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Effects of the β-Carboline Abecarnil on Epileptic Activity, EEG, Sleep and Behavior of Rats

A. M. L. COENEN,*[1]
D. N. STEPHENS† AND E. L. J. M. VAN LUIJTELAAR*

*Department of Psychology, University of Nijmegen, P.O. Box 9104, 6500 HE Nijmegen, The Netherlands
†Department of Neuropsychopharmacology, Schering A.G., Berlin, Germany

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COENEN, A. M. L., D. N. STEPHENS AND E. L. J. M. VAN LUIJTELAAR. *Effects of the β-carboline abecarnil on epileptic activity, EEG, sleep and behavior of rats.* PHARMACOL BIOCHEM BEHAV **42**(3) 401–405, 1992. – The profile of the anxiolytic β-carboline isopropyl 6-benzyloxy-4-methoxymethyl β-carboline-3-carboxylate (abecarnil; ZK 112 119), a partial agonist at benzodiazepine receptors, was determined in two experiments. In the first, abecarnil was given to WAG/Rij rats; these rats generate spontaneously occurring spike-wave discharges and are regarded as a model for absence epilepsy. Effects were measured on epileptic activity, together with those on the spectral content of the background electroencephalograph (EEG), as well as on ongoing behavior. In a second experiment, effects on sleep and behavior were investigated in Wistar rats. It was found that, similarly to classical benzodiazepines, abecarnil possessed a strong antiepileptic character and also changed the background EEG to more high-frequency waves and less spindle activity. It also produced more immobile behavior. Abecarnil induced only small, marginally significant increases in slow-wave sleep while reducing REM sleep as a proportion of total sleep. It also reduced the number of REM periods. These observations are consistent with the proposed partial agonist activity of abecarnil, a drug with interesting therapeutic implications.

Abecarnil ZK 112 119 β-Carboline Absence epilepsy EEG Sleep Behavior Rat

ISOPROPYL 6-benzyloxy-4-methoxymethyl β-carboline-3-carboxylate (Abecarnil; ZK 112 119) is a β-carboline acting at central benzodiazepine receptors to produce potent anticonvulsant and anxiolytic effects in animal models. In rats, the half-life of abecarnil is about 2 h reaching a peak level within 30 min after intraperitoneal application and with no evidence on active metabolites (14). In contrast to classical benzodiazepines such as diazepam, abecarnil has only weak sedative and muscle relaxant effects (14,15). Since abecarnil is metabolically stable (14), with acceptable pharmacokinetic properties in animals as well as humans (9,10), it is a promising drug, lacking some important side effects of the classical benzodiazepines. Importantly, tolerance appears to develop rather slowly in comparison to benzodiazepines in anticonvulsant models and withdrawal effects following chronic treatment are less severe than after treatment with benzodiazepines (11).

In the present article, the effects of abecarnil on spontaneously occurring epilepsy in rats, on the background electroencephalogram (EEG), and on sleep are reported. The latter is important since drowsiness and sleepiness are unwanted side effects of the benzodiazepines when used as anxiolytics. From preliminary work, it was already clear that the hypnotic effects of abecarnil are less pronounced than those of the full benzodiazepine agonist diazepam (18). Furthermore, it was found that the partial agonist ZK 91296 has only an anticonvulsive effect without effects on EEG and behavior. This means that the effects on the EEG could be separated from the anticonvulsive effects; whether this is also true for the anxiolytic effects remains in question.

The behavioral nature of abecarnil was characterized in two experiments. In the first, rats of the WAG/Rij strain, all sharing the characteristic of absence-like epilepsy, were used (17). These, as well as those of a similar strain, have proven useful in demonstrating bidirectional effects of different β-carbolines on spontaneous petit mal-like epileptic discharges in the rat (5,12). Effects of abecarnil on spike-wave activity were investigated, as well as on spectral content of the background EEG and on spontaneous behavior. In the second study, using Wistar rats, the effects on sleep were established, whereby sleep was divided into slow-wave and REM sleep.

[1] To whom requests for reprints should be addressed.

METHOD

Experiment 1: Effects of Abecarnil on Epileptic Activity, EEG, and Behavior

Male and female rats of the WAG/Rij strain obtained from the breeding facility of the University of Nijmegen were used. The weights of animals ranged between 190–380 g (age 13–19 weeks). Several weeks before the actual start of the experiment, rats were housed singly and maintained on a 12 L: 12 D cycle, with lights on at 2200 h. During the dark period, a small red bulb of 2–3 lux allowed behavioral observations of animals. Standard rat food and water were continuously available. A tripolar cortical electrode set (Plastics One, Roanoke, VA, model MS 333-2A) was permanently implanted under deep surgical anesthesia (pentobarbital, 50 mg/kg). Coordinates with skull surface flat were for the first electrode A 2.0 and L 3.5 and for the second A −6.0 and L 4.0; the earth electrode was located above the cerebellum. EEG activity, measured between 1–70 Hz, was registered on a polygraph (Elema-Schönander) and on magnetic tape.

After recovery from surgery for a minimum of 2 weeks and habituation to the experimental procedures, experiments started. At 0900 h on the experimental day, animals were connected to the recording cables. When attached to these leads, a metal spring connected to a commutator allowed free movement of animals. Baseline EEG measures were made from 1200–1300 h. Then, rats were injected intraperitoneally with abecarnil or solvent, the latter being bidest to which 5% Tween-80 was added. Abecarnil was given in a volume of 1 ml per 400 g body weight in dosages of 0, 0.16, 0.4, 1.0, and 2.5 mg/kg. The number of subjects in all groups was eight; groups were labeled according to their doses. After injection, recordings continued for four h.

The behavior of rats was observed continuously for a period of half an hour, beginning 2 h after injection when the plasma level of abecarnil is still high. Behavioral categories recognized were: voluntary behavior (walking, rearing, sniffing, and digging), automatic behavior (grooming, eating, and drinking), and immobile behavior (sitting, lying, and standing still) (3,19). Epileptic activity was established by measuring the number, mean duration, and total duration of spike-wave discharges according to criteria described elsewhere (5,17). Changes in the EEG spectral content were determined in the delta (2–4 Hz), theta (6–10 Hz), spindle (11–14 Hz), and beta (15–30 Hz) bands (5,16). Spectral analysis was assessed on the background EEG accompanying passive wakefulness during the observation period, whereby paroxysms were excluded. The spectral content of the EEG was established for a period between 75–100 s. Next, a mean spectrogram was constructed for each animal; this mean spectrogram was normalized (mean = 0; variance = 1) to compensate for different amplifications between animals. This gives a *Z*-score. Finally, effects on behavior during the half-hour observation period were determined by measuring the total number of seconds passed by in each of the three behavioral categories.

Experiment 2: Effects of Abecarnil on Sleep

In this experiment, the hypnotic effects of abecarnil were studied. Sleep was investigated in the dark period of the light-dark cycle. This was done because an eventual increase in sleep caused by the benzodiazepine-agonistic activity of abecarnil is easier to find in this period than in the light period, in which sleep is common. Subjects were 32 male rats of the random-bred Wistar strain, with body weights between 340–410 g and aged about 5 months. Rats were housed individually under a 12 L: 12 D cycle with lights on at 1900 h. Food and water were permanently available. Under deep surgical anesthesia (pentobarbital, 50 mg/kg, IP), animals were equipped with a tripolar cortical electrode set. EEG electrode locations were the same as in the first experiment, whereas a bipolar (EMG) electrode set was placed over the neck muscles. After at least 2 weeks of recovery from surgery, animals were habituated to the experimental procedures. EEG (1–70 Hz) and electromyogram (EMG) (70–700 Hz) registrations were made on an Elema-Schönander polygraph with a paper speed of 1.0 cm/s.

Predrug (baseline) recordings started at 9:00 a.m. and lasted for 1 h. Animals were then intraperitoneally injected with the solvent (bidest with 5% Tween-80, group 0) or 0.16, 0.4, or 1.0 mg/kg abecarnil. Doses were given in a volume of 1 ml/400 g body weight. Each dose group consisted of eight rats, which were used only once. Postinjection registrations lasted 4 h. Sleep and wakefulness were scored according to conventional criteria: wakefulness (W) is characterized by a small-amplitude, fast-frequency EEG together with a high-amplitude and/or a rapidly changing EMG; slow-wave sleep (SWS) by a large voltage; slow-wave EEG with a moderate and relatively constant EMG; whereas REM sleep is characterized by a low-voltage, high-frequency EEG, often with clear theta, and a low-amplitude EMG. Variables, all of which were determined for the whole period of 4 h, were: total sleep (TS) time, SWS time, the number of SWS periods whereby interrupts of shorter than 5 s were allowed, the mean duration of these periods, and, finally, the sleep latency.

Results from both experiments were analyzed with a one-way analysis of variance (ANOVA) with drug as factor. Subsequent posthoc tests were performed with Duncan's multiple-range tests. p values of <0.05 were taken as significant.

RESULTS

Experiment 1

Spike-wave discharges. During the 1-h baseline period, no differences were noted between the five groups in the number, $F(4, 35) = 0.44$, NS, mean duration, $F(4, 35) = 0.66$, NS, nor total duration of spike-wave discharges, $F(4, 35) = 0.38$, NS (Fig. 1). During the first postinjection hour, a significant dose-related effect was found in the number of spike-wave discharges, $F(4, 35) = 5.40$, $p < 0.01$. The posthoc comparisons showed that group 2.5 had less discharges than groups 1.0, 0.4, 0.16, and 0; group 1.0 less than 0.16 and 0; and group 0.4 less than group 0. During the second hour, a significant dose effect for the number of spike-wave discharges emerged, $F(4, 35) = 4.40$, $p < 0.01$. Groups 2.5 and 1.0 showed less discharges than groups 0.16 and 0. During the third and fourth hour, differences between groups were no longer present. No differences in the mean duration of spike-wave discharges were detected in the first postinjection hour, but both during the second, $F(4, 35) = 7.75$, $p < 0.001$, and third hour, $F(4, 35) = 3.87$, $p < 0.01$, abecarnil reduced the duration of spike-wave discharges. Posthoc analysis showed that during the second hour groups 2.5, 1.0, and 0.4 had a shorter mean duration of discharges than groups 0.16 and 0, whereas during the third hour groups 2.5 and 1.0 had also a shorter duration than group 0. No effects were found during the fourth hour.

During the first three postinjection hours, effects of different doses on the total time of spike-wave discharges were seen:

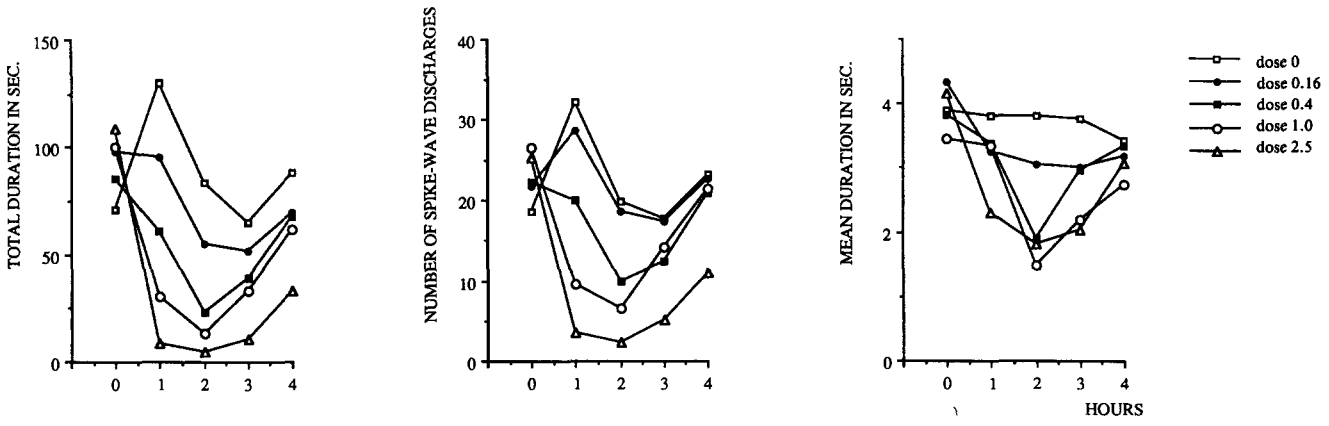

FIG. 1. Effects of abecarnil on (left) total duration, (middle) number, and (right) mean duration of spike-wave discharges. All data are given per hour whereby 0 is the baseline hour and 1, 2, 3, and 4 the postinjection hours, respectively. Abecarnil is given in five doses (0, 0.16, 0.4, 1.0, and 2.5 mg/kg). For statistics, see text.

first hour, $F(4, 35) = 5.52$, $p < 0.01$, second hour, $F(4, 35) = 5.94$, $p < 0.001$, and third hour, $F(4, 35) = 2.69$, $p < 0.05$. Duncan's tests showed that in the first hour there was less time with spike-wave discharges in groups 2.5, 1.0, and 0.4 than in group 0 and less time with discharges in groups 2.5 and 1.0 compared to group 0.16. In the second hour, similar differences between groups were found, whereas in the third hour only group 2.5 had a shorter total time with spike-wave discharges than group 0. All differences had disappeared by the fourth hour. The ED_{50} of abecarnil, in reducing the number of spike-wave discharges in the second hour, was 0.4 mg/kg. This was calculated from the regression line between the log of the dose and the relative reduction in the number of spike-wave discharges.

An enhancement in the number of spike-wave discharges was noticed in the first hour after injection with the solvent only; this was statistically confirmed with a *t*-test for correlated means. The number of spike-wave discharges in the first postinjection hour was significantly increased compared to baseline, $t = (7)2.84, p < 0.05$.

Spectral content of EEG. Analysis of the spectral content of the EEG (Fig. 2) was carried out in the background EEG accompanying passive wakefulness. All aberrant EEG activity was thereby excluded. Numerous significant differences were found in this analysis, although in the delta-band only one significance was detected. Group 0.16 showed more delta activity than group 1.0, $F(4, 35) = 2.65$, $p < 0.05$. A clear dose-response relationship was established in the theta-band, $F(4, 35) = 81.83$, $p < 0.0001$, in which group 0 had more theta than groups 0.16, 0.4, 1.0, and 2.5; group 0.16 more than groups 0.4, 1.0, and 2.5; and group 0.4 more than groups 1.0 and 2.5. In the spindle-band, $F(4, 35) = 4.47$, $p < 0.01$, group 1.0 had more power than groups 0.4, 0.16, and 0. In the beta-band, again a clear drug effect was found, $F(4, 35) = 28.94$, $p < 0.0001$, groups 2.5 and 1.0 showing more beta than groups 0.16 and 0; group 1.0 more than groups 0.4, 0.16, and 0, and group 0.4 more than groups 0.16 and 0.

Behavioral observations. With respect to behavior observed during the half-hour observation period (Fig. 3), no dose-related effects in exploratory behavior were observed, although group 0.16 showed more of this type of behavior than all other groups, $F(4, 35) = 5.12$, $p < 0.01$. For automatic and immobile behavior, more effects were found. For automatic behavior, a significant dose effect was detected, $F(4, 35) = 4.66$, $p < 0.01$, whereby groups 0 and 0.16 differed from groups 0.4, 1.0, and 2.5: The higher dose groups showed less automatic behavior (feeding, drinking, grooming). Abecarnil also increased immobile behavior, $F(4, 35) = 9.03$, $p < 0.01$, groups 0.4, 1.0, and 2.5 being more immobile than groups 0 and 0.16.

Experiment 2

Effects on sleep. In the baseline hour, there were no differences between the four groups. Rats slept 35.7% of total time, of which 29.8% was SWS and 5.9% REM sleep. The results of the 4 postinjection h are given in Table 1. In the 4 h following drug injection, no significant differences were found on SWS parameters. Marginally significant changes were detected in TS time, $F(3, 28) = 2.58$, $p < 0.1$, in SWS time,

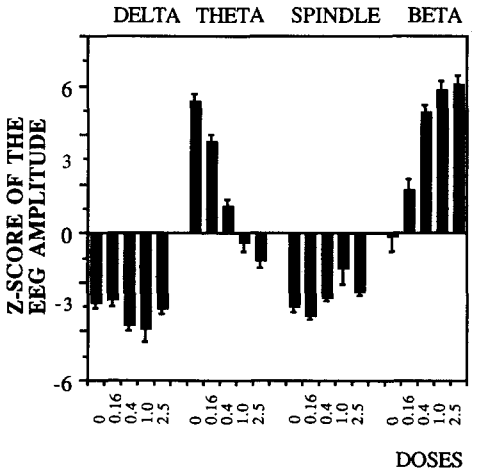

FIG. 2. Effects of abecarnil on EEG amplitude, expressed in the Z-score. Data (means and SEM) are given for the (left) delta-band (2–4 Hz), (left middle) theta-band (6–10 Hz), (right middle) spindle-band (11–14 Hz), (right) beta-band (15–30 Hz). The compound is given in five doses (0, 0.16, 0.4, 1.0, and 2.5 mg/kg). Statistics can be found in the Results section.

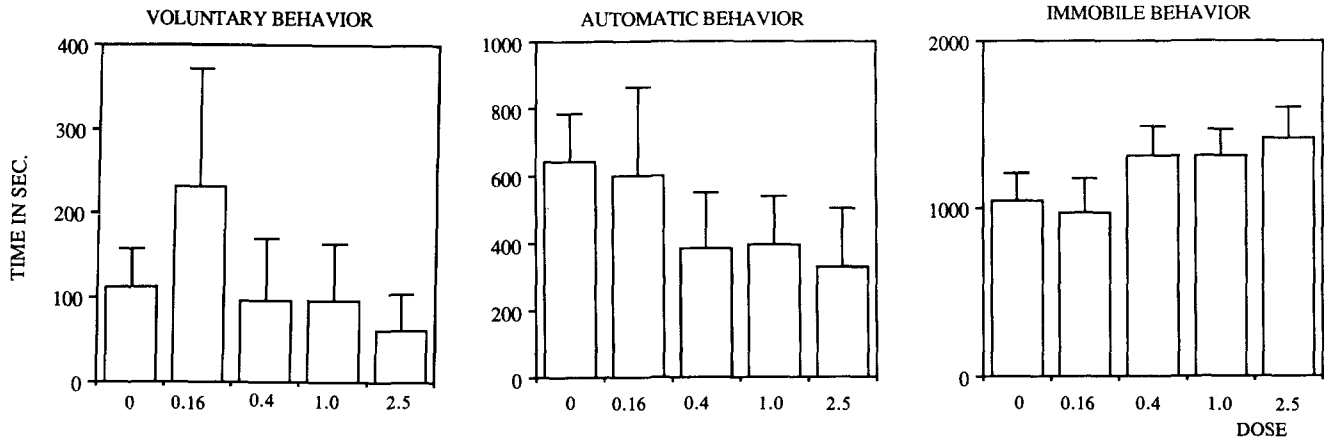

FIG. 3. Effects of abecarnil on (left vertical series of graphs) voluntary behavior, (middle series) automatic behavior, (right series) immobile behavior. Data are given in means and SD. The compound is given in five doses (0, 01.6, 0.4, 1.0, and 2.5 mg/kg). Statistics are given in the Results section.

$F(3, 28) = 2.60$, $p < 0.1$, and in the mean duration of SWS periods, $F(3, 28) = 2.32$, $p < 0.1$. No effects were found on the mean duration of sleep periods or the sleep latency.

The only significant findings were with respect to parameters of REM sleep. REM sleep time showed, due to large variations, a nonsignificant, dose-related tendency to decrease, but this became significant when REM sleep time was expressed as the percentage of total sleep time (REM efficiency), $F(3, 28) = 3.46$, $p < 0.05$. Group 1.0 had a smaller percentage REM efficiency than groups 0.16 and 0. The number of REM periods also decreased significantly, $F(3, 28) = 4.68$, $p < 0.01$, in a dose-related way (groups 1.0 and 0.4 had less periods than group 0.16). No significant effects emerged on the mean duration of the REM periods.

DISCUSSION

Abecarnil dose dependently reduced epileptic activity, whether measured as number, mean duration, or total duration of spike-wave discharges. The ED_{50} for reducing the number of spike-wave discharges was about 0.4 mg/kg, indicating that abecarnil is twice as potent as diazepam with respect to its antiepileptic properties. Abecarnil has similar effects on the spectral content of the EEG as diazepam, favoring the higher EEG frequencies as beta-waves, while decreasing the lower frequencies, mainly the theta-waves. This is not only in agreement with the EEG effects of diazepam (5) but also with other classical benzodiazepines so far described (1,8). As was found in a previous study, an increase in the number and in total duration of spike-wave discharges was found in the solvent group in comparison to the baseline hour (5). This effect was only found in the first hour and, as pointed out previously, may be related to a proconvulsant activity of the solvent, Tween-80 (5).

At the higher doses, abecarnil decreased automatic and increased immobile behavior. When compared to the effects of diazepam on these behaviors, the effects of abecarnil are smaller (5). These behavioral effects are well known for the benzodiazepines and are generally interpreted as sedation. Whether this interpretation is also correct for abecarnil cannot be proven yet, but together with the fact that small effects on sleep were noted this viewpoint seems acceptable. Not fitting into the general picture of abecarnil was the finding that the lowest dose (0.16 mg/kg) had an activating effect. Presum-

TABLE 1

EFFECTS OF ABECARNIL ON THE VARIOUS SLEEP CHARACTERISTICS IN THE POSTTREATMENT RECORDING PERIOD OF 4 h IN MEANS AND SEM ($n = 8$)

	Dose Abecarnil (mg/kg)				
	0	0.16	0.4	1.0	
TS (in seconds)	4886 ± 630	6156 ± 434	5124 ± 469	6124 ± 521	
SWS (in seconds)	4348 ± 497	5690 ± 407	4789 ± 436	5908 ± 486	
REM sleep (in seconds)	538 ± 144	466 ± 98	335 ± 56	216 ± 71	
Percentage REM/TS	9.5 ± 12.1	7.6 ± 1.4	6.5 ± 0.8	3.3 ± 1.0	$p < 0.05$
Number of SWS periods	63.0 ± 6.9	56.3 ± 3.2	51.5 ± 5.6	60.7 ± 5.4	
Mean duration of SWS periods	69.0 ± 2.9	102.0 ± 7.1	104.0 ± 16.0	103.0 ± 13	
Number of REM periods	7.0 ± 1.8	10.0 ± 2.2	4.0 ± 0.6	2.9 ± 0.6	$p < 0.01$
Mean duration of REM periods	73.1 ± 10.9	50.1 ± 9.4	83.7 ± 9.1	65.0 ± 13.5	
Sleep latency	1233 ± 302	1317 ± 301	1335 ± 334	1034 ± 36	

ably, this was a chance finding since wakefulness was not markedly different and in a replication study this effect was, as expected, not found.

In the second experiment, particular attention was devoted to the hypnotic activity of abecarnil. The amounts of TS, SWS, and REM sleep obtained in the preinjection baseline hour, correspond well to earlier reports on sleep in the dark period (2,4,16) and the proportion of REM time was also in good agreement with percentages mentioned in the literature (4). Postinjection sleep percentages tended to be lower than preinjection ones for group 0, presumably as a result of the sleep-disturbing effects of the injection.

More important are the comparisons between groups after administration of abecarnil. Only marginal differences were found on SWS parameters. A slight increase of this type of sleep appeared, accompanied by a reduction in both the number of REM episodes and the amount of REM when calculated as a proportion of total sleep time.

In an earlier study (18), we compared the effects on sleep of abecarnil (1 mg/kg) with diazepam (5 mg/kg). These doses were selected for the reason of having identical antiepileptic effects in WAG/Rij rats. In this study, abecarnil increased sleep too, but to a lesser degree than diazepam. A safe conclusion with respect to the effects of abecarnil on sleep in the rat must therefore be that these exist but, except on REM sleep, are relatively insignificant. Although it cannot be ruled out that higher doses of abecarnil might have exerted stronger effects on sleep, it should be noted that in models of anxiolytic and anticonvulsant activity abecarnil is up to 10 times more potent than diazepam (14,15). On the basis of the present study, clinically useful hypnotic properties for abecarnil are unlikely.

Nevertheless, the general profile of abecarnil in the present study was more similar to a full benzodiazepine agonist than to another β-carboline partial agonist, ZK 91 296 (5). In contrast to this latter β-carboline, abecarnil exerted strong antiepileptic properties in our spontaneously epileptic rats, together with a powerful action on the EEG spectrum. With ZK 91296, these actions were separated (5). It should be worthwhile to investigate whether the spectral changes are related to either the anxiolytic effects or perhaps the amnesic effects of the full benzodiazepines (6,7,13) and whether all these effects undergo tolerance.

Previous studies also observed full agonist-like effects on abecarnil in animal models predictive of anxiolytic and anticonvulsant activity (14,15), but accompanied by effects more consistent with a partial agonist profile in tests of sedative and ataxic actions (14,15). Taking these observations together with the mild effects on behavior and sleep in the present study, it will be clear that abecarnil is a promising drug in which a good anxiolytic and antiepileptic activity is relatively dissociated from effects on behavioral and EEG measures of sedation.

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