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Anticonvulsant Action of the β-Carboline Abecarnil: Studies in Rodents and Baboon, *Papio papio*

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ABSTRACT

Abecarnil (ZK 112119; isopropyl-6-benzyloxy-4-methoxymethyl-β-carboxylate) is a metabolically stable β-carboline derivative with potent anxiolytic and few sedative and ataxic effects in rodents. The anticonvulsant and muscle relaxant actions of abecarnil have been evaluated in mice, rats, gerbils and baboons. Abecarnil raised the threshold for tonic electroconvulsions in mice after corneal but not after auricular application, had no effect on maximal electroshock-induced tonic convulsions triggered by either method, protected mice against the tonic hindlimb extension in PTZ-, picrotoxin- and 3-mercaptopropionate-induced seizures and blocked clonus after PTZ, DMCM (methyl-4-ethyl-6,7-dimethoxy-9H-pyrido-(3,4-*b*)-indol-3-carboxylate) and 3-mercaptopropionate. Abecarnil had no effect on convulsions induced by bicuculline and strychnine. Furthermore, abecarnil blocked kindled seizures after chronic administration of PTZ and FG 7142 (β-carboline-3-carboxylic acid methylamide) and protected mice and rats against limbic convulsions induced by pilocarpine. Severity and afterdischarge duration of amygdala-kindled seizures were reduced in rats treated with abecarnil. Abecarnil also antagonized selectively convulsions induced by i.c.v. administration of kainate, but not those triggered by N-methyl-D-aspartate or quisqualate. In genetic models of reflex epilepsy, abecarnil was effective against sound-induced convulsions in DBA/2 mice, against air blast-induced generalized seizures in gerbils and against myoclonus in baboons *Papio papio*. The anticonvulsant effect of abecarnil in a PTZ seizure model in mice was potentiated by ethosuximide, whereas no significant potentiation was found with diazepam, clonazepam, diphenylhydantoin, carbamazepine and phenobarbital. Electromyographic monitoring in an etorphine model of muscle rigidity in rats showed no or little muscle relaxant effect of abecarnil. These findings document a distinctive profile of anticonvulsant action of abecarnil in rodents and primates.

An important accomplishment permitting progress in the field of BZ research was the discovery of specific binding sites for BZ ligands in the brain (Möhler and Okada, 1977; Squires and Braestrup, 1977) and the advent of a multidomain theory for the GABA receptor effector complex (Polc *et al.*, 1982). Biochemical, electrophysiological and behavioral analyses have shown a continuum of actions of BZ ligands extending from agonism through antagonism to inverse agonism, depending on the direction of their modulation of GABA effects (*e.g.* Chapman *et al.*, 1987; Jensen *et al.*, 1987; Polc, 1988; Stephens *et al.*, 1987).

The identification of β-carboline-3-carboxylate derivatives as high affinity ligands of BZ receptors (Braestrup *et al.*, 1980, 1982) prompted new synthetic activities toward designing compounds with improved pharmacological profiles (*e.g.* anxioselectivity). Our previous results with certain β-carboline derivatives have demonstrated that their functional effect is less than that predicted from their receptor affinity, suggesting partial agonist properties (Jensen *et al.*, 1983; Schneider *et al.*, 1989). Such knowledge has provided a correlate to function and enabled the design of novel compounds.

Isopropyl-6-benzyloxy-4-methoxymethyl-β-carboline-3-carboxylate (ZK 112119; abecarnil) is a metabolically stable β-carboline derivative binding with high affinity to BZ receptors and showing high anxiolytic potential in rodent tests with no or minor sedation and motor impairment, and virtual absence of ethanol potentiation (Stephens *et al.*, 1990). Here we report on the anticonvulsant profile of abecarnil 1) in a number of models of chemically or electrically induced seizures in rodents; 2) in PTZ, β-carboline-3-carboxylic acid methylamide (FG 7142) and amygdala kindling in rats and mice; 3) in genetically

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ABBREVIATIONS: BZ, benzodiazepine; GABA, γ-aminobutyrate; PTZ, pentylenetetrazol; EMG, electromyogram; MES_T, maximal electroshock seizure threshold; MES, maximal electroshock seizures; NMDA, N-methyl-D-aspartate; ILS, intermittent light stimulation; DMCM, methyl-4-ethyl-6,7-dimethoxy-9H-pyrido-(3,4-*b*)-indole-3-carboxylate.

seizure-prone gerbils and audiogenic mice; 4) and in photically induced epilepsy in the Senegalese baboon, *Papio papio*. To obtain information on a possible muscle relaxant action of abecarnil its action was tested by EMG in an etorphine model of muscle rigidity in rats.

We found that abecarnil possesses a high potency against chemically induced seizures in rodents and in genetic models of reflex epilepsy in rodents and baboons, and is devoid of or has very little muscle relaxant effect in the etorphine model of muscle rigidity in rats. The anticonvulsant and muscle relaxant characteristics of abecarnil promise advances in the understanding of the function of BZ receptor in seizure control and may portend a new class of therapeutic agents.

Materials and Methods

Animals. Experiments were carried out on male NMRI-Han mice, 18 to 22 g in weight, male DBA/2J BOM mice, 8 to 12 g in weight, gerbils of both sexes, 40 to 70 g in weight, and Wistar rats of both sexes, 220 to 250 g in weight, supplied by the Department of Animal Breeding (Schering AG, Berlin, F.R.G.), the Zentralinstitut für Versuchstierzucht (Hannover, F.R.G.), Winkelmann Versuchstierzuchtanstalt (Borchen, F.R.G.) and Bolmholtgaard (Copenhagen, Denmark). The animals were housed under environmentally controlled conditions (6:00 A.M.–6:00 P.M. light/dark cycle; 22–24°C; 45–55% humidity) and permitted free access to food (Sniff, Versuchstierdiäten GmbH, Soest, F.R.G.) and water. The behavioral observations and monitoring of the EMG took place between 9:00 A.M. and 5:00 P.M. Experimental groups were selected randomly and consisted of 4 to 15 animals.

Time Course of Action of Abecarnil

PTZ-induced clonic seizures in mice. PTZ (15 mg/ml) was infused into a tail vein of unrestrained mice until a clonic convulsion was elicited. The clonic seizure represented the endpoint of the test and was used as a criterion to indicate a convulsive response. The dose of PTZ (in milligrams per kilogram) required to elicit clonic seizures (minimal convulsant dose) in mice subjected to abecarnil in the dose of 2 mg/kg was estimated 5, 10, 20 and 30 min and 1, 2, 4 and 8 hr after i.p. administration and the mean ± S.E.M. was calculated for groups of five to six animals. Statistical analysis of the data was made by means of two-way analysis of variance with drug and time as two independent factors (Armitage, 1971).

Electroconvulsions and Chemically Induced Seizures in Mice and Rats

MES_T (tonic extension of the hindlimbs) in mice. Groups of 10 mice were given solvent or experimental drugs i.p. 30 min before the electrical stimulation (50 Hz, 0.2 sec) by corneal or auricular electrodes. The threshold current intensity, defined as the current inducing tonic hindlimb extension in 50% of the mice, was determined by the up and down method of Dixon and Mood (1948) for each dose of the drug. At least 10 mice were tested at a current ± 20% of the threshold. Daily controls showed basal seizure threshold at 12 mA (10–14) for corneal and 9.3 mA (8.5–10) for auricular current application. The ED_{100} was the dose resulting in 100% increase of the threshold estimated by computer analysis (corresponding to 24 or 18.6 mA, respectively) (Armitage, 1971).

MES in mice. MES seizures (tonic extension of the hindlimbs) were triggered in mice with a current intensity 5 to 7 times that required to induce maximal tonic extension threshold seizures (Piredda *et al.*, 1985). Groups of 6 to 10 mice were given solvent or experimental drugs i.p. 30 min before the electrical stimulation (50 mA, 50 Hz, 0.2 sec) by corneal or auricular electrodes. The basal maximal MES_T was determined at 7.8 mA (6.9–8.9) for corneal and 10 mA (9–11) for auricular current application. The anticonvulsive potency of abecarnil and diazepam was estimated as ED_{50} (in milligrams per kilogram) against tonic seizures according to Litchfield and Wilcoxon (1949). Thirty six to 50 mice were used to calculate ED_{50} values and their 95% CL.

Chemical convulsions in mice and rats. Chemical convulsions were induced by PTZ (150 mg/kg), DMCM (15 mg/kg), bicuculline (3.5 mg/kg), picrotoxin (10 mg/kg), 3-mercaptopropionic acid (40 mg/kg), strychnine (10 mg/kg) or pilocarpine (380 mg/kg). All chemoconvulsants were injected s.c. except 3-mercaptopropionic acid and pilocarpine which were administered i.p. The animals were subsequently placed singly into perspex cages (20 × 15 × 10 cm) and observed for 2 hr after pilocarpine and for 30 min after the other convulsants. The criterion used to indicate convulsant responses was either clonic or tonic seizure (or status epilepticus in pilocarpine seizures). The anticonvulsant potency of abecarnil or diazepam was evaluated as ED_{50} (in milligrams per kilogram) against seizures. Sixteen to 70 mice were used to calculate ED_{50} values and their 95% CL according to Litchfield and Wilcoxon (1949).

Chemical convulsions in mice after i.c.v. injections of excitatory amino acids. The compounds were delivered in a volume of 5 µl into the lateral brain ventricle of mice according to the technique described elsewhere (Turski *et al.*, 1985). The injection cannula (Hamilton microsyringe type 75 N) fitted with a nylon stop to attain a depth of 3.2 mm was placed perpendicular to the surface of the skull. The flow rate of the drug solution was 1 µl/5 sec. The animals were restrained gently during the period of the injection. The mice were injected only once and were observed subsequently for the occurrence of clonic seizures within 30 min. The dose of convulsant required to induce a seizure response in 50% of the mice (CD_{50}; convulsant dose) was determined by computer analysis of data obtained from four to five experiments with different doses. The incidence of seizure response (probit transformed percentages) was plotted *vs.* log dose of the convulsant administered. The CD_{50} and the CLs were estimated by means of linear regression analysis. NMDA and kainate were used in the dose of 1 nmol and quisqualate in the dose of 100 nmol (which approximated CD_{100} for clonic seizures), for determining the anticonvulsant potency of abecarnil and diazepam. The ED_{50} values for abecarnil and diazepam against clonic seizures triggered by i.c.v. administration of the excitatory amino acids were determined 30 min after systemic (i.p.) administration of abecarnil or diazepam. The data from the convulsant tests were analysed statistically according to Litchfield and Wilcoxon (1949).

Audiogenic convulsions. Male DBA/2J BOM mice were exposed to sound stimulation with a tone of 111 dB and 14 kHz for 30 sec, 30 min after i.p. administration of the drugs tested. The animals were placed individually in a wooden, sound-insulated box (25 × 22 × 15 cm) and the sound stimulation begun immediately after transfer to the test box. The sound triggers in DBA/2 mice a characteristic sequence of seizure-response consisting of wild running, clonus, tonus and, frequently, respiratory arrest. The control and drug- treated mice were scored for incidence of the tonic hindlimb extension (tonus). The dose of the drug required to block tonic convulsions in 50% of the DBA/2J BOM mice (ED_{50}) was determined using linear regression analysis (Armitage, 1971).

Air blast-induced generalized seizures in gerbils. From the age of 5 to 8 weeks the gerbils were exposed once weekly to a blast of compressed air (average pressure, 5 bars) aimed at the back of the animal for 10 sec. Seizures induced by such treatment were classified by means of a scoring scale of Loskota *et al.* (1974). Gerbils were used for drug testing when they had developed a constant seizure pattern (stage 4–5; generalized tonic/clonic seizures; age 10–12 weeks) for at least 3 consecutive weeks. Animals were exposed to the air blast 30 min after i.p. administration of the drugs. The dose of the drug required to block the convulsant response to an air blast (stage 0) in 50% of the gerbils (ED_{50}) was determined using linear regression analysis (Armitage, 1971).

Kindling

PTZ kindling. Kindled seizures were induced by s.c. injections of 40 mg/kg of PTZ every 2nd day (Ito *et al.*, 1977; Andrews *et al.*, 1989). A 0 to 4 scoring scale was used to classify the development of convul-

sions. In this scale 0 represents no convulsive behavior, 1 represents head twitches or wet dog shakes, 2 represents myoclonic jerks, 3 represents rearing with forelimb clonus and 4 represents generalized clonic seizures. The animals were considered kindled when three consecutive stage 4 seizures were observed after PTZ and these animals were used repeatedly for drug testing. The animals were injected with the test drug i.p. 30 min before s.c. injection of PTZ and observed for 30 min for the occurrence of seizures. The dose of the drug required to block the convulsant response to PTZ (stage 0 kindling) in 50% of the rats (ED_{50}) was determined using linear regression analysis (Armitage, 1971).

FG 7142 kindling. Kindled seizures were induced in mice by i.p. injections of 40 mg/kg of FG 7142 once daily (Little *et al.*, 1984). Convulsions were characterized by initial occurrence of exophthalmos, vocalization and myoclonic jerks followed by forelimb clonus. Subsequently, animals showed upright posture with clonus of the forelimbs or running fits (Stephens and Weidmann, 1989). The animals were considered kindled when three consecutive clonic seizures were observed after FG 7142 and these animals were used repeatedly for drug testing. The animals were injected with the test drug i.p. 30 min before i.p. administration of FG 7142 and observed for 45 min for the occurrence of seizures. Those animals which had not kindled within 16 treatments were discarded. The dose of the drug required to block the convulsant response to FG 7142 in 50% of mice (ED_{50}) was determined using linear regression analysis (Armitage, 1971).

Amygdala kindling. For kindling of the right anterior amygdaloid nucleus, rats were implanted stereotaxically with bipolar electrodes under pentobarbital anesthesia (Nembutal; Ceva, Neuilly-sur-Seine, France; 50 mg/kg i.p.) (Löscher and Schwark, 1985). The constant current stimulation (500 μA, 1 msec, monophasic square-wave pulses, 50 Hz for 1 sec) was delivered to the amygdala once a day until 10 stage 5 seizures were recorded. The following variables were measured in fully kindled rats for determination of anticonvulsant efficacy of drugs: 1) *seizure latency*, the length of time between stimulation of the amygdala and the first sign of behavioral seizure activity (usually eye closure); 2) *seizure severity*, the severity of behavioral seizures graded according to Racine (1972); 3) *seizure duration*, the time between the first sign and the disappearance of all signs of behavioral seizure activity; and 4) *afterdischarge duration*, the duration of electrographic seizure activity recorded from the amygdala (with an amplitude of at least twice the amplitude of the prestimulus recording and a frequency greater than 1 Hz). The data from the amygdala kindling were analyzed statistically by means of Wilcoxon rank tests (Armitage, 1971).

Seizures in Baboons

Photically induced seizures. Experiments were performed in five adolescent Senegalese baboons, *Papio papio*, 4 to 9 kg in weight, habituated to the experimental procedure. Animals were seated in primate chairs and the myoclonic responses to ILS were graded according to Meldrum and Horton (1971). One hour after the control photic stimulation the animals received an i.v. injection of the drug and were subsequently tested with intermittent photic stimulation after 15 min and then at hourly intervals. Myoclonic responses and behavioral alterations were observed for 5 hr after the injection of solvent, abecarnil or diazepam. In each baboon at least 1 week elapsed between successive drug tests.

Muscle Tone

Electromyographic procedure. EMG activity was recorded from the gastrocnemius muscle of rats subjected to i.p. injection of etorphine in the dose of 20 μg/kg with pairs of Teflon-insulated stainless-steel wire electrodes (Cooner Wire, Chabworth, CA, AS 632 SS) inserted percutaneously into the muscle. The rats were placed separately in ventilated perspex boxes and their hindlimbs, secured gently with adhesive tape, extended through slots in the bottom of the boxes. The electrical signals were amplified, band-pass filtered (5 Hz-10 kHz) and rectified. The EMG was recorded continuously and the average integrated activity determined over 5-min periods. The control EMG activity was measured in two 5-min periods at an interval of 5 min. The mean value calculated from these measurements served as a reference for the determination of changes in the EMG after the administration of drugs or solvent. Changes in the activity in the EMG were expressed as a fraction of the mean activity before and after the administration of the drug under study. The data from the EMG experiments were analyzed by means of the Mann-Whitney U test (Armitage, 1971).

Drugs

PTZ, picrotoxin, strychnine nitrate, 3-mercaptopropionic acid, pilocarpine hydrochloride, methylscopolamine nitrate (Sigma Chemical Co., St. Louis, MO), etorphine (Reckitt & Colman, Densom Lane, Hull, U.K.), phenobarbital (Merck, Darmstadt, F.R.G.) and ethosuximide (Gödecke, Berlin, F.R.G.) were brought into solution with saline, whereas bicuculline was dissolved in a drop of glacial acetic acid and made up with saline being subsequently adjusted to pH 4.3–4.5 with 1 N NaOH. DMCM (Schering AG, Berlin, F.R.G.) was dissolved in 0.01 N HCl. NMDA was obtained from Tocris Chemicals (Buckhurst Hill, Essex, U.K.). Kainic acid and quisqualic acid were purchased from Sigma. The excitatory amino acids were brought into solution with a minimum quantity of 1 N NaOH and the final volume was made up with saline, with the pH being adjusted to 7.35 by adding 0.2 N HCl. Abecarnil (ZK 112119; Schering AG), FG 7142 (Ferrosan, Soeborg, Denmark), diazepam and clonazepam (Hoffmann-La Roche, Basle, Switzerland), diphenylhydantoin (Desitin, Hamburg, F.R.G.) and carbamazepine (Sigma) were suspended in 5% Cremophor EL in saline (BASF, Ludwigshafen, F.R.G).

Abecarnil and diazepam were administered i.p. or p.o. 30 min before convulsant tests. For interaction tests clonazepam, phenobarbital, carbamazepine and ethosuximide were injected 30 min and diphenylhydantoin 60 min before either abecarnil or diazepam. EMG was recorded immediately after i.p. administration of abecarnil or diazepam over the period of 90 min. In baboons, abecarnil or diazepam were given i.v. 45 min after the control test with ILS, *i.e.*, 15 min before the first postdrug exposure to ILS.

Statistics. The experimental data were analyzed statistically by means of two-factor analysis of variance, Mann-Whitney U test, Wilcoxon signed rank test for paired replicates (Armitage, 1971) and according to the Litchfield and Wilcoxon method (Litchfield and Wilcoxon, 1949).

Results

Time course of anticonvulsant action of abecarnil. The effect of abecarnil on the threshold for clonic seizures after i.v. administration of PTZ in mice was time-dependent. The elevation of the threshold for clonic seizures induced by the dose of 2 mg/kg was maximal 20 to 30 min after i.p. administration of the drug. This anticonvulsant effect remained unchanged until 2 hr postinjection and then abated gradually within the following 6 hr (fig. 1).

MES_T. Abecarnil elevated the threshold for maximal tonic extension seizures in mice by 100% at a dose of 14.53 mg/kg after corneal application of the current; after auricular application the drug remained without effect on susceptibility of mice to MES up to a dose of 100 mg/kg. Treatment with diazepam resulted in the elevation of the threshold by 100% at a dose of 6.25 mg/kg after corneal and at a dose of 3.85 mg/kg after auricular application of the current (table 1).

MES. Abecarnil (up to the dose of 100 mg/kg) failed to block tonic extension of the hindlimbs in mice subjected to MES, whereas diazepam protected against such seizures with an ED_{50} of 5.6 mg/kg after corneal and 12.25 mg/kg after auricular application of the current (table 1).

Audiogenic seizures. Abecarnil blocked hindlimb exten-

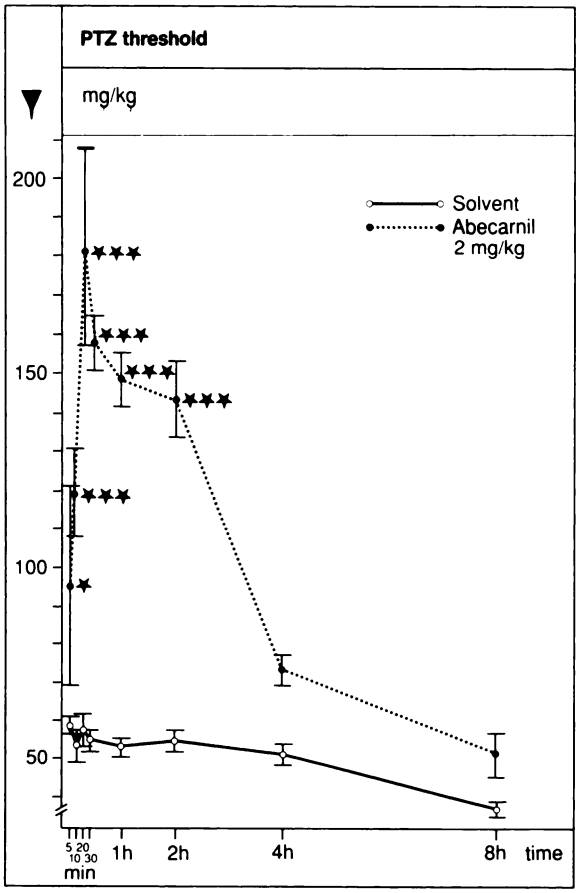

Fig. 1. Time course of anticonvulsant action of abecarnil on clonic seizures induced in mice by i.v. administration of PTZ. Abscissa scale: time (minutes or hours); ordinate scale: minimal convulsant dose of PTZ (milligrams per kilogram). Results are presented as mean values (± S.E.M.) for five to six mice. The seizure threshold in mice treated with abecarnil was elevated markedly as compared to vehicle-treated mice, $F(1,74) = 189.42$ ($P < .001$). Significant main effect over time was also recorded, $F(7,74) = 12.25$ ($P < .001$). Significant interaction effect revealed that treatment with abecarnil and vehicle differed in their effects over time, $F(7,74) = 8.61$ ($P < .001$). Treatment comparisons (contrasts) were performed within framework of the 2-way analysis of variance, ★$P < .05$; ★★★$P < .001$ *vs.* vehicle-treated mice.

sion in DBA/2J BOM mice subjected to sound stimulation with an ED_{50} of 0.0034 mg/kg, whereas diazepam was effective with an ED_{50} of 0.33 mg/kg (table 1).

Air blast-induced generalized seizures in gerbils. Abecarnil blocked generalized tonic/clonic convulsions in gerbils subjected to air blast stimulation 30 min after i.p. administration with an ED_{50} of 0.076 mg/kg. Diazepam showed similar anticonvulsant potential and protected gerbils from such seizures with an ED_{50} of 0.22 mg/kg (table 1).

Chemical convulsions (table 1). Clonic seizures induced by chemoconvulsants were blocked differentially by abecarnil. Clonic seizures induced by DMCM were antagonized with an ED_{50} of 0.04 mg/kg, whereas those triggered by PTZ were blocked with an ED_{50} of 0.43 mg/kg. Abecarnil also protected mice against clonic convulsions induced by 3-mercaptopropionic acid. In contrast, clonic seizures produced by bicuculline, picrotoxin and strychnine remained unaffected by abecarnil up to a dose of 100 mg/kg whereas diazepam was active in these tests. In rats, abecarnil blocked PTZ-induced clonic convulsions with an ED_{50} of 0.29 mg/kg. The profile of anticonvulsant action of diazepam was characterized by nonselective antagonism of clonic seizures induced by the chemoconvulsants. Clonic seizures induced by DMCM and PTZ in mice were blocked with ED_{50} values of 0.53 and 1.15 mg/kg, respectively. Mice subjected to the convulsant action of bicuculline and picrotoxin were protected by diazepam with ED_{50} values of 6.72 and 6.39 mg/kg, respectively. Clonic seizures induced by 3-mercaptopropionic acid and strychnine were also blocked by diazepam with ED_{50} values of 0.22 and 4.28 mg/kg, respectively. In rats, diazepam blocked clonic seizures induced by PTZ with an ED_{50} of 1.86 mg/kg.

Abecarnil protected mice against tonic convulsions induced by PTZ (ED_{50}, 12 mg/kg), picrotoxin (4.39 mg/kg) and 3-mercaptopropionic acid (0.02 mg/kg) and remained inactive (>100 mg/kg) against tonic seizures triggered by either bicuculline or strychnine. Diazepam protected mice against tonic seizures induced by all convulsants tested.

The seizures induced by pilocarpine in mice were blocked by abecarnil with an ED_{50} of 0.13 mg/kg and by diazepam with an ED_{50} of 0.15 mg/kg. A similar potency of protective activity of the two drugs was detected in pilocarpine seizures in rats (ED_{50} for abecarnil was 0.04 mg/kg whereas ED_{50} for diazepam was 0.18 mg/kg).

An anticonvulsant action of abecarnil was also seen in mice subjected to p.o. administration of the drug. Clonic convulsions triggered by PTZ were blocked with an ED_{50} of 1.44 mg/kg and tonic with an ED_{50} of 0.22 mg/kg. Diazepam, when administered p.o., blocked clonic seizures induced by PTZ with an ED_{50} of 1.38 mg/kg and tonic with an ED_{50} of 0.69 mg/kg.

Chemical kindling (table 2). Seizures kindled by repeated treatment of rats with PTZ (seizure stages 1–4) were blocked by abecarnil with an ED_{50} of 0.46 mg/kg whereas diazepam antagonized such seizures with an ED_{50} of 1.74 mg/kg. Both abecarnil and diazepam also prevented kindled seizures (generalized clonic) induced by repeated treatment of mice with FG 7142 (ED_{50}, 0.03 and 1.26 mg/kg, respectively).

Amygdala kindling (table 3). Convulsions kindled by repeated electrical stimulation of the amygdala in rats were ameliorated by abecarnil at a dose of 5 mg/kg. The severity of the kindled seizures and afterdischarge duration in abecarnil-treated rats was significantly less than in control animals, whereas the latency and duration of seizures remained unchanged. Similar effects were seen in rats subjected to the treatment with diazepam (5 mg/kg). Diazepam reduced markedly the severity and duration of kindled seizures, and shortened duration of afterdischarges. The latency of kindled seizures remained unaffected by diazepam treatment.

Seizures induced by excitatory amino acids (table 4). Abecarnil protected mice from clonic seizures induced by kainic acid with an ED_{50} of 2.34 mg/kg, whereas remaining ineffective (up to 100 mg/kg) against convulsions induced by i.c.v. administration of either NMDA or quisqualate. Diazepam nonpreferentially blocked seizures induced by NMDA, kainate and quisqualate (ED_{50}, 4.36, 2.21 and 7.82 mg/kg, respectively).

Interaction with antiepileptic drugs (table 5). Pretreatment of mice with antiepileptic drugs (diazepam, clonazepam, phenobarbital and carbamazepine 30 min and diphenylhydantoin 60 min before abecarnil) in doses which did not by themselves affect the susceptibility of mice to PTZ-induced clonic convulsions had no significant effect on the ED_{50} of abecarnil. However, pretreatment with ethosuximide (30 min) shifted the ED_{50} of abecarnil to the left (potency ratio, 3.0; $P < .001$, Litchfield and Wilcoxon, 1949). The ED_{50} values of diazepam

TABLE 1

Efficacy of abecarnil and diazepam against electrically and chemically induced convulsions, against audiogenic seizures and against air blast-induced seizures in mice, rats and gerbils

Abecarnil and diazepam were administered i.p. 30 min before convulsive tests. The data represent anticonvulsant potency expressed as ED_{50} values (and their 95% CL) estimated from probit-log dosage regression curves. *n*, number of animals. AB-S, air blast-induced seizures; AS, audiogenic seizures; BIC, bicuculline; 3-MP, 3-mercaptopropionic acid; PIL, pilocarpine; PTX, picrotoxin; STR, strychnine.

Treatment (dose)	Abecarnil ED_{50}		*n*	Diazepam ED_{50}		*n*
	Clonic seizures	Tonic seizures		Clonic seizures	Tonic seizures	
	mg/kg			*mg/kg*		
Mice						
MES (corneal)		>100	30		5.60 (4.81–6.50)	50
MES (auricular)		>100	18		12.25 (8.51–14.38)	36
MES_T† (corneal)		14.53 (9.58–22.1)	40		6.25 (4.60–8.50)	40
MES_T† (auricular)		>100	70		3.85 (2.68–5.53)	55
AS		0.0034 (0.0015–0.008)	30		0.33 (0.22–0.48)	30
DMCM (15 mg/kg i.p.)	0.04 (0.01–0.14)		25	0.53 (0.23–1.16)		25
PTZ (150 mg/kg s.c.)	0.43 (0.30–0.63)	0.12 (0.09–0.17)	64	1.15 (0.85–1.56)	0.44 (0.34–0.55)	36
PTZ* (150 mg/kg s.c.)	1.44 (0.81–2.89)	0.22 (0.13–0.48)	35	1.38 (0.96–2.79)	0.69 (0.36–0.79)	35
BIC (5 mg/kg s.c.)	>100	>100	69	6.72 (4.06–14.1)	3.62 (2.09–6.39)	34
PTX (10 mg/kg s.c.)	>100	4.39 (0.20–11.9)	50	6.39 (2.70–13.2)	0.31 (0.04–0.95)	45
3-MP (40 mg/kg i.p.)	0.03 (0.01–0.17)	0.02 (0.01–0.03)	24	0.22 (0.06–0.71)	0.04 (0.0002–0.40)	24
STR (1.5 mg/kg s.c.)	>100	>100	15	4.28 (2.24–8.33)	2.71 (1.39–5.81)	20
PIL** (380 mg/kg i.p.)		0.13 (0.002–0.9)	35		0.15 (0.001–0.70)	25
Rats						
PTZ (150 mg/kg s.c.)	0.29 (0.16–0.55)	0.09 (0.06–0.55)	25	1.86 (1.17–2.97)	1.01 (0.68–1.50)	19
PIL** (380 mg/kg i.p.)		0.04 (0.004–0.1)	42		0.18 (0.06–0.44)	30
Gerbils						
AB-S***		0.076 (0.046–0.099)	32		0.22 (0.14–0.35)	28

* Abecarnil and diazepam were administered p.o. 30 min before s.c. injection of PTZ; ** the criterion used to indicate convulsant response was status epilepticus defined as continuous seizure [stage 4/5 according to Racine (1972)] persisting for a period of at least 30 min before spontaneous termination; *** the criterion used to indicate convulsant response to an air blast was generalized clonic/tonic seizure [stage 4/5 according to Loskota *et al.* (1974)]; † The dose elevating the threshold for MES by 100%. The threshold for MES induced by auricular stimulation was estimated as 9.3 mA (8.5–10; $n = 36$), whereas that for MES induced by corneal stimulation was 12 mA (10–14; $n = 10$).

TABLE 2

Effect of abecarnil and diazepam on chemical kindling with PTZ and β-carboline-3-carboxylic acid methylamide (FG 7142)

The table data represent anticonvulsant potency expressed as ED_{50} values (and their 95% CL) estimated from probit-log dosage regression curves. *n*, number of animals.

Treatment (dose)	ED_{50} *vs.* Kindled Seizures				
	Abecarnil	*n*	Diazepam	*n*	
	mg/kg				
PTZ* (40 mg/kg s.c.)	0.46 (0.15–0.72)	20	1.74 (1.27–2.36)	24	
FG 7142** (40 mg/kg i.p.)	0.03 (0.002–0.07)	36	1.26 (0.78–1.62)	30	

* Kindled seizures were induced in rats by s.c. injections of 40 mg/kg of PTZ every 2nd day. The rats were injected with the test drug i.p. 30 min before s.c. injection of PTZ and observed for 30 min for the occurrence of seizures; ** kindled seizures (generalized clonic) were induced in mice by i.p. injections of 40 mg/kg of FG 7142 once daily. The mice were injected with the test drug i.p. 30 min before i.p. administration of FG 7142 and observed for 45 min for the occurrence of seizures.

TABLE 3

Effect of abecarnil and diazepam on electrical kindling of the amygdala in female rats

The table data represent anticonvulsant potency expressed as severity, latency and duration of motor seizures, and afterdischarge duration in kindled rats. *n*, number of animals.

Treatment (dose)	Motor seizures			Afterdischarge	
	Severity	Latency	Duration	Duration	*n*
		sec	*sec*	*sec*	
Control	5	1.8 ± 0.5	41.3 ± 2.5	40.7 ± 2.6	7
Abecarnil (5 mg/kg i.p.)	2.4 ± 0.4**	2.1 ± 0.5	37.1 ± 3.2	19.0 ± 4.5*	7
Control	5	2.5 ± 0.4	51.0 ± 7.4	59.2 ± 10.8	5
Diazepam (5 mg/kg i.p.)	1*	4.3 ± 0.6	11.4 ± 2.6*	11.8 ± 3.1*	5

Kindled seizures were induced in rats by electrical stimulation of the amygdala (constant current, 500 μV, 1 msec, monophasic square-wave pulses, 50 Hz, 1 sec) by means of stereotaxically implanted bipolar electrodes. The stimulation of the amygdala was performed 30 min after i.p. administration of drugs. Control readings were taken 2 to 3 days before drug testing. * $P < .05$; ** $P < .02$ *vs.* control; Wilcoxon signed rank test for paired replicates.

under similar experimental conditions were shifted nonselectively to the left by all antiepileptic drugs tested. Abecarnil remained without significant effect on the ED_{50} of diazepam.

Photically induced seizures in baboons. Five baboons were given solvent or abecarnil in doses of 0.033, 0.1, 0.33 and 1 mg/kg i.v. in random order. After the dose of 0.033 mg/kg of abecarnil a substantial protective effect was observed 5 and 60 min after the injection (fig. 2A). Complete protection against photically induced myoclonus was observed at these time intervals after abecarnil, 0.1 mg/kg, and some protection appeared to continue up to 5 hr postdrug (fig. 2B). Increasing the dose progressively increased the duration of anticonvulsant effect

TABLE 4

Protective efficacy of abecarnil and diazepam against convulsions induced by excitatory amino acids in mice

Male NMRI mice, 18 to 22 g in weight, randomly assigned to experimental groups of four to eight animals, were injected i.c.v. with NMDA (1 nmol), kainate (KA; 1 nmol) or quisqualate (QA; 100 nmol) 30 min after i.p. administration of the drugs tested. For NMDA, KA and QA doses were chosen to give approximately equiactive convulsant action (ED_{100}). Table data represent the anticonvulsant potency expressed as ED_{50} values (and their 95% CLs) against clonic seizures estimated from probit-log dosage regression curves. *n*, number of animals.

Treatment (dose)	Abecarnil		Diazepam	
	ED_{50}			
	Clonic seizures	*n*	Clonic seizures	*n*
	mg/kg		*mg/kg*	
NMDA (1 nmol)	>100	8	4.36 (3.47–5.47)	16
KA (1 nmol)	2.34 (0.7–12.78)	24	2.21 (1.97–2.47)	20
QA (100 nmol)	>100	8	7.82 (4.17–13.5)	24

(fig. 2, C and D). After the administration of abecarnil in baboons no acute motor abnormalities were observed at any dose tested. There was no nystagmus or ataxia, or evidence of disturbed balance. Responsiveness did not appear altered after abecarnil, 0.033 to 0.33 mg/kg. However, after abecarnil, 1 mg/kg, animals would not accept a banana or other food offered. This absence of the normal positive response to food lasted for about 1 hr. The anticonvulsant effect of diazepam in photosensitive baboon *Papio papio* is seen at 0.5 to 1 mg/kg and has been described elsewhere (Meldrum *et al.*, 1975).

Electromyography. Etorphine, 20 μg/kg, induced long-lasting increase in the muscle tone in rats after systemic administration (fig. 3A). Maximal increase in the muscle tone was registered within 20 min postinjection (fig. 3A). Within 60 to 80 min the increased activity in the EMG abated slowly and faded away 100 min after administration of etorphine (fig. 3A). The administration of solvent did not affect the time course and intensity of the action of etorphine in the EMG (fig. 3A). Systemic (i.p.) administration of abecarnil, 30 mg/kg, 10 min after etorphine had no effect on muscle tone in the following 90 min (fig. 3A). Abecarnil, 100 mg/kg, slightly lowered muscle tone 30 to 50 min after administration (fig. 3A). Systemic (i.p.) injection of diazepam in etorphine-treated rats dose- and time-dependently lowered the muscle tone (fig. 3B). Diazepam, 1 mg/kg, had no effect on muscle tone in etorphine-treated rats (fig. 3B), whereas 2 mg/kg decreased muscle tone for 20 min after the injection reaching maximum effect at 20 to 25 min (fig. 3B). The administration of diazepam in the dose of 5 mg/kg decreased muscle tone for 45 min with an apparent maximum effect between 20 and 45 min after administration (fig. 3B).

Discussion

The present results highlight a distinctive profile of anticonvulsant action of the β-carboline derivative abecarnil in rodents and baboons. Abecarnil was highly potent against clonic and/or tonic seizures induced by PTZ, DMCM and 3-mercaptopropionic acid, and had no effect on convulsions induced by bicuculline and strychnine. Clonic seizures induced by picrotoxin remained unaffected whereas tonic convulsions were blocked by abecarnil. Chemically kindled seizures triggered by chronic administration of PTZ to rats or FG 7142 to mice as

TABLE 5

Effect of combined treatment of abecarnil or diazepam with antiepileptic drugs on the susceptibility of mice to clonic seizures induced by PTZ

Male NMRI mice, 18 to 22 g in weight, randomly assigned to experimental groups of four to eight animals, were injected s.c. with PTZ (150 mg/kg) 30 min after i.p. administration of abecarnil or diazepam with antiepileptic drugs. For antiepileptic drugs doses were chosen to give no anticonvulsant action against PTZ. The table data represent anticonvulsive potency expressed as ED_{50} values (and their 95% CLs) estimated from probit-log dosage regression curves. Relative potency ratios were determined for either abecarnil or diazepam alone, and in the presence of respective antiepileptic drug in the constant dose. The data were statistically analyzed according to Litchfield and Wilcoxon (1949). *n*, number of animals.

Treatment (dose)	Abecarnil		
	ED_{50}		
	Abecarnil	*n*	Potency ratio
mg/kg	*mg/kg*		*mg/kg*
Control	0.44 (0.29–0.80)	60	1.0
Diazepam, 0.2	0.26 (0.20–0.41)	49	1.54 (0.97–2.56)
Clonazepam, 0.02	0.29 (0.20–0.41)	50	1.45 (0.88–2.56)
Abecarnil, 0.05			
Phenobarbital, 5	0.36 (0.24–0.51)	45	1.18 (0.72–2.09)
Diphenylhydantoin, 8	0.30 (0.22–0.41)	55	1.38 (0.87–2.34)
Carbamazepine, 10	0.36 (0.25–0.50)	44	1.18 (0.73–2.10)
Ethosuximide, 100	0.15 (0.09–0.23)	56	3.00 (1.77–5.73)***

Treatment (dose)	Diazepam		
	ED_{50}		
	Diazepam	*n*	Potency ratio
mg/kg	*mg/kg*		*mg/kg*
Control	1.16 (0.85–1.72)	36	1.0
Diazepam, 0.2			
Clonazepam, 0.02	0.67 (0.46–0.92)	55	1.73 (1.14–2.79)*
Abecarnil, 0.05	0.74 (0.58–0.97)	44	1.47 (0.98–2.22)
Phenobarbital, 5	0.55 (0.33–0.86)	32	2.11 (1.29–3.69)*
Diphenylhydantoin, 8	0.52 (0.40–0.68)	44	2.16 (1.45–3.30)*
Carbamazepine, 10	0.55 (0.31–0.88)	39	2.11 (1.29–3.73)*
Ethosuximide, 100	0.67 (0.47–1.00)	32	1.69 (1.05–2.78)*

* $P < .05$; *** $P < .001$ *vs.* respective control groups, Litchfield and Wilcoxon (1949).

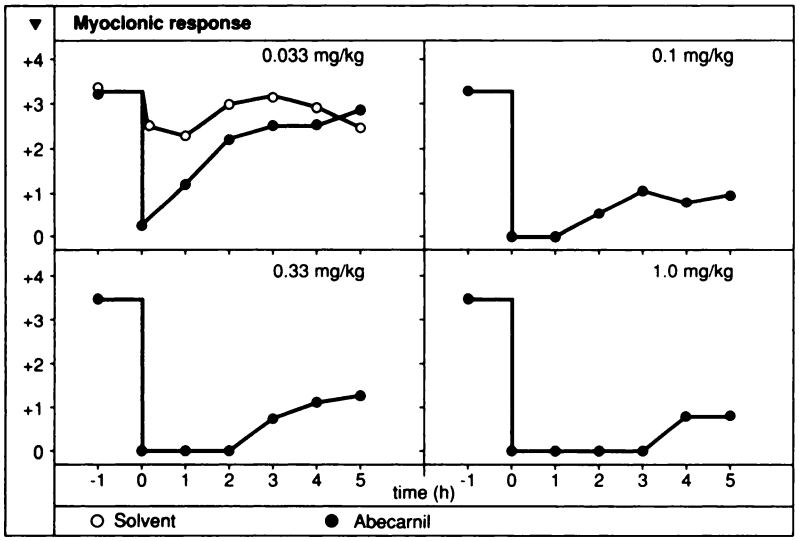

Fig. 2. Suppression of myoclonic responses to photic stimulation in the *Papio papio* baboon after the i.v. administration of abecarnil. Ordinate scores indicate response to standardized stroboscopic stimulation scored as (0) no response; (1) myoclonus of eyelids; (2) myoclonus of muscles of face and neck; (3) myoclonus of all 4 limbs; and (4) myoclonus continuing beyond the end of photic stimulation. Each point represents the mean score for five baboons at the time before or after the injection of solvent or abecarnil.

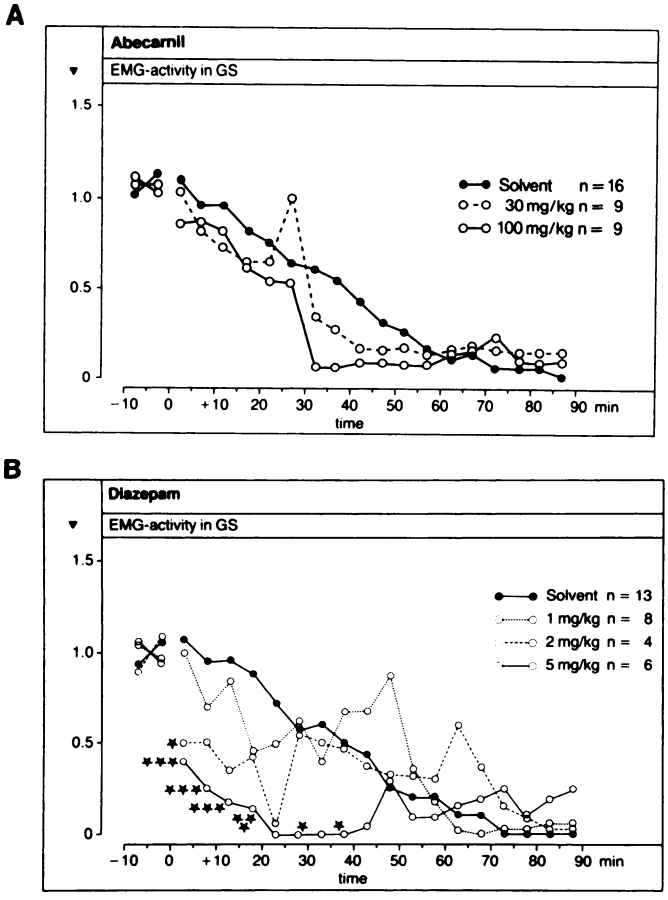

Fig. 3. Time course of action and dose-relationship of abecarnil (A) and diazepam (B) on electromyogram (EMG) activity in the gastrocnemius muscle (GS) of rats treated with etorphine, 20 μg/kg. Abecarnil and diazepam were administered i.p. 10 min after i.p. injection of etorphine. Abscissa scale: time (minutes); ordinate scale: normalized EMG activity in the GS. Results are presented as median values for 4 to 16 animals. ★$\alpha < .05$; ★★$\alpha < .01$; ★★★$\alpha < .005$ *vs.* solvent-treated rats, Mann-Whitney U test.

well as electrically kindled amygdala seizures in rats were sensitive to the anticonvulsant action of abecarnil. These observations are paralleled by the extremely high potency of abecarnil against limbic seizures induced in rodents by a cholinergic agonist, pilocarpine. Seizures induced by intracerebral application of excitatory amino acids were affected differentially by systemic administration of abecarnil: clonic convulsions induced in mice by kainic acid but not those triggered by NMDA or quisqualic acid were blocked. Experimental work with electroconvulsions showed that abecarnil moderately elevated the threshold for maximal tonic extension seizures after corneal but not after auricular application of the current. In contrast, the tonic extension seizures induced by supramaximal stimulation (MES) remained unaffected by abecarnil. In genetic models of reflex epilepsy abecarnil protected DBA/2J mice from audiogenic seizures (tonic hindlimb extension), seizure-prone gerbils from air blast-induced clonic/tonic seizures and baboons from photically induced convulsions. The anticonvulsant action of abecarnil was detected at doses that neither lowered muscle tone as evidenced by electromyographic monitoring in etorphine-treated rats nor disturbed motor coordination (neurotoxicity) as evidenced by unchanged performance of mice in rota-rod or chimney test (Stephens *et al.*, 1990).

This profile of anticonvulsant action of abecarnil differs from that of diazepam in terms of both potency and selectivity. The most noteworthy differences between abecarnil and diazepam in the selectivity of their anticonvulsant action were detected in seizures induced by electroshock, bicuculline, picrotoxin, strychnine and excitatory amino acids. In terms of potency, major differences were observed in DMCM- and sound-induced convulsions.

Diazepam and other BZs elevate the threshold for maximal tonic extension seizures triggered by electroshock and block tonic extension of the hindlimbs induced by MES in rodents, although the doses required to prevent such seizures are substantially higher than those required to protect against chemoconvulsions (Haefely *et al.*, 1981; Meldrum and Chapman, 1986). No difference in the anticonvulsant potency of diazepam in electroshock-induced seizures is observed after corneal or auricular application of the current. Lesion studies have shown that corneal application of the current involves forebrain and brainstem structures, whereas auricular application of the elec-

trical stimulus triggers a convulsive response mainly by activating the brainstem (Browning, 1987; Gale and Browning, 1988). The clear-cut difference in elevating the threshold for electroshock seizures by abecarnil after corneal and auricular application of current suggests that abecarnil acts within the forebrain to achieve its anticonvulsant effect in this seizure model. This inference is apparently supported by the fact that abecarnil is inactive against strychnine-induced seizures, whose generation is dependent on the intact spinal cord and brainstem (Dusser de Barenne, 1933; Somjen, 1987). In contrast, diazepam is active against strychnine convulsions. Another piece of evidence in line with a hypothesis of a primarily forbrain action of abecarnil is its unusual potency against DMCM, pilocarpine and chemically kindled seizures, all of which are regarded as seizures of forebrain origin (Ito *et al.*, 1977; Little *et al.*, 1984; Petersen, 1983; Turski *et al.*, 1983b).

An important mechanistic contribution to the forebrain site(s) hypothesis of anticonvulsant action of abecarnil comes also from EMG monitoring in etorphine-treated rats. Muscle rigidity induced by opiates is of both spinal and forebrain origin and is suppressed by diazepam (Turski *et al.*, 1983a). By contrast, abecarnil showed little or no effect on increased muscle tone induced by etorphine suggesting that spinal and brainstem actions of the opiate remained unaffected.

Another important issue represents the apparent ineffectiveness of abecarnil in protecting mice against MES-induced convulsions. Such observations suggest that abecarnil acts to increase seizure threshold and has little effect on seizure spread. However, this obvious inference should be interpreted with particular care, because abecarnil has been found to inhibit tonic extension seizures induced by PTZ, which is said to be equivalent to blocking MES-induced seizures (Piredda *et al.*, 1985).

Convulsions triggered by bicuculline were hardly affected by abecarnil, nor was the clonus induced by picrotoxin. In contrast, diazepam blocked efficiently seizures induced by either drug. The explanation of this apparent discrepancy may be based on the fact that running/bouncing clonus with opisthotonus and hindlimb extension induced by bicuculline is of brainstem origin (Browning, 1987) or, alternatively, that abecarnil activates domains of the BZ/GABA receptor complexes in a way which is different from that of diazepam. The latter possibility may be supported by lack of effects of abecarnil upon clonic convulsions in mice subjected to picrotoxin.

Although the hypothesis of forebrain site(s) of anticonvulsant action of abecarnil is both attractive and supported by the *in vivo* data from experimental models of seizures, the proof of this inference using electrophysiological methods is required.

An alternative explanation of apparent potency differences between abecarnil and diazepam in several seizure tests may be the interaction of both compounds at the BZ receptor (Bowling and DeLorenzo, 1982; DeLorenzo, 1986). Diazepam and other BZs interact with both nanomolar and micromolar BZ receptors (Bowling and DeLorenzo, 1982). The binding of BZs to the nanomolar BZ receptor correlates with the inhibition of PTZ seizures (Paul *et al.*, 1979), whereas that to micromolar BZ receptor correlates well with inhibition of MES-induced convulsions (Bowling and DeLorenzo, 1982). It may be that binding capacity of abecarnil to the micromolar BZ receptor is less than that of diazepam, which could also explain some potency differences between both compounds at least in the inhibition of MES-induced seizures.

The partial agonist characteristics of abecarnil may provide a further explanation for potency differences between it and diazepam. Several biochemical and behavioral data are consistent with such a view (Stephens *et al.*, 1990) and the activity of abecarnil in some seizure tests is also consistent with this explanation. Table 6 lists the approximate receptor occupancies of diazepam and abecarnil at doses equivalent to their ED_{50} values in certain of the convulsant tests using mice. From table 6 it is clear that for convulsions induced by picrotoxin, by pilocarpine and by kainic acid, abecarnil requires to occupy more receptors then does diazepam to achieve an equivalent effect. This is in keeping with a partial agonist action of the β-carboline. It is also noticeable that in those tests in which diazepam requires a receptor occupancy greater than about 50% at its ED_{50} value, abecarnil is ineffective, again presumably because even at near 100% receptor occupancy, abecarnil has too low an efficacy to be active in these models. These results are also consistent with a partial agonist action.

Nevertheless, in certain models, *viz.* seizures induced by audiogenic stimulation or by DMCM, abecarnil achieves its effects at receptor occupancies similar to or considerably lower than those required by diazepam. In these models, abecarnil's action cannot be accounted for by a partial agonist hypothesis, and some form of selective action must be postulated, possibly reflecting a heterogeneity of the BZ/GABA receptor complexes (Pritchett *et al.*, 1989).

The important difference between diazepam and abecarnil in their potency against DMCM-induced and audiogenic seizures has been reported previously for other β-carbolines (Jensen *et al.*, 1983; Petersen, 1983), but the explanation of these observations is not clear.

The anticonvulsant potency of abecarnil in the photosensitive baboon *Papio papio* is significantly greater than that of diazepam (minimal dose for full suppression of myoclonus = 0.1 mg/kg). Abecarnil is also more potent than the two β-

TABLE 6

Receptor occupancies (forebrain) at ED_{50} values in several convulsion models in the mouse

ED_{50} values are taken from tables 1 and 4 and receptor occupancies calculated from data presented in Stephens *et al.* (1990). C, clonic; T, tonic; AUR, auricular; COR, corneal. For further explanations *cf.* tables 1 and 4.

	Diazepam		Abecarnil	
	Effective dose	% Receptor occupancy	Effective dose	% Receptor occupancy
	mg/kg		*mg/kg*	
3-MP (T)	0.04	1	0.02	4
3-MP (C)	0.22	7	0.03	6
AS	0.33	10	0.0034	1
DMCM (C)	0.53	15	0.04	7
PIL	0.15	5	0.13	21
PTZ (T)	0.44	13	0.12	19
PTZ (C)	1.15	28	0.43	46
PTX (T)	0.31	9	4.39	90
MES (COR)	6.25	68	14.53	97
MES (AUR)	3.85	56	>100	>99
STR (T)	2.71	47	>100	>99
STR (C)	4.28	59	>100	>99
BIC (T)	3.62	55	>100	>99
BIC (C)	6.72	69	>100	>99
PTX (T)	6.39	68	>100	>99
KA (C)	2.21	42	2.34	82
NMDA (C)	4.36	59	>100	>99
QA (C)	7.82	72	>100	>99

carbolines, ZK 91296 and ZK 93423, previously tested in this model (Meldrum *et al.*, 1983, 1986). No clinical signs of muscle relaxation were detected in baboons after abecarnil administration whereas diazepam induced loss of muscle tone at dosages required to sustain anticonvulsant action for 1 to 2 hr (Meldrum *et al.*, 1975).

Another important finding of this study represents an observation on the potentiation of the anticonvulsant action of abecarnil in the PTZ seizure test in mice (clonic convulsions) by ethosuximide. No such effect was detected after the administration of phenobarbital, diphenylhydantoin or carbamazepine. The anticonvulsant action of diazepam in the same convulsant model was potentiated by diphenylhydantoin, phenobarbital, carbamazepine and ethosuximide, but not by abecarnil. At present, no explanation for the potentiating effect of ethosuximide on the anticonvulsant action of abecarnil in the PTZ seizure test can be offered. The potentiation of the anticonvulsant effect of diazepam by diphenylhydantoin may be due to an increase in the binding capacity of the BZ to respective receptors after a pretreatment with diphenylhydantoin (Gallager *et al.*, 1980). Such an interaction has not been reported for other antiepileptic drugs.

In summary, this report documents a distinctive profile of anticonvulsant action of a metabolically stable β-carboline, abecarnil. Inasmuch as the therapeutic safety of abecarnil measured as the ratio between anticonvulsant and muscle relaxant action is more favorable than that of diazepam, it can be predicted that abecarnil might offer a useful alternative to available antiepileptic treatments.

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