency was decreased by adrafinil treatment. A statistically significant interaction between treatment and dose, $F(3, 24) = 3.19$, $p = 0.0418$, indicates that the effect of adrafinil varied with dose (Fig. 4). Tukey's HSD multiple comparisons test revealed that the urination effect was marginally significant at the 40 mg/kg dose level ($p = 0.052$) only.

Test Order, Experience, and Individual Differences

The experimental protocol required that half of the animals be treated first with adrafinil and the other half with a

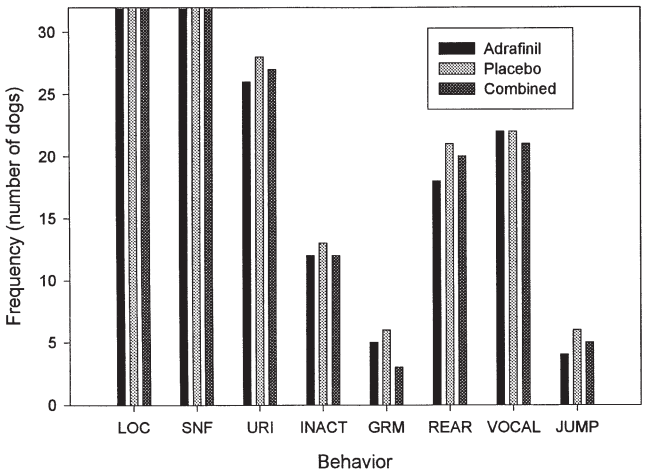

FIG. 1. Frequency of occurrence of behaviors displayed by the dogs. The number of dogs that exhibited the behavior on at least 50% of the adrafinil tests, placebo tests, or combining both sets of tests, are plotted. Only those behaviors with a high frequency of occurrence were analyzed. The behaviors recorded were locomotion (LOC), sniffing (SNF), urination (URI), inactivity (INACT), grooming (GRM), rearing (REAR), vocalizing (VOCAL), and jumping (JUMP).

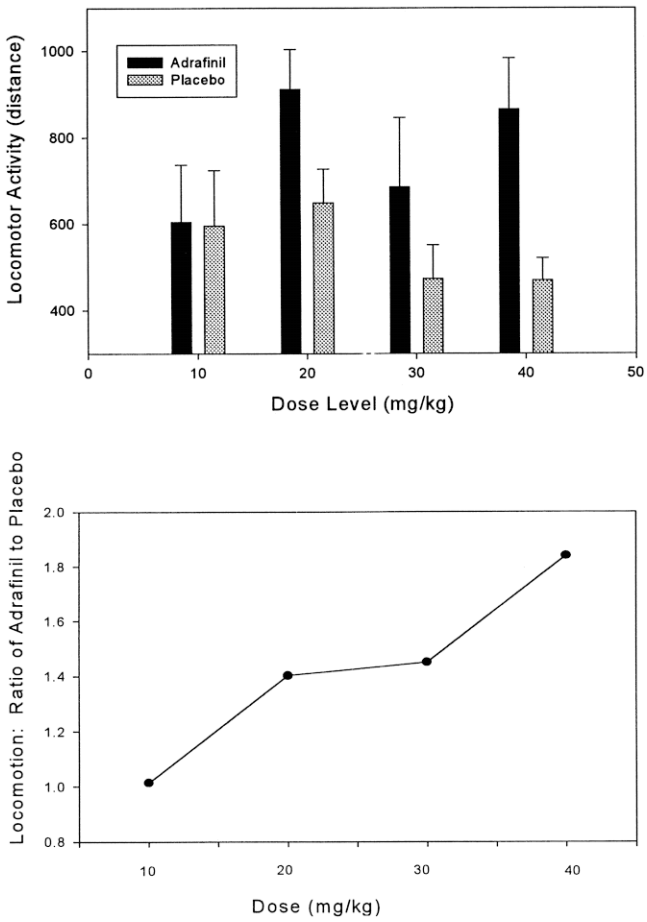

FIG. 2. Dose–response effect of adrafinil on locomotion. The top graph shows the mean total locomotion scores under adrafinil and placebo conditions. The error bars represent standard errors. The bottom shows the dose–response relationship. The scores were calculated by taking the ratio of the mean locomotion score under adrafinil and dividing it by the mean locomotor activity score under the placebo control condition.

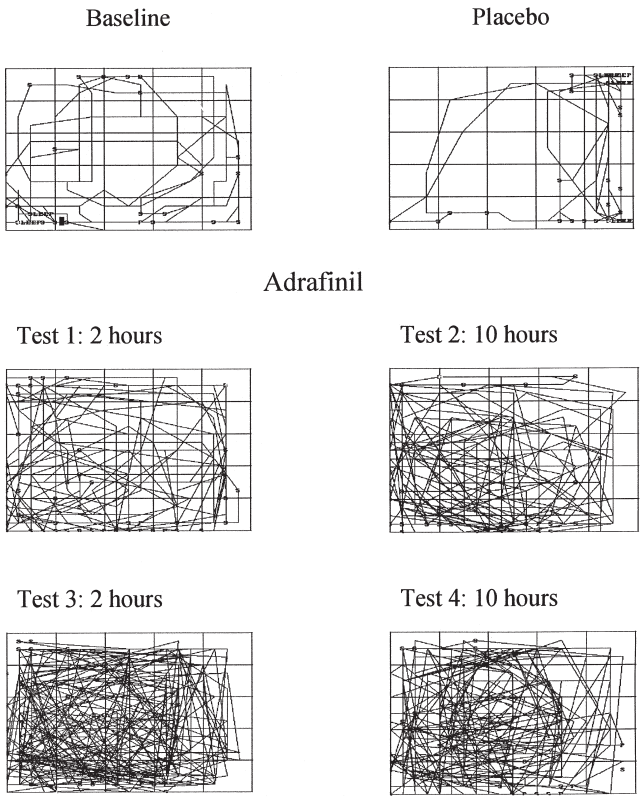

FIG. 3. Computer generated activity patterns for a representative subject under baseline, adrafinil, and placebo control conditions. The activity patterns reflect a tracing of all movements of the animal during a 10-min session. The patterns are idiosyncratic. This subject, for example, tended to stay in the periphery of the room, and the same general pattern was seen under both adrafinil and control conditions. The symbols indicate the occurrence and location of sniffing (S), grooming (G), urination (P), and inactivity (Sleep).

placebo control. The importance of test order was revealed by a significant interaction between test order and treatment for the locomotion measure, $F(1, 24) = 6.0250$, $p = 0.0217$. This effect was due, in part, to a smaller effect on locomotion in the animals subjected to the control treatment first.

Prior experience with adrafinil was another factor that contributed to the test order effect. In their first placebo control session, the group tested first under adrafinil showed a statistically significant increase in locomotor activity over both baseline sessions (Fig. 5). The analysis was based on a t-test for correlated means; for baseline 1 vs. placebo 1, $p = 0.018$; for baseline 2 vs. placebo 2, $p = 0.04$. There was also a high and significant correlation ($r = 0.863$, $p < 0.05$) between the increase in activity under the drug condition and the increase in activity under the placebo condition.

Inspection of the individual data revealed considerable intersubject variability, which was not obviously related to the dose level. To better characterize this variability, difference scores were calculated by subtracting the mean baseline score from each of the four test scores obtained under adrafinil. At each test interval (2 and 10 h), dogs that showed an increase in locomotor activity over their baseline mean on both test sessions were classified as positive responders. Nonresponders did not show a consistent change, while negative responders showed a decrease in activity at one of the intervals. The results of this analysis are shown in Table 1. We also looked at the animals' response over all four sessions. Sixty-nine percent of the dogs could be characterized as responders, 16% were nonresponders, and another 16% were negative responders.

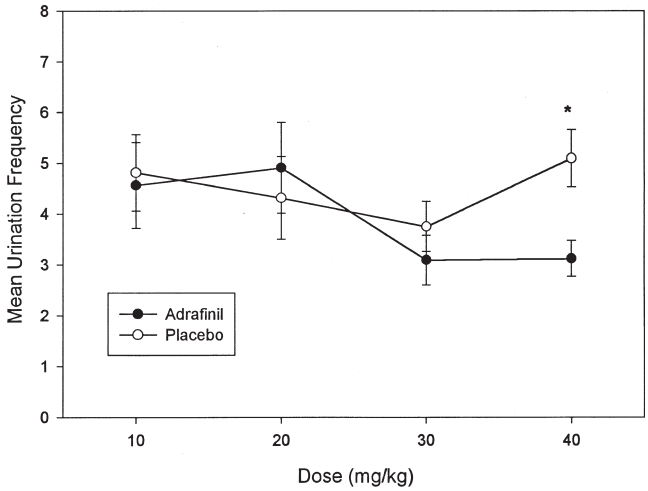

FIG. 4. Effect of adrafinil treatment on urination frequency. Mean urination frequency plotted against dose level reveals that urination was decreased at the highest dose (40 mg/kg) only.

Stereotyped behavior was not observed with adrafinil treatment in this study, with one exception: one animal exhibited repetitive circling behavior and head-jerking motions. This particular animal showed an increase in activity but its activity pattern was altered by adrafinil treatment.

Plasma Levels of Adrafinil, Modafinil, and CRL40467

Serum levels of adrafinil and metabolites varied as a function of dose, drug-test interval, and duration of treatment.

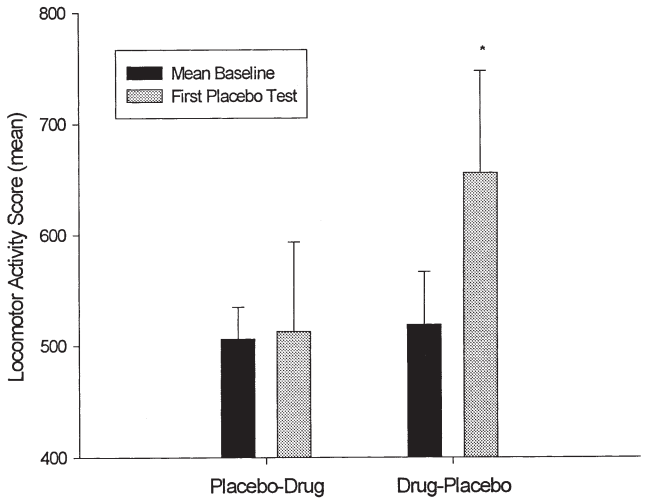

FIG. 5. Effect of test order on locomotion under the placebo control condition. This shows that when the placebo test occurred before the treatment (placebo-drug), activity under the placebo condition did not differ from activity under baseline. When the placebo followed the treatment phase, activity under placebo was significantly greater than activity under baseline.

TABLE 1

FREQUENCY OF ANIMALS SHOWING NO EFFECT OR NEGATIVE EFFECT UNDER ADRAFINIL AND PLACEBO CONTROL CONDITION

	+ Responders		− Responders		No effect	
Dose	2 Hours	10 Hours	2 Hours	10 Hours	2 Hours	10 Hours
Adrafinil						
10	5	5	3	2	0	1
20	7	7	0	0	1	1
30	5	5	2	1	1	2
40	7	7	0	0	1	1
Total	24	24	5	3	3	5
Placebo						
10	4	3	2	1	2	4
20	5	6	1	0	2	2
30	3	2	3	3	2	3
40	2	3	2	4	4	1
Total	14	14	8	8	10	10

The effect on each of the three substances was analyzed using a three-way ANOVA with dose as a between-subject variable. For adrafinil, significant main effects were obtained for dose, $F(3, 27) = 7.38$, $p = 0.00009$, drug-test interval, $F(1, 27) = 65.19$, $p = 0.0000$, and duration of treatment, $F(1, 27) = 12.48$, $p = 0.0015$. There was also a significant interaction between dose and drug-test interval, $F(1, 3) = 11.85$, $p = 0.003$. The origin of these effects is shown in Fig. 6. Two hours following treatment, adrafinil showed the expected dose dependent function. At 10 h, however, levels were higher in the 10 mg/kg group than in the 40 mg/kg group, but not significantly. In addition, serum levels overall were higher on the second test day with adrafinil (day 9 of treatment) then on the first.

Figures 7 and 8 show that the results for modafinil and CRL40467 were different. At 2 h test day 2 levels of modafinil were lower than test day 1, although the differences were only marginally significant, $F(1, 28) = 3.89$, $p = 0.0754$, while CRL40467 levels were similar on both sessions.

As shown in Table 2, we also looked at the correlations with locomotion. Serum levels of adrafinil, modafinil, and CRL40467 2 h following dosing were all correlated with locomotion, although only modafinil showed a significant correlation on both of the 2-h tests. There were no significant positive correlations at 10 h following treatment. In fact, levels of all three metabolites were negatively correlated with the change in activity.

Finally, we compared the serum levels of adrafinil, modafinil, and CRL40467 in animals deemed to be responders with those classified as nonresponders. The results are shown in Fig. 9. Nonresponders had lower levels of metabolites compared to responders, at 2 h, but not at 10 h. This was supported by statistically significant interactions between drug-test interval and response to activity test for both adrafinil, $F(1, 29) = 4.28$, $p = 0.0474$, and modafinil, $F(1, 30) = 4.48$, $p = 0.0448$. In the case of CRL40467, the difference was not statistically significant. We also did an analysis of covariance with dose as a covariate. This did not affect the results, suggesting that differences in concentrations were not simply attributable to differences in dose.

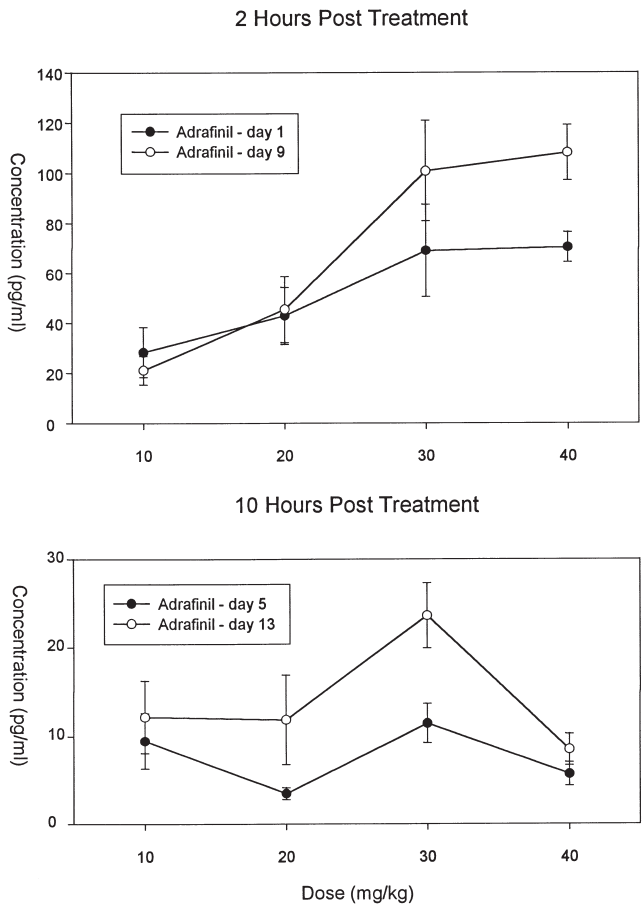

FIG. 6. Serum levels of adrafinil are plotted as a function of dose at 2 h (top) and 10 h (bottom) following treatment.

DISCUSSION

The present results provide the first evidence that adrafinil can enhance behavioral activity in aged animals. Aged dogs show a dose dependent increase in open field locomotion, with a dose of 20 mg/kg being the minimum effective dose. This activity-enhancing effect of adrafinil was long lasting, persisting for at least 10 h after treatment.

This is the only work we know of examining the effects of adrafinil in canines. The basic finding of an increase in activity is consistent with findings from other species. The dose–response data, however, indicate marked differences between canines and other species in the effectiveness of adrafinil. Milhaud and Klein (19) reported that a dose of 60 mg/kg was effective in producing activity in monkeys. Studies with rodents have typically used doses ranging from 30 to 256 mg/kg (4,24). The origins of these differences probably relate to species differences in metabolism. This is an area that requires further research.

Another novel aspect of the present study was the use of a direct observation procedure to quantify behavioral activity. This provides us with a behavioral profile and quantitative measures of each behavior displayed by the dogs. This enabled us to identify behavioral profiles produced by drug treatment. All previous studies have used automated procedures.

As mentioned previously, controversy exists in the current literature as to whether adrafinil actually does cause an in-

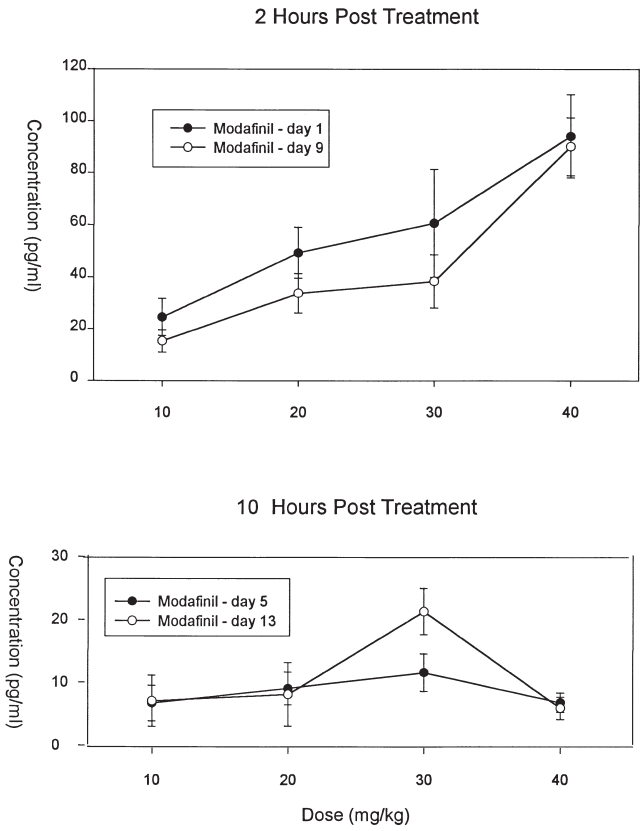

FIG. 7. Serum levels of modafinil are plotted as a function of dose at 2 h (top) and 10 h (bottom) following treatment.

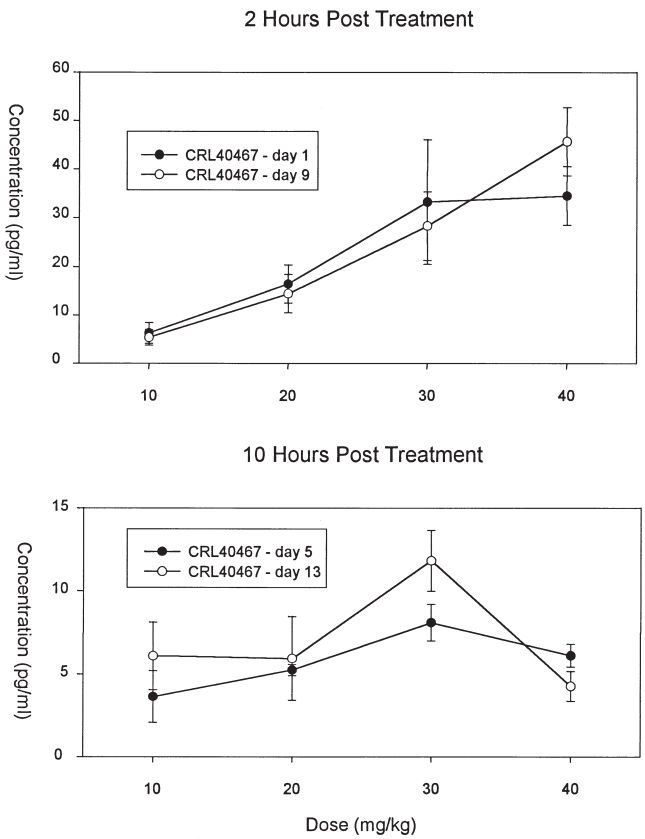

FIG. 8. Serum levels of CRL40467 are plotted as a function of dose at 2 h (top) and 10 h (bottom) following treatment.

crease in behavioral activity or whether the increase in activity is actually due to an increase in wake time (7). The present results indicate that the major factor that contributes to the increase is locomotor activity. Only locomotor activity showed a consistent increase. Other behaviors such as sniffing, inactivity, rearing, grooming, vocalization, and jumping did not show any consistent effects. Urination frequency, however, was decreased at the highest dose level.

A crossover design was used in this experiment, and this resulted in a significant effect of test order. Dogs that were tested first with adrafinil showed a greater effect, and greater overall activity than did dogs tested first with placebo. Habituation to the testing room may have contributed to this effect. The placebo first group had six sessions in the test room before being tested under adrafinil (two baseline and four placebo control).

Another factor that contributed to the test order effect was higher activity levels on the first placebo test session, relative to baseline, for the group tested first under adrafinil. This result was unexpected. Prior to their initial placebo test, the dogs had had six open-field activity sessions, and this should have led to habituation to the testing room. The activity increase effect is not likely to be due to residual effects of adrafinil (13,19). This high activity score under the placebo condition may reflect a type of conditioned locomotor effect. Conditioned increases in locomotion have been induced by morphine and locomotion was the only behavior affected (20,34). A conditioned effect could occur if administration of adrafinil had reinforcing properties. In fact, modafinil has been shown to serve as a positive reinforcer for drug self-administration in monkeys (10).

The apparent reinforcing effects of adrafinil may be partly attributable to the presence of the active metabolite modafinil. However, the positively reinforcing properties of modafinil occur at very high doses only, which are much higher than those seen in the present study. The reinforcing effects of adrafinil could be linked to dopaminergic involvement in view of the substantial evidence linking brain dopamine to reward. The involvement of dopamine is likely to be indirect however. There is evidence to show that adrafinil may indirectly affect dopamine levels through inhibition of GABAergic neurons (8,18). We suggest that the dopaminergic system is involved in the mechanism of action of adrafinil indirectly. A plausible mechanism of action could involve adrafinil inhibiting GABAergic neurons through alpha-1 activation, which would then release the inhibition on the dopaminergic neurons.

TABLE 2

CORRELATIONS OF SERUM LEVELS OF ADRAFINIL, MODAFINIL, AND CRL40467 WITH LOCOMOTION

Test Session	Adrafinil	Modafinil	CRL
1–2 hour post	0.34	0.38*	0.32
2–10 hour post	−0.25	−0.07	−0.18
3–2 hour post	0.39*	0.48*	0.42*
4–10 hour post	−0.52*	−0.17	−0.35

*Significant at 0.05 level.

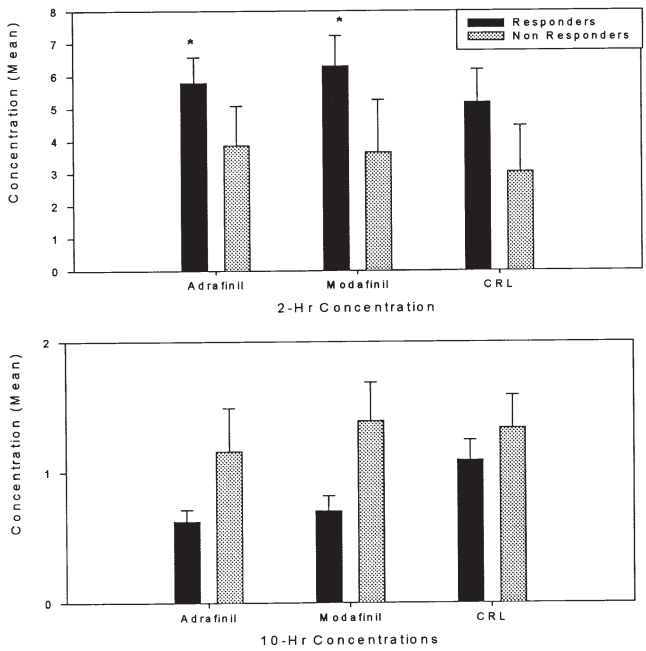

FIG. 9. Plasma levels of adrafinil, modafinil, and CRL40467 are compared in responders and nonresponders.

The present study also looked at the effect of repeated administration of adrafinil over 14 consecutive days. We found no evidence of adaptation to the activity inducing effects of adrafinil; to the contrary, the locomotor facilitation effect increased with repeated administration. This observation is consistent with findings of Milhaud and Klein (19) using monkeys. This is the first report we know of that shows that the effects of adrafinil do not habituate with repeated administration. Most other studies have looked at the effects of short-term administrations.

We also obtained serum levels of adrafinil and metabolites (modafinil and CRL40467) following each activity test. Serum levels of adrafinil and modafinil at 2 h posttreatment showed the expected dose-dependent increase, but this was not the case at 10 h posttreatment. In fact, at 10 h, levels of adrafinil in the 40 mg/kg group were actually lower than levels in the 10 mg/kg group. This unexpected observation raises the possibility of adrafinil itself having an effect on the rate of metabolism and elimination. This conjecture is supported by evidence that modafinil causes an increase in levels of glutamine synthetase. This enzyme is responsible for glutamine production from glutamate and ammonia, in the brain (33). Glutamine serves as an energy substrate and a neurotransmitter in the brain. Further, inositiol, aspartate, and the creatine-phosphocreatine pool are increased by modafinil treatment (23). A general increase in brain metabolism is an alternative to the hypothesis of adrafinil affecting neurotransmitter systems directly.

We also found that serum levels of adrafinil 2 h following treatment increased over repeated dosing, while the effect of repeated dosing on modafinil levels was the opposite; serum levels decreased. This suggests that adrafinil itself is an active agent and is responsible for the behavioral effects observed.

Not all animals showed increased locomotion in the open-field test following administration of adrafinil; we were able to distinguish a small group of nonresponders from a larger group of responders. We also found differences between responders and nonresponders in serum concentrations of adrafinil and modafinil; responders showed higher levels at 2 h than nonresponders and lower levels at 10 h. This suggests differences in rate of metabolism. These observations raise the possibility that individual differences in rate of metabolism of adrafinil can account for individual differences in the animals' behavioral response.

Increased locomotor activity was generally unaccompanied by stereotypical behavior. In the present model of canine activity, stereotypy is accompanied by a change in activity pattern resulting in repetitive movement and a marked decrease in other measures of investigatory behavior such as sniffing (12). We did not see behavioral stereotypy in adrafinil treated dogs, with one exception. The exception was a dog that exhibited repetitive circling and head jerking behaviors during the testing period while receiving adrafinil. Its activity pattern was changed by adrafinil treatment by becoming restricted to a smaller portion of the test room, which is characteristic of stereotypy (12). This particular animal was receiving 30 mg/kg of adrafinil.

We also observed a decrease in urination. This was unexpected. Adrafinil is a putative noradrenergic agonist and brain adrenergic fibers are known to control micturition in the dog (5,22). Decreased drinking is another possible explanation for the urination effect. Modafinil has also been shown to cause a decrease in fluid intake in rats (21). It is also notable that we only saw a clear affect on urination at the highest dose of 40 mg/kg. This raises the possibility of the behavioral activating effect and the effect on urination involving separate mechanisms, with urination having a higher threshold level of activation (17).

In summary, adrafinil increases locomotor activity in most aged beagle dogs. This effect varies with dose, with 20 mg/kg being the minimum effective. The locomotor facilitating effects of adrafinil persist for at least 10 h, and do not diminish over 14 days of treatment. Urination frequency may be decreased, and sniffing frequency is transiently increased during the first few days of treatment. This study also revealed that there are individual differences in the responses to drug administration which appear to be linked to differences in metabolism.

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