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# Long-term abecarnil administration produces tolerance and withdrawal signs in the rat

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**Abstract**

Abecarnil is a non-benzodiazepine that possesses anxiolytic and anticonvulsant properties. Conflicting reports of tolerance and withdrawal signs following chronic abecarnil administration have emerged from animal studies using different species and different models of tolerance and dependence. This study used a radiotelemetric method to examine any emergence of tolerance and abstinence signs in the rat following long-term abecarnil administration. Hooded Wistar rats, $n = 6$ per group, were administered either abecarnil (8 mg/kg/bidaily, i.p.) or vehicle for 24 days. Locomotor activity, body temperature and electromyographic (EMG) activity were measured daily immediately following abecarnil administration. Tolerance to the abecarnil-induced muscle relaxant effects and decreased locomotor activity developed within 21 days. Administration of the benzodiazepine antagonist flumazenil (25 mg/kg), 18 h after abecarnil cessation, precipitated abstinence signs that included decreases in body temperature, and large increases in locomotor activity and muscle tone. Moreover, continuous recording of these measures over the 4 days after flumazenil administration indicated a prolonged increase in daytime locomotor activity, suggestive of spontaneous withdrawal. These data support earlier findings that reported signs of tolerance during administration of abecarnil and abstinence signs following abecarnil cessation. 

*Keywords:* Radiotelemetry; Abecarnil; Withdrawal, precipitated

## 1. Introduction

Abecarnil is a β carboline derivative with a high affinity for $GABA_A$ receptors that shows promise as a new treatment for use in anxiety disorders and some forms of epilepsy. Abecarnil has not yet been approved for marketing, but clinical trials have provided evidence for the efficacy of low-dose abecarnil, with few side effects, in the short-term treatment of generalised anxiety disorder (Ballenger et al., 1991; Lydiard et al., 1997; Pollack et al., 1997; Small and Bystritsky, 1997).

In addition to efficacy and minimal side effects, two important criteria for the acceptance of new anxiolytic and anticonvulsant drugs are minimal or no tolerance and no major discontinuation signs upon drug cessation. Some animal studies have indicated that abecarnil meets these criteria (Löscher and Honack, 1992; Löscher et al., 1990; Steppuhn et al., 1993; Natolino et al., 1996). Steppuhn et al. (1993) compared electroencephalographic (EEG) activity, electromyographic (EMG) activity and locomotor activity in mice following cessation of abecarnil (6 mg/kg/day for 12 days) or diazepam (12 mg/kg/day for 12 days) administration. Diazepam cessation induced severe muscle spasms, seizures and increased locomotor activity, with a maximal effect 4 days after discontinuation. In contrast, abecarnil was reported to induce few abstinence signs, with no observed seizures or changes in EEG or EMG activity. Similarly in dogs, chronic low-dose abecarnil administration (4 mg/kg/day, for 6 weeks) resulted in little evidence of tolerance and only relatively mild precipitated withdrawal signs when compared to diazepam (Löscher and Honack, 1992; Löscher et al., 1990).

There is, however, evidence of tolerance to the anticonvulsant effects of abecarnil (Rundfeldt et al., 1995; Jackson et al., 1996; Löscher et al., 1996). In a study comparing the anticonvulsant efficacy of diazepam, bretazenil and abecarnil in mice, Rundfeldt et al. (1995) suggested that the seizure model used was critically important when

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assessing tolerance development. These researchers found that high-dose abecarnil administration (10 mg/kg, bidaily for 6 days) resulted in tolerance to its anticonvulsant effects in the electroshock seizure model, but none to its effects in the pentylenetetrazole-induced seizure model. In an extension of this research, Löscher et al. (1996) examined the ability of abecarnil to increase the threshold for both pentylenetetrazole and electroshock induced seizures. In this latter experiment, mice were chronically treated with abecarnil (10 mg/kg, bidaily) for a total of 4 weeks. After 1 week of treatment with abecarnil, mice experienced a moderate loss of anticonvulsant activity, and after 4 weeks, there was a marked loss of anticonvulsant activity. In addition, pentylenetetrazole seizure thresholds were significantly decreased, indicating withdrawal excitability, during the 7 days following abecarnil cessation. These researchers concluded that as an anticonvulsant, abecarnil offers no clear advantages over conventional benzodiazepines. Furthermore, they concluded that for evaluation of tolerance and dependence, these non-benzodiazepine compounds should be administered for longer than the 1–2 weeks commonly used in such studies.

As abecarnil tolerance was evident after 4 weeks of drug administration in the Löscher et al. (1996) study, it is important to re-evaluate tolerance and withdrawal using similar treatment regimes. The earlier studies in dogs (Löscher and Honack, 1992; Löscher et al., 1990) found little evidence of withdrawal after 6 weeks exposure, but these studies used low doses of abecarnil that did not produce motor impairment. Steppuhn et al. (1993) found no evidence of withdrawal signs, but their mice were administered abecarnil for only 12 days. Thus, to date, only some abecarnil withdrawal signs have been evaluated in a study combining moderately high dosing levels and prolonged administration.

The aim of the present study was to use a radiotelemetric method in the rat to examine the ability of abecarnil, administered over a period of 24 days, to induce tolerance and withdrawal signs. Radiotelemetry allowed concurrent measurement of locomotor activity, EMG and body temperature both during and after abecarnil administration. A previous study had shown that all three parameters changed as a result of benzodiazepine administration, that tolerance developed to these effects and that marked changes occurred during withdrawal (Elliot and White, 2000). The benzodiazepine antagonist flumazenil was administered 18 h after the last dose of abecarnil to examine the emergence of any abecarnil abstinence signs. Flumazenil displaces benzodiazepines from the $GABA_A$/benzodiazepine receptor complex to precipitate rapid benzodiazepine withdrawal (Rosenberg and Chiu, 1982). It has also been reported by Löscher and Honack (1992) to precipitate mild abecarnil abstinence signs, including hot foot walking, hyperexcitability and rigid postures, similar to those observed in dogs after diazepam cessation. Thus, it was anticipated that if abecarnil discontinuation signs were to emerge in the present study, then they would be evident shortly after flumazenil administration. In addition, to obtain evidence of any spontaneous withdrawal, data were collected over the 4 days after flumazenil administration.

## 2. Materials and methods

### 2.1. Subjects

Male Hooded Wistar rats (Gilles Plains, Adelaide) weighing 250–300 g, were singly caged in a temperature ($21 \pm 2$°C) and humidity controlled room at the Institute of Medical and Veterinary Sciences (IMVS) animal house and maintained on a 12-h light–dark cycle from 0700 h. All animals were treated with due care and respect in accordance with the guidelines of the IMVS Animal Ethics Committee. The experimental protocols were performed with approval from the IMVS Animal Ethics Committee.

### 2.2. Drugs and drug preparation

Abecarnil (gift from Schering, Germany) was dissolved in peanut oil (8 mg/ml). Tribrissen antibiotic (trimethoprim 80 mg/ml and sulfadiazine 400 mg/ml, Jurox, Siverwater, NSW) 0.5 ml/kg/day was administered after surgery. Flumazenil (25 mg/kg, gift from Hoffman La-Roche, Basel, Switzerland) was suspended in 4% Tween 80 (BDH; Sigma, Castle Hill, NSW). All drugs were injected in a 1 ml/kg volume by the i.p. route. All surgical procedures were performed using gas anaesthesia (fluothane, nitrous oxide and oxygen mixture).

### 2.3. Experimental design and procedures

Rats, $n = 6$ per group, were surgically implanted with radiotransmitters (Data Sciences, St. Paul, MN) 1 week after habituation in their home cages. Each transmitter was placed in the abdomen and two electrodes were sutured into the left thigh muscle. Following surgery rats were given daily i.p. injections of Tribrissen antibiotic for 5 days to minimise the risk of infection. All animals were habituated to handling immediately after recovery from surgery. However, during the experiments handling was restricted to weighing, injecting, and activating and deactivating the radiotransmitters by passing a magnet underneath the rats' abdomens.

One group of rats was administered abecarnil (8 mg/kg, i.p.) and the other group abecarnil vehicle at 0900 and 1700 h daily for 24 days. Data were collected over 70 min after the morning dose of abecarnil on days 1–7, 14 and 21. On day 25, flumazenil (25 mg/kg) was administered to all rats followed immediately by 70 min of data collection after which data were then recorded at 30-min inter-

vals for the following 18 days. The dose of abecarnil and route of administration were comparable to those used by Löscher et al. (1996) in their study using mice. The dose of flumazenil was the same as that used to precipitate lorazepam abstinence signs in an earlier study.

For data collection, each cage was placed on top of a receiver that was connected to a 486 DX computer. EMG bursts were sampled at 600 Hz for 2 s/min. Each sample was low cut filtered at 50 Hz, to eliminate movement interference, and fully rectified. Consecutive 1-min EMG bursts were then averaged over 10 min to give one data point for every 10 min. Core body temperature and locomotor activity data were also recorded and processed by the radiotelemetry system.

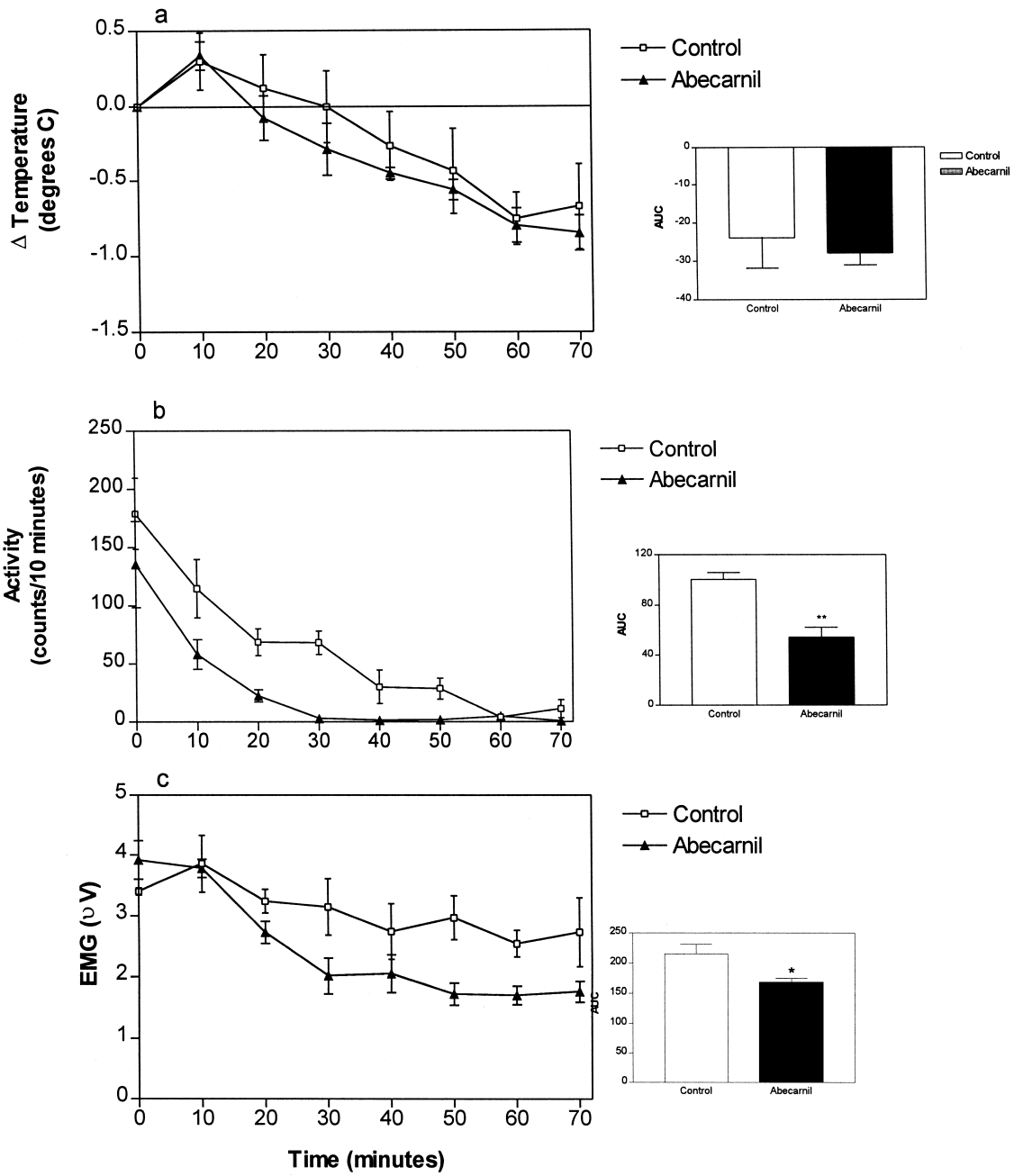


Fig. 1. (a) Acute changes in body temperature after i.p. administration of vehicle 1 ml/kg (□, control) or abecarnil 8 mg/kg (▲). Student's unpaired *t*-test of area under the curve (expressed as °C times minutes) over 70 min indicated no significant drug effect. All values are represented as means ± S.E.M., $n = 6$ per group. (b) Changes in activity after i.p. administration of vehicle 1 ml/kg (□, control) or abecarnil 8 mg/kg (▲). Student's unpaired *t*-test of area under the curve (expressed as log counts times minutes) over 70 min indicated a significant drug effect. $^{**}P < 0.01$ compared to vehicle administered controls. All values are represented as mean ± S.E.M., $n = 6$ per group. (c) Changes in EMG after i.p. administration of vehicle 1 ml/kg (□, control) or abecarnil 8 mg/kg (▲). Student's unpaired *t*-test of area under the curve (expressed as μV times minutes) over 70 min indicated a significant drug effect. $^{*}P < 0.05$ compared to vehicle administered controls. All values are represented as means ± S.E.M., $n = 6$ per group.

### 2.4. Data analysis

For each parameter the acute effects of abecarnil and vehicle administration and the effects of flumazenil were expressed as the mean ± S.E.M. of each 10-min time period over 70 min. These data were then analyzed by calculating the area under the curve (AUC) for each rat. Comparisons between abecarnil and control (vehicle-treated) groups were made using unpaired Student's *t*-tests for activity, temperature and EMG. To preserve homogeneity of variance activity counts were log transformed for statistical analyses. Activity data gathered during spontaneous withdrawal are represented as the proportion of total daytime activity (total daytime counts/total 24 h

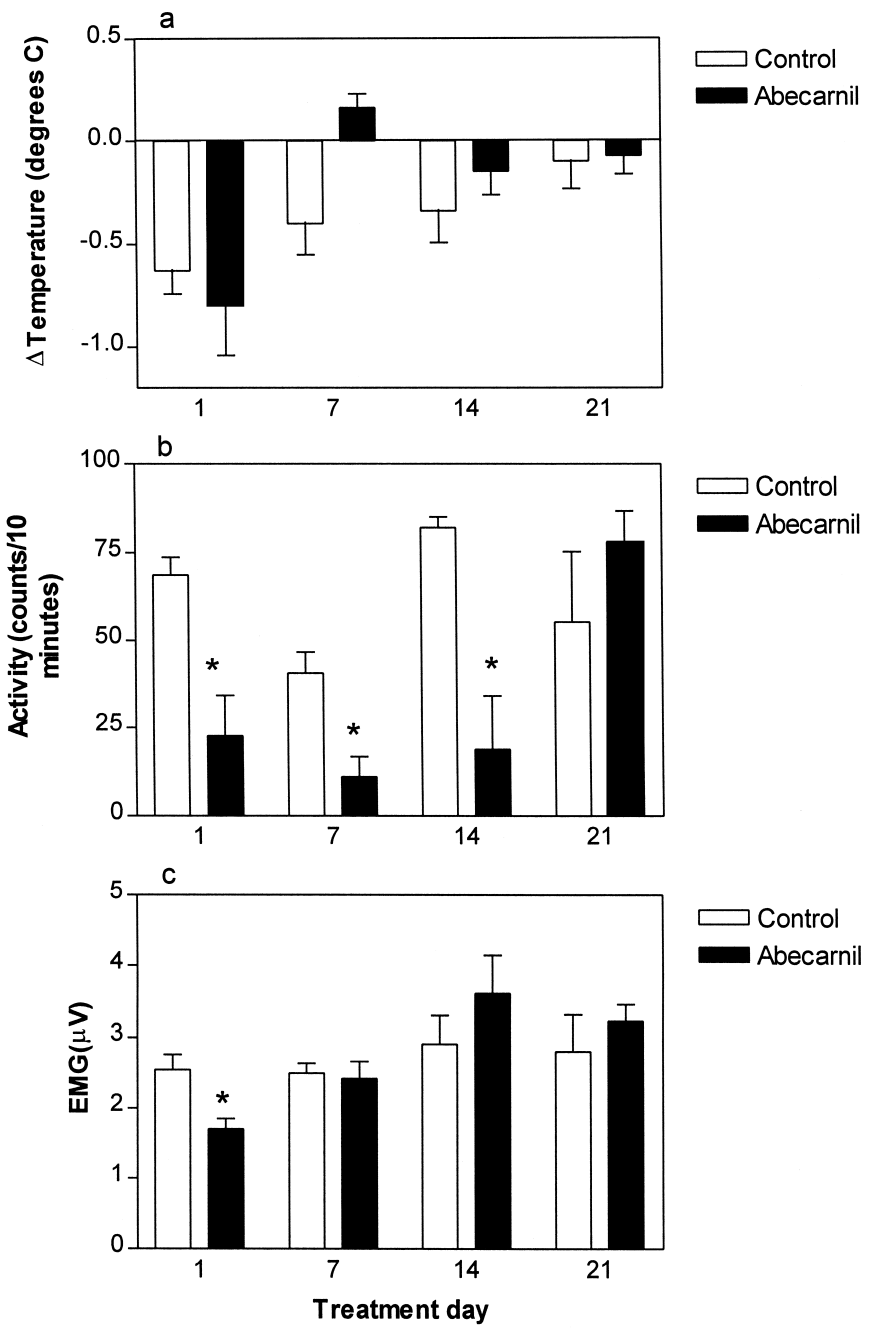


Fig. 2. (a) Acquisition of tolerance to the acute hypothermic effects of abecarnil 8 mg/kg. Body temperatures were recorded 60 min after drug administration. All values are represented as means ± S.E.M., $n = 6$ per group. (b) Acquisition of tolerance to the acute sedative effects of abecarnil 8 mg/kg. Activity was recorded 20 min after drug administration. $^{*}P < 0.05$, compared to vehicle administered controls. All values are represented as means ± S.E.M., $n = 6$ per group. (c) Acquisition of tolerance to the acute muscle relaxant effects of abecarnil 8 mg/kg. EMG was recorded 20 min after drug administration. $^{*}P < 0.05$, compared to vehicle-administered controls. All values are represented as means ± S.E.M., $n = 6$ per group.

counts) recorded during the lights on period (0700–1900 h) on withdrawal days 1, 2, 3, and 4. EMG represents data gathered between 0900 and 1200 h daily, on withdrawal days 1, 2, 3, and 4. All statistical analyses were performed using the GraphPad Prism package.

## 3. Results

Fig. 1 shows the time course for each of the three parameters over the 70-min recording period following the first administration of abecarnil. Data for vehicle treated

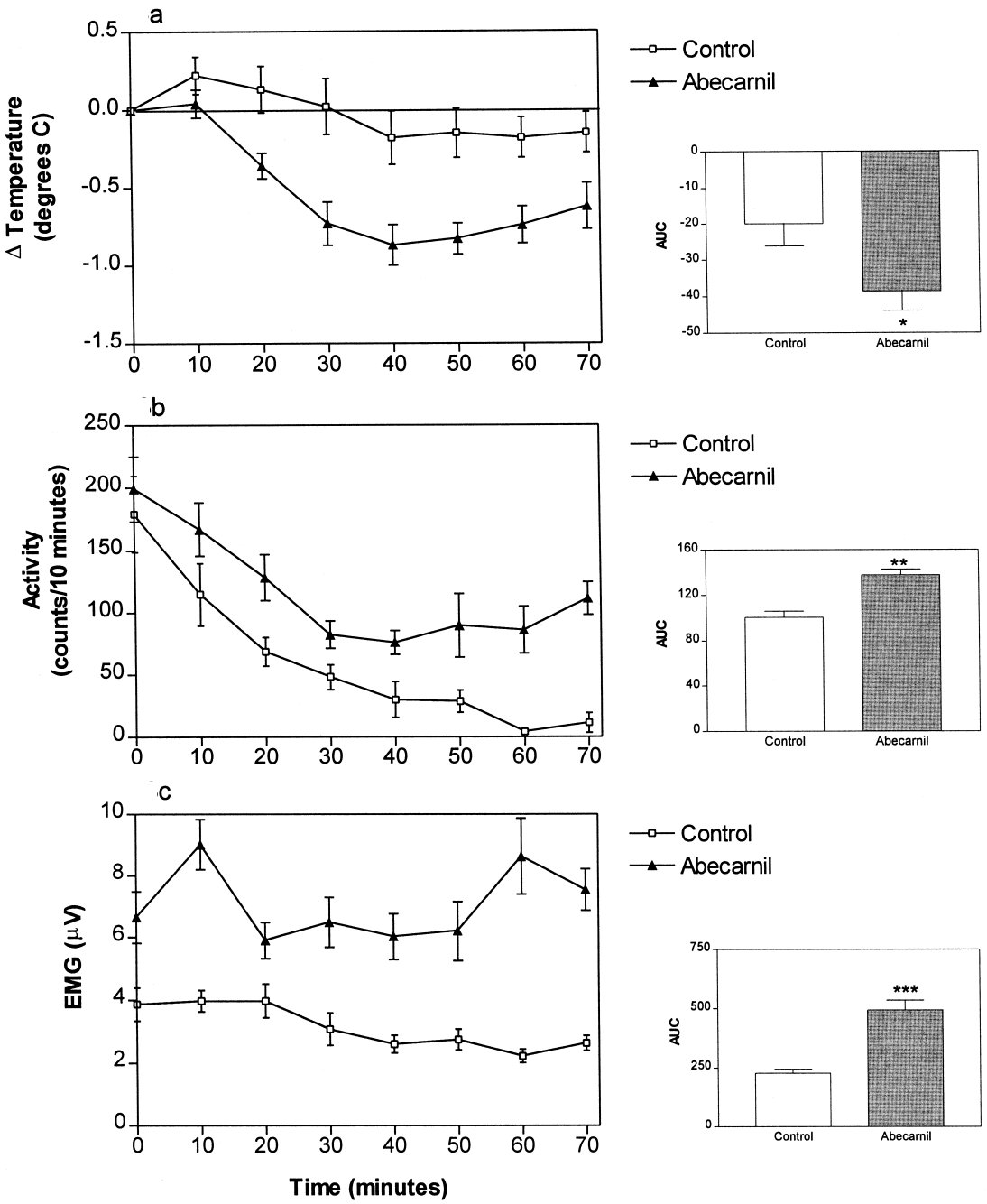


Fig. 3. (a) Changes in body temperature after i.p. administration of flumazenil (25 mg/kg) following 24 days of treatment with vehicle (□ control) or abecarnil 8 mg/kg (▲). Student's unpaired *t*-test of area under the curve (expressed as °C times minutes) over 70 min indicated a significant withdrawal effect. $^{*}P < 0.05$, compared to vehicle administered controls. All values are represented as means ± S.E.M., $n = 6$ per group. (b) Changes in activity after i.p. administration of flumazenil (25 mg/kg) following 24 days of treatment with vehicle (□, control) or abecarnil 8 mg/kg (▲). Student's unpaired *t*-test of area under the curve (expressed as log counts times minutes) over 70 min indicated a significant withdrawal effect. $^{**}P < 0.01$, compared to vehicle administered controls. All values are represented as means ± S.E.M., $n = 6$ per group. (c) Changes in EMG after i.p. administration of flumazenil (25 mg/kg) following 24 days of treatment with vehicle (□, control) or abecarnil 8 mg/kg (▲). Student's unpaired *t*-test of area under the curve (expressed as μV times minutes) over 70 min indicated a significant withdrawal effect. $^{***}P < 0.001$ compared to vehicle administered controls. All values are represented as means ± S.E.M., $n = 6$ per group.

control animals are also shown and for both groups the area under the time–effect curve is graphed. On the first day of treatment, abecarnil administration resulted in significant decreases in activity ($P < 0.01$) and muscle tone ($P < 0.05$), compared to vehicle administered controls (Fig. 1b and c). Activity and EMG decreases were maximal 30 and 60 min, respectively, after abecarnil administration with the greatest rate of decline occurring within the first 30 min of recording. Control animals showed a gradual decline in activity over time, whereas the corresponding EMG values showed a similar but smaller decrease. Body temperatures for both vehicle and abecarnil treated rats declined over the 70-min recording period, but there were no statistically significant differences between the two treatment groups.

Rats were administered abecarnil (8 mg/kg, s.c.) or vehicle bidaily for 24 days. Tolerance was evaluated by comparing the peak effect of the morning dose of abecarnil on treatment days 1, 7, 14 and 21. Fig. 2 shows body temperature values at 60 min, and activity and EMG at 30 min, after drug or vehicle administration (peak effect). There were no statistically significant changes in body temperatures in either treatment group throughout the 24 days of testing (Fig. 2a). Tolerance to the abecarnil-induced decrease in locomotor activity developed slowly and did not emerge until the third week of treatment ($P < 0.05$, Fig. 2b). In contrast, tolerance to the mild muscle relaxant action of abecarnil administration developed rapidly and was evident within 7 days of treatment ($P < 0.05$, Fig. 2c). Controls administered peanut oil vehicle recorded no statistically significant changes in body temperature, activity or muscle relaxation over the 24 days of treatment (Fig. 2a–c).

After 24 days of abecarnil pretreatment the benzodiazepine antagonist flumazenil (25 mg/kg, i.p.) was administered to evaluate precipitated abecarnil abstinence signs. Fig. 3 illustrates changes in body temperature, locomotor activity and EMG over the 70 min immediately following flumazenil administration. A significant decrease in body temperature was recorded in rats pretreated with abecarnil compared to those pretreated with vehicle ($P < 0.05$) (Fig. 3a). This decrease in body temperature was maximal 40 min after flumazenil administration and remained below control levels throughout the recording period. Compared to vehicle treated controls, both activity ($P < 0.01$) and EMG ($P < 0.05$) values were elevated significantly in abecarnil administered rats (Fig. 3b and c). The difference in activity levels was maximal 70 min after flumazenil administration and at this end time point the activity levels appeared to be still increasing. In abecarnil-pretreated rats, EMG levels rose rapidly, reaching a maximal level within 10 min of flumazenil administration, and then remained elevated throughout the recording session. Vehicle-pretreated controls recorded minor decreases in body temperature, activity and EMG following flumazenil administration. However, these changes were smaller in magnitude than those observed on the first day of vehicle administration and presumably attributable to habituation to the bidaily injecting regime.

Signs of withdrawal over the 4 days following cessation of abecarnil and vehicle are shown in Fig. 4. In vehicle-treated controls, daytime activity levels (expressed as a proportion of total 24-h activity) accounted for approximately 30% of the total activity in any one 24-h period. However, after abecarnil cessation daytime activity levels rose to 55% of the total 24-h activity (Fig. 4a), with night-time activity levels lower than those recorded by controls. This elevated daytime activity peaked on withdrawal day three (Fig. 4a) and remained elevated for a further 2 weeks after abecarnil cessation (data not shown). During these same 4 days, EMG levels in the abecarnil treated rats were moderately higher than those recorded in controls, attaining statistical significance ($P < 0.05$) on the third day of withdrawal (Fig. 4b). No other statistically significant differences between groups were observed in the 4 days post-treatment.

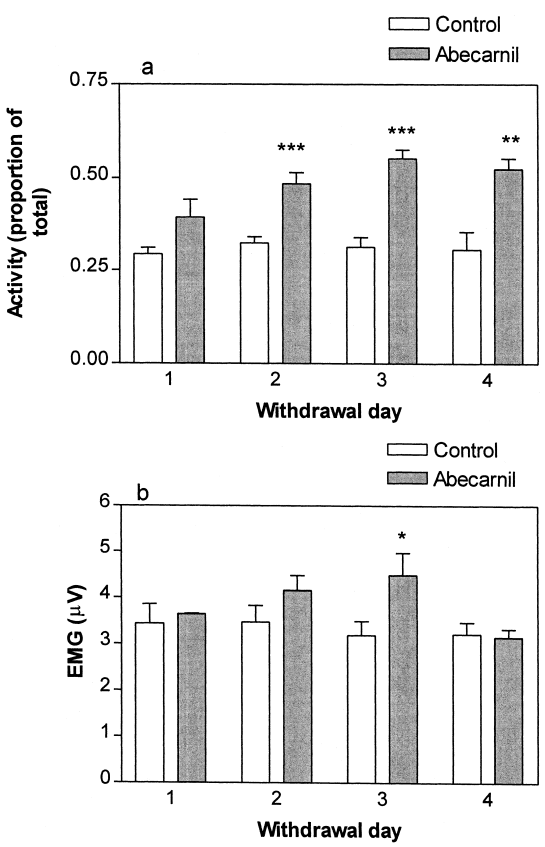


Fig. 4. (a) Daytime activity, as a proportion of total daily activity, over the 4 days after abecarnil and vehicle discontinuation. $^{***}P < 0.001$, $^{**}P < 0.01$ compared to vehicle treated controls. All values are represented as means ± S.E.M., $n = 6$ per group. (b) EMG levels of rats (between 0900 and 1200 h) over the 4 days after abecarnil and vehicle discontinuation. $^{*}P < 0.05$, compared to vehicle-treated controls. All values are represented as means ± S.E.M., $n = 6$ per group.

## 4. Discussion

The main findings from the present study were that chronic administration of high doses of abecarnil over 24 days produced tolerance and abstinence signs. These findings are consistent with those reported by Löscher et al. (1996) using a high dose of abecarnil in mice (10 mg/kg bidaily, i.p., for 4 weeks), but are not in accord with their earlier study (Löscher et al., 1990) where a low dose of abecarnil (4 mg/kg, s.c., for 6 weeks) failed to elicit withdrawal signs in dogs.

In the present study, acute administration of abecarnil (8 mg/kg, i.p.) produced a marked decrease in locomotor activity and mild muscle relaxation, with no accompanying significant changes in body temperatures over the 70 min of recording. These decreases in locomotor activity and muscle tone are consistent with the rotarod failure, ataxia and sedation scores reported by Löscher et al. (1996) following acute abecarnil administration (10 mg/kg). However, the modest decrease in body temperature in the present study contrasts to the 2°C decrease in body temperature of mice, reported by these same researchers, 30 min after administration of abecarnil. The difference between this marked hypothermia in mice and the results reported here probably reflects the smaller dose used in the present study, although species difference and the methodology itself cannot be discounted. Using rectal probes, Miller and O'Callaghan (1996) reported that restraint stress decreased body temperatures in mice by approximately 1.5°C. Thus, the difference between the temperatures reported here and those reported by Löscher et al. (1996) may have resulted from the different methods used to measure body temperature: radiotelemetry is restraint free, while the rectal probe method used by Löscher et al. (1996) necessitates some restraint.

On the first day of administration, the peak sedative and muscle relaxant effects of abecarnil were observed after approximately 30 min. These acute effects persisted over the remaining 40 min of data collection. This time course of acute effects is similar to that reported by Turski and Stephens (1993), who examined the ability of i.v. and i.p. abecarnil to decrease muscle tone and suppress flexor reflexes in genetically spastic mice. They reported a maximal i.v. effect of abecarnil after 20 min, and this effect persisted for at least 2 h. Interestingly, they found no corresponding decrease in EMG activity or suppression of flexor reflexes after i.p. abecarnil in these mice. Why this should be so is uncertain, although the authors did suggest that abecarnil may be acting as a partial agonist with low intrinsic efficacy at spinal cord receptors.

Tolerance developed rapidly (within 7 days) to abecarnil's mild muscle relaxant effect, but tolerance to the reduced locomotor activity levels took 3 weeks to develop. It is interesting to note that if the shorter treatment time used in previous experiments had been adhered to, then tolerance to the sedative effect of abecarnil would not have been observed. The time taken for tolerance to develop to abecarnil's locomotor depressant actions was similar to that reported by Löscher et al. (1996) for changes in seizure threshold, but longer than the 7–14 days they reported for the development of tolerance to abecarnil's sedative, motor impairing and hypothermic actions. Again, both methodological and species differences could account for the variation in findings. Löscher et al. used subjective observer-rated scales of ataxia and sedation, and rotarod failure scores, to assess the development of tolerance, and this may account for much of the difference in time for tolerance to develop in these two studies.

In abecarnil-pretreated rats, administration of flumazenil (25 mg/kg) precipitated abstinence signs that included decreases in body temperature and concomitant increases in activity and EMG. The magnitude of the temperature decreases was approximately half that observed in an earlier study using lorazepam, whereas the increases in EMG and activity values were similar in magnitude (Elliot and White, 2000). This result is in agreement with that of Löscher and Honack (1992) who reported qualitatively similar abstinence signs in both abecarnil and diazepam pretreated dogs, following flumazenil (20 mg/kg, i.v.) administration. The main difference between the two groups in the Honack and Loscher study was that the total abecarnil abstinence score was approximately half the total diazepam abstinence score. The present results, taken together with the study reported by Löscher and Honack (1992), suggest that the withdrawal syndromes associated with benzodiazepines and abecarnil are qualitatively similar, but there may be quantitative differences across measures.

Cessation of abecarnil administration resulted in large increases in the proportion of total activity occurring during daytime (i.e. the normal quiescent period for rats). These data reflect significant disruption to the normal diurnal activity pattern of the animals and the duration of increased daily activity (ratio of total activity) was unexpectedly protracted. Pinna et al. (1997) reported anxiety, seizures and increases in muscle tone for up to 14 days after alprazolam cessation, but total exploratory activity was not reported. In a study of lorazepam dependence, van der Laan et al. (1992) reported increases in daytime activity that lasted until withdrawal days 7–9. Thus, it appears that abecarnil cessation produced spontaneous withdrawal signs that persisted for as long as those reported following cessation of benzodiazepines.

The development of complete tolerance to the acute effects of abecarnil, together with the ability of flumazenil to precipitate large withdrawal effects and the presence of spontaneous withdrawal, imply that some major neurochemical changes, akin to those observed after benzodiazepine discontinuation, had occurred in these rats. As no receptor binding studies were performed on these animals, it is only possible to speculate about the nature of any such changes. In a study of $GABA_A$ receptor mRNA levels in

rats pretreated with abecarnil (6 mg/kg/day, s.c.) or diazepam (15 mg/kg/day, s.c.), Holt et al. (1996) reported a significant decrease in cortex $\beta_2$ and $\gamma_2$ mRNA and a small but non-significant decrease in $\alpha_1$ mRNA expression in both groups after 14 days. However, in diazepam-treated rats, they reported an additional increase in $\alpha_3$, $\alpha_4$, $\alpha_5$ and $\beta_1$ mRNA. Thus, chronic administration of abecarnil produced a subset of the constellation of changes elicited by chronic diazepam administration.

In humans, chromosomal mapping studies have shown that $GABA_A$ receptor subunit genes tend to be arranged in clusters and that, in some cases, clustered genes are co-regulated. The $\alpha_1$, $\beta_2$ and $\gamma_2$ subunit genes are located on human chromosome 5q32–5q33 (Wilcox et al., 1992). Further, it has been established that $GABA_A$ receptor gene sequences are highly conserved in vertebrates (Lasham et al., 1991). Thus, although this has not been established in rats, it would seem reasonable that the genes encoding $\alpha_1$, $\beta_2$ and $\gamma_2$ subunits would also cluster on the rat chromosome equivalent to human chromosome 5. Theoretically, it is possible that $\alpha_1$, $\beta_2$ and $\gamma_2$ subunit mRNA expression may be co-regulated, particularly given the decreased expression of $\beta_2$ and $\gamma_2$ subunit transcripts reported by Holt et al. (1996) after chronic treatment with abecarnil. Hence, prolonged treatment with moderate to high doses of abecarnil, as administered in the present study, may result in decreased expression of $\alpha_1$, $\beta_2$ and $\gamma_2$ subunit mRNA transcripts and ultimately a decrease in $GABA_A$ receptor subtypes that are associated with abecarnil binding. A decrease in $GABA_A$ receptor numbers, or a conformational change in $GABA_A$ receptors that altered abecarnil binding, could account for the tolerance and subsequent withdrawal signs observed in these animals.

The reason for the discrepancy in the findings of different researchers relating to abecarnil's ability to induce an abstinence syndrome is unclear. Apart from species differences, pharmacokinetic differences may, in part, provide another explanation. Abecarnil is a highly lipid soluble compound that requires an organic solvent such as peanut oil for administration. Pharmacokinetic studies in animals show that abecarnil is rapidly and almost completely absorbed after i.p. (5 mg/kg), oral (10 mg/kg) or i.v. (1 mg/kg) administration and no accumulation occurs with multiple oral or i.v. daily dosing for up to 30 days (Krause and Mengel, 1990). However, Löscher et al. (1990) reported that, in dogs, abecarnil was eliminated rapidly following i.v. or p.o. administration, but elimination was substantially delayed after s.c. administration. This latter study indicated that s.c. abecarnil administration resulted in slow absorption from the injection sites, with elimination taking at least 16 days after cessation of dosing. Thus, when administered s.c., abecarnil may accumulate at the injection site and provide a pool of drug that is available for absorption long after daily dosing has ceased. However, when administered i.p., as in the present study and that by Löscher et al. (1996), no such pool of abecarnil would be available to ameliorate withdrawal signs.

In conclusion, this study demonstrated the development of tolerance to the locomotor activity depressant and mild muscle relaxant actions of abecarnil and the emergence of both flumazenil-precipitated and spontaneous withdrawal signs following abecarnil cessation. These data suggest that long-term use of abecarnil may have limited advantages over the classic benzodiazepines.

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