Purification and *N*-terminal partial sequence of anti-epilepsy peptide from venom of the scorpion *Buthus martensii* Karsch

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An anti-epilepsy peptide (AEP) was isolated and purified from venom of the scorpion *Buthus martensii* Karsch. The purification procedure included CM-Sephadex C-50 chromatography, gel filtration on Sephadex G-50 and DEAE-Sephadex A-50 chromatography. Its homogeneity was demonstrated by pH 4.3 polyacrylamide-disc-gel electrophoresis, focusing electrophoresis and SDS/polyacrylamide-disc-gel electrophoresis. The M_r of this peptide, calculated from measurements in SDS/15%-polyacrylamide-disc-gel and SDS/20%-polyacrylamide-disc-gel electrophoresis, is 8300. The isoelectric point is 8.52 by pH 8–9.5-range isoelectric focusing. No haemorrhagic or toxic activities were found. No toxicity was found even after the dose reached 28 mg/kg. The pharmacological tests showed that the AEP had no effect on heart rate, blood pressure or electrocardiogram, but strongly inhibited epilepsy induced by coriaria lactone and cephaloridine. The fluorescence spectrum showed that the peptide has a strong emission peak at 337 nm. Amino acid analysis suggested that the AEP is composed of 66 residues from 18 amino acids and has an M_r of 8290. The sequence of the first 50 *N*-terminal residues is as follows: Asp-Gly-Tyr-Ile-Arg-Gly-Ser-Asp-Asn-Cys-Lys-Val-Ser-Cys-Leu-Leu-Gly-Asn-Glu-Gly-Cys-Asn-Lys-Glu-Cys-Arg-Ala-Tyr-Gly-Ala-Ser-Tyr-Gly-Tyr-Cys-Trp-Thr-Val-Lys-Leu-Ala-Gln-Asp-Cys-Glu-Gly-Leu-Pro-Asp-Thr.

INTRODUCTION

Since Miranda and co-workers isolated and purified neurotoxins from venoms of the scorpions *Buthus occitanus* and *Androctonus australis* (Miranda *et al.*, 1964a) and studied the relationship between the structure and function of the toxins (Miranda *et al.*, 1964b), progress in this field has been rapid. So far, at least 50 kinds of mammalian neurotoxins and ten kinds of insect toxins have been purified from scorpion venoms (Tu, 1977; Rochat *et al.*, 1979; Possani, 1984). The primary and tertiary structures of some toxins as well as the relationship between structure and function have been determined (Fontecilla-Camps *et al.*, 1982; Arseniev *et al.*, 1983; Pashkov *et al.*, 1986). The mammalian neurotoxins are known to inhibit specifically the Na⁺ channel, and they have been used to study that Na⁺ channel (Ovchinnikov & Grishin, 1982). A proteinase inhibitor that specifically inhibits pepsin and kallikrein, and a histamine-releasing factor that is composed of 33 amino acid residues, have been purified (Chhatwal & Habermann, 1982). Separation and partial characterization of smooth-muscle-contraction material in the venom of the scorpion *Heterometrus bengalensis* has also been reported (Kar *et al.*, 1983).

In China, scorpions have been used in treatment of convulsion and epilepsy by Chinese traditional doctors since the Sung Dynasty (A.D. 960–1279) (Zhou, 1984). In the past seven years 15 kinds of mammalian neurotoxins have been purified (Zhou, 1983a; Ji & Xu, 1983; Ji *et al.*, 1984; Zhou & Mao, 1985a; Wang & Lei, 1985; Zhou *et al.*, 1988) from venom of the scorpion *Buthus martensii* Karsch, which is widely distributed in China.

The complete amino acid sequence of one toxin has been determined (Liu *et al.*, 1987). A hyaluronidase has also been purified from the same species (Zhou & Mao, 1985b). The present paper describes the first purification of anti-epilepsy peptide (AEP) from venom of the scorpion *Buthus martensii* Karsch and its subsequent characterization. A comparison of primary structures between AEP and mammalian neurotoxins was also made.

EXPERIMENTAL

Materials

Fresh venom from the scorpion *Buthus martensii* Karsch from China was obtained and freeze-dried as previously described (Zhou, 1983b). CM-Sephadex C-50, Sephadex G-50, Sephadex G-10 and DEAE-Sephadex A-50 were obtained from Pharmacia Biotechnology, Uppsala, Sweden. Ampholines were purchased from LKB Produkter, Stockholm, Sweden. Trypsin, Zn²⁺-free insulin, ribonuclease and cytochrome *c* used as M_r markers in electrophoresis were from Boehringer Mannheim, Mannheim, Germany. Bovine serum albumin was obtained from Sigma Chemical Co., Poole, Dorset, U.K. Acrylamide and *NN'*-methylenebisacrylamide were obtained from Fluka AG, Buchs, Switzerland, and cephaloridine (δ -form) was obtained from Glaxo Laboratories, Greenford, Middx., U.K. Coriaria lactone was purchased from the Pharmaceutical Factory of Sichua Medical College, Chendu, China. The other reagents and chemicals were analytically pure. Trifluoroacetic acid was purchased from Servier

Abbreviation used: AEP, anti-epilepsy peptide.

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Laboratories, Fulmer, France. 4-Dimethylaminoazobenzene 4'-isothiocyanate, phenyl isothiocyanate and Polyamide film were obtained from Pierce and Warriner, Chester, Cheshire, U.K. Dithioerythritol was obtained from Calbiochem-Behring Corp., Watford, Herts., U.K.

Assay of anti-epilepsy activity

This was carried out as described by Chai *et al.* (1983). Thirtytwo 170–300 g albino male rats were divided into four groups. The head of each rat was fastened firmly in a head-holder, after being intravenously anaesthetized with sodium pentobarbital. A long midline incision was made in the scalp, the skin was retracted, and the temporal muscles were detached and reflected, exposing the calvarium to be dried. A large part of each temporal lobe was carefully excised. For electroencephalogram recording, two disposable electrodes were made from 1.5 cm lengths of 0.2 mm-diameter needle. When a normal electroencephalogram appeared, the rats in the control group were intramuscularly administered with coriaria lactone (2.5 mg/kg) or cephaloridine (1000 mg/kg) (Kamei & Tasaka, 1981; Kamei & Sunami, 1983), and for test groups the rats were intravenously administered with AEP (different doses) or diazepam (7.5 mg/kg) or crude venom (0.3 mg/kg) followed by coriaria lactone (2.5 mg/kg) or cephaloridine (1000 mg/kg). The inhibitions in the electroencephalograms and individual behaviour were observed carefully.

Determination of protein

Protein concentration was generally determined by the Folin method (Lowry *et al.*, 1951), with bovine serum albumin as standard.

Purification of AEP from venom of *Buthus martensii* Karsch

All operations were carried out at 4–5 °C. All buffers and gel liquids were degassed before use.

(1) CM-Sephadex C-50 column chromatography. A 2 g portion of *Buthus martensii* venom dissolved in 15 ml of 0.01 M- $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ buffer, pH 6.4, was centrifuged at 18000 *g* for 20 min at 4 °C. The soluble venom was chromatographed on a CM-Sephadex C-50 (115 cm $\times$ 4.5 cm) column equilibrated with the same phosphate buffer. Some 45% of the protein did not bind to the column. The proteins retained were eluted by a step gradient of NaCl in the phosphate buffer, the elution being done with 1500 ml of 0.25 M-NaCl followed by 1400 ml of 0.5 M-NaCl. The flow rate was 36 ml/h, and 9 ml fractions were collected.

(2) Gel filtration on Sephadex G-50. This was carried out on a column (110 cm $\times$ 1.6 cm) of Sephadex G-50 equilibrated with 0.1 M- $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ buffer, pH 8.0. The fractions of AEP obtained from CM-Sephadex were freeze-dried to powder and redissolved in 5 ml of distilled water. Five runs were performed, in each of which a 1.0 ml sample was applied and eluted with the same buffer at a flow rate of 20 ml/h. Active fractions were pooled and dialysed against 4 litres of 0.01 M- $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ buffer, pH 8.0.

(3) DEAE-Sephadex A-50 column chromatography. The dialysed eluate from the Sephadex G-50 column

was applied to a DEAE-Sephadex A-50 column (20 cm $\times$ 1.6 cm) equilibrated with 0.01 M- $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ buffer, pH 8.0. The sample solution (35 ml) was applied at a flow rate of 24 ml/h to the column, which was eluted with a step gradient of NaCl in the phosphate buffer, elution being done with 120 ml of 0.01 M-NaCl and 50 ml of 0.25 M-NaCl followed by 100 ml of 0.5 M-NaCl. The flow rate was 24 ml/h and 6 ml fractions were collected.

Further purification of AEP

(1) Reverse-phase h.p.l.c. on ODS-C₁₈. Freeze-dried AEP, after chromatography on CM-Sephadex C-50, Sephadex G-50 and DEAE-Sephadex A-50, was dissolved in 0.1% (v/v) trifluoroacetic acid to a concentration of 10 mg/ml and purified further by reversed-phase h.p.l.c. on an ODS-C₁₈ column (4 mm $\times$ 200 mm) (LKB Produkter). A 100 μ l sample was applied to the column, which had previously been equilibrated with 30% (v/v) acetonitrile in 0.1% (v/v) trifluoroacetic acid. After washing for 8 min at a flow rate of 0.4 ml/min, elution was with a gradient of 30–80% (v/v) acetonitrile in 0.1% (v/v) trifluoroacetic acid at the same flow rate. Fractions (0.4 ml) were collected, and peptides or proteins were detected at a 280 nm fixed wavelength. Of the five peaks obtained from chromatography, peak 3 possessed the anti-epilepsy activity. Total recovery of protein was 80%.

(2) Size-exclusion h.p.l.c. on TSK G2000 SW. A 25 μ l sample with high anti-epilepsy activity, as shown in Fig. 5, was subjected to size-exclusion chromatography on a TSK G2000 SW column (4 mm $\times$ 300 mm) (LKB Produkter), which had previously been equilibrated with 60% (v/v) acetonitrile in 0.1% (v/v) trifluoroacetic acid. Two protein peaks were obtained, of which peak 1 possessed the anti-epileptic activity. Total recovery of protein was 92%. The preparation was freeze-dried for subsequent study.

Amino acid composition

Amino acid analysis was carried out essentially as described by Liu & Chang (1971). The sample was hydrolysed by 2% (v/v) tryptamine/4 M-toluene-*p*-sulphonic acid at 110 °C for 24 h and then the preparation was analysed by using a 121-MB Beckman amino acid analyser.

Reduction and S-carboxymethylation of AEP

This was performed essentially as previously described by Crestfield *et al.* (1963).

Determination of N-terminal partial sequence of AEP

Reduced and S-carboxymethylated AEP was subjected to Edman automatic sequential degradation (Edman & Begg, 1967). Portions (10 nmol and 40 nmol) of reduced and S-carboxymethylated AEP were dissolved in 0.2 M-acetic acid (30 μ l and 100 μ l respectively) and added to Biobrene films, which were applied to the reaction chamber of a model 470-A protein gas-phase sequencer (Applied Biosystems). The phenylthiocarbamoyl-amino acid produced at each cycle was converted automatically and identified in a model 120-A amino acid phenylthiohydantoin analyser (Applied Biosystems). Each h.p.l.c. chromatogram of amino acid phenylthiohydantoin output by the instrument was analysed according to the

retention times of standard amino acid phenylthiohydantoin derivatives. Forty cycles were performed with the sample of low concentration and 70 cycles with the sample of high concentration.

Polyacrylamide-slab-gel electrophoresis

Polyacrylamide-gel electrophoresis was carried out as described by Davis (1964). Gel electrophoresis in the presence of SDS was performed on Tris/glycine-buffered 15% or 20% (w/v) polyacrylamide slab gels (Anderson *et al.*, 1973). Gels were stained for protein with Coomassie Brilliant Blue G-250 and for glycoprotein with Schiff reagent (Glossmann & Neville, 1971).

Isoelectric focusing

This was performed with the electrofocusing gel-electrophoresis system (Haglund, 1971). The precast gels encompassed the pH ranges 3.5–10 and 8.0–9.5. A mixture of ten standard proteins of pI 3.6–10.2 (FMC Corp., Rockland, ME, U.S.A.) and pure AEP were electrofocused and then stained with Coomassie Brilliant Blue R.

Thermostability

Pure AEP preparations were heated in 0.01 M- $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$ buffer, pH 6.4, for 30 min at temperatures from 40 to 80 °C. Relative activity was determined by using the anti-epilepsy assay.

Spectra

Absorption spectra of AEP were recorded on a Philips PU 8800 spectrometer, and fluorescence spectra of AEP were recorded on a Hitachi 650-60 fluorescent spectrometer.

Measurement of LD_{50} and haemorrhagic activity

The LD_{50} of AEP was measured as previously described by Dixon & Mood (1948). The determination of haemorrhagic activity was carried out as described by Kondo *et al.* (1960).

Effects on heart rate, electrocardiogram, blood pressure and breath rate

Rabbits (1.8–2.8 kg) were intravenously anaesthetized with sodium pentobarbital, and then pure AEP was administered intravenously (0.72–1.42 mg/kg). The blood pressure in the common carotid artery was determined with a pressure sensor, the breath rate was determined with a thermocouple sensor, and the ECG was measured. A multipurpose polygraph (made by Nihon Konden, Japan) was used to record the physiological curves.

RESULTS

Purification of AEP

Of the 18 protein peaks obtained from CM-Sephadex C-50 chromatography, only peaks 1–5 had no toxicity to mice (Fig. 1), which means most of the toxic proteins or peptides present in the crude venom were cationic at pH 6.4 and did bind to the cation-exchanger resin. AEP activity was in peak 4. It was convenient that this procedure removed toxic fractions. In gel filtration on a Sephadex G-50 column, the position of AEP, which was pooled, was in peak 3 (Fig. 2), showing that it was a protein with low M_r . AEP was found in peak 1 from DEAE-Sephadex A-50 (Fig. 3).

The content of AEP in crude venom was low, and only 6.1 mg of pure AEP was obtained from 2000 mg of *B. martensii* venom. The purification of AEP is summarized in Table 1, and the protein recovery was typically 0.2–0.5%, representing an apparent purification of 5–8-fold over the active fraction from CM-Sephadex C-50 chromatography (Table 2). Purified AEP was essentially homogeneous, as judged by pH 8.3 polyacrylamide-slab-gel electrophoresis, isoelectric focusing and SDS/polyacrylamide-gel electrophoresis (Fig. 4). The material represented in the band in SDS/polyacrylamide-gel electrophoresis stained with Coomassie Brilliant Blue C-250 but did not stain with Schiff reagent. From this result it was concluded that AEP is not a glycoprotein.

Fig. 1. Chromatography of crude venom from the scorpion *Buthus martensii* Karsch on CM-Sephadex C-50.

The freeze-dried venom (2000 mg) was applied to a CM-Sephadex C-50 column as described in the Experimental section. -----, NaCl gradient.

Fig. 2. Gel filtration of partially purified AEP on Sephadex G-50

Details are given in the text.

Table 1. Purification of AEP from venom of the scorpion *Buthus martensii*

For full experimental details see the text.

Step	Protein (mg)	Yield of protein (%)
Crude venom	2000	100
CM-Sephadex C-50	65.0	3.25
Sephadex G-50	13.0	0.650
DEAE-Sephadex A-50	6.1	0.305

Purity and N-terminal of AEP

AEP was further purified by reverse-phase h.p.l.c. on an ODS-C₁₈ column with removal of trace contaminants (Fig. 5). In size-exclusion h.p.l.c., two protein peaks were obtained, of which peak 1 had the anti-epilepsy activity with 92% recovery of protein (Fig. 6), and assay of anti-

Fig. 3. Chromatography of partially purified AEP on DEAE-Sephadex A-50

The partially purified AEP from Fig. 2 was applied to a DEAE-Sephadex A-50 column as described in the text. -----, NaCl gradient.

epilepsy activity demonstrated that the preparation possessed 3-fold improvement in activity over the active fraction from DEAE-Sephadex A-50 chromatography.

Physicochemical characterization of AEP

Analysis of amino acid composition of AEP. Analysis of amino acid composition of AEP showed that AEP is composed of 66 residues, being particularly rich in aspartic acid (nine residues) and glycine (nine residues) but containing only one residue of methionine and one of tryptophan, and no phenylalanine or histidine (Table 3). The M_r of AEP, calculated from the amino acid composition, is 8290, which is in agreement with that measured from electrophoresis in the presence of SDS in 15% and 20% (w/v) polyacrylamide slab gels.

Reduction and S-carboxymethylation of AEP. Purified AEP was reduced by dithioerythritol in 6 M-guanidinium chloride and was treated with iodoacetic acid to produce S-carboxymethylated AEP, which was desalted by molecular-sieve h.p.l.c. on a TSK G2000SW column.

Table 2. Biological assay of AEP activity during purification

When a normal electroencephalogram appeared, the rats in the control group were intramuscularly administered with 0.01 M-Na₂HPO₄/NaH₂PO₄ buffer, pH 6.4, followed by coriaria lactone (2.5 mg/kg), and for test groups the rats were intravenously administered with different eluents containing AEP followed by coriaria lactone (2.5 mg/kg). For full experimental details see the text.

Step	AEP dosage (mg/kg)	Mean time to seizure (min)	Severity of epilepsy			Death rate (%)	Mean time to recovery (min)
			Minor	Mild	Severe		
CM-Sephadex C-50	1.46	16 ($P < 0.01$)		80	20	0	70 ($P < 0.01$)
Sephadex G-50	0.763	16 ($P < 0.02$)	10	90		0	66 ($P < 0.1$)
DEAE-Sephadex A-50	0.284	18 ($P < 0.01$)	19	81		0	64 ($P < 0.01$)
Control		5		20	80	100*	

* Rats died 86 min after coriaria lactone administration.

Fig. 4. SDS/polyacrylamide-slab-gel electrophoresis of purified AEP

Tracks 1 and 2, purified AEP after chromatography on DEAE-Sephadex A-50; track 3, crude venom.

N-Terminal partial sequence of AEP. By using gas-phase automatic degradation with a 470-A sequencer and 120-A amino acid phenylthiohydantoin analyser (Applied Biosystems), the sequence of the first 50 *N*-terminal residues was determined as shown in Fig. 7. The same first 30 *N*-terminal residues were also identified from the sequence analysis of the smaller quantity of AEP.

M_r and pI of AEP. The M_r of AEP was 8300 ($\pm 10\%$) as determined by electrophoresis in the presence of SDS in 15% and 20% (w/v) polyacrylamide slab gels, with trypsin, ribonuclease, cytochrome *c* and insulin as standards. The M_r of AEP calculated from the amino acid composition is 8290. The isoelectric points of AEP from measurements in isoelectric focusing in ranges of pH 3.5–10 and pH 8.0–9.5 are 8.66 and 8.52 respectively. These values are consistent with the behaviour of AEP on ion-exchange resins.

Assessment of AEP thermostability. The AEP was completely stable up to 80 °C. The assay of anti-epilepsy activity indicated that heat-treated AEP had an activity almost equal to that of untreated AEP.

Spectrum of AEP. The absorption spectrum of AEP exhibited an absorption peak at 279 nm. The fluorescence spectrum exhibited an emission peak at 337 nm and an excitation peak at 280 nm.

Pharmacological characterization of AEP

Anti-epilepsy activity. The animal model of epilepsy induced by coriaria lactone or cephaloridine is a new

Fig. 5. Reverse-phase h.p.l.c. of purified AEP on an ODS-C₁₈ column

Details are given in the text. ----, Acetonitrile gradient in 0.1% trifluoroacetic acid.

Fig. 6. Size-exclusion h.p.l.c. of purified AEP on a TSK G2000 SW column

The AEP from peak III of Fig. 5 was applied to a TSK G2000 SW column as described in the Experimental section.

Table 3. Amino acid composition of AEP

The results are expressed as the number of residues per molecule of peptide, without correction for destruction or incomplete hydrolysis. Values less than 4.5 nmol are judged insignificant. Cysteine was determined as the *S*-carboxymethyl derivative. For full experimental details see the text.

Amino acid	Amino acid composition	
	(nmol)	(residues/molecule)
Asx	82.4	9
Thr	16.4	2
Ser	43.1	5
Glx	46.4	5
Tyr	35.9	4
Gly	82.8	9
Ala	35.1	4
CyS	33.0	4
Val	36.0	4
Met	9.8	1
Ile	18.4	2
Leu	43.6	5
Pro	8.7	1
Phe	3.2	0
Lys	45.6	5
His	2.5	0
Trp	10.7	1
Arg	25.2	3
Total		66
M_r		8290*

* Even though the calculated value for half-cystine is 4, not 6, 6 cysteine residues were found in sequencing; however, since Cys is difficult to determine in amino acid analysis, it is not unexpected that analysis shows a lower value. So the M_r of AEP is calculated in accordance with 6 half-cystine residues.

Asp-Gly-Tyr-Ile-Arg-Gly-Ser-Asp-Asn-Cys-Lys-Val-Ser-
 Cys-Leu-Leu-Gly-Asn-Glu-Gly-Tyr-Cys-Asn-Lys-Glu-Cys-Arg-
 Ala-Tyr-Gly-Ala-Ser-Tyr-Gly-Tyr-Cys-Trp-Thr-Val-Lys-
 Leu-Ala-Gln-Asp-Cys-Glu-Gly-Leu-Pro-Asp-Thr

Fig. 7. N-Terminal partial sequence of AEP

The initial sequencing yield for the peptide was 94%, and the repetitive yield was 96%. For full experimental details see the text.

method and easy to control. At an adequate dose, epilepsy can be induced in half an hour. But there are no strict one-to-one correlations regarding the electroencephalogram and behaviour, only relative degrees of correlation. We had to choose mean time to seizure, severity of epilepsy, death rate and mean time to recovery as end points. The tests showed that AEP has remarkable effects on inhibition of epilepsy and muscle tic, at least 26 times that of diazepam ($P < 0.01$) (Table 4). As purification of AEP proceeded, its relative activity rose rapidly (Table 2).

Toxicity and haemorrhagic activity. The determination of toxicity and haemorrhagic activity of AEP showed

Table 4. Effects of control, diazepam, scorpion venom and AEP against epilepsy induced by coriaria lactone and cephaloridine

For full experimental details see the text.

Group	Dosage		Mean time to seizure		Severity of epilepsy			Death rate (%)	Mean time to recovery	
	(mg/kg)	(μ mol/kg)	(min)	(% increase)	Minor	Mild	Severe		(min)	(% decrease)
Coriaria lactone-induced epilepsy										
Control			6			20	80	100†		
Diazepam	7.5	2.6	15	150 ($P < 0.05$)		100		0	61	21.0 ($P < 0.01$)
Scorpion venom	0.3	—	35	480 ($P < 0.02$)	20	80		25	55	34.5 ($P < 0.02$)
AEP	0.284*	0.034	19	217 ($P < 0.1$)	20	80		0	65	23.6 ($P < 0.02$)
Cephaloridine-induced epilepsy										
Control			10			100		0	65	
Diazepam	7.5	2.6	25	150 ($P < 0.02$)	60	40		0	47	27.7 ($P < 0.1$)
Scorpion venom	0.3	—	18	80 ($P < 0.2$)	40	60		0	58	10.7 ($P > 0.2$)
AEP	0.284*	0.034	15	50 ($P > 0.2$)	40	60		0	41	36.9 ($P < 0.05$)

* AEP dosage means total protein administered.

† Rats died 84 min after coriaria lactone administration.

that toxicity was not found even after the dose to mice reached 28 mg/kg body wt.

General pharmacological effects on experimental animals. The tests indicated that when animals were intravenously injected with AEP (0.72–1.42 mg/kg) the heart rate of the rabbit was decreased by 7% ($P < 0.01$) of the original rate, arrhythmia and change of breath rate were not found, and blood pressure was only raised a little; when the animals were ventricularly injected, the heart rate and blood pressure were decreased by 8.8% ($P < 0.05$) and 9.4% ($P < 0.01$) respectively, and there was no effect on breath rate and rhythm.

DISCUSSION

Work on peptides or proteins with anti-epilepsy activity is rare to date. We are the first to report on the

AEP from venom of the Chinese scorpion *Buthus martensii* Karsch.

The experimental model of epilepsy that we chose is a new one. It responds sensitively to drugs and is very convenient to control, so that we obtained reliable results during the assay of AEP. When diazepam was used for comparison, the action of AEP against epilepsy was very strong, i.e. 76 times as strong as diazepam on a molar basis, or 26 times on a weight basis.

Generally speaking, some nerve diseases such as epilepsy and convulsions have definite foci in the brain, and the epilepsy models induced would also be accompanied by some cortical lesions in the brain. If the drug acts against epilepsy, it must have the capability to pass through the blood–brain barrier. In blood, hydrophobic molecules such as O_2 , CO_2 and ethanol can pass through it, but hydrophilic or polar molecules, such as penicillin, cannot do so. The pharmacological tests

AaH I	Lys-Arg-Asp-Gly-Tyr-Ile-Val-Tyr-Pro-Asn-Asn-Cys-Val-Tyr-His-Cys-Val-Pro-Pro-Cys-Asp-Gly-Leu-Cys-	
AaH I'	Lys-Arg-Asp-Gly-Tyr-Ile-Val-Tyr-Pro-Asn-Asn-Cys-Val-Tyr-His-Cys-Ile-Pro-Pro-Cys-Asp-Gly-Leu-Cys-	
AaH II	Val-Lys-Asp-Gly-Tyr-Ile-Val-Asp-Asp-Val-Asn-Cys-Thr-Tyr-Phe-Cys-Gly-Arg-Asn-Ala-Tyr-Cys-Asn-Glu-	
AaH III	Val-Lys-Asp-Gly-Tyr-Ile-Val-Asn-Ser-Lys-Asn-Cys-Val-Tyr-His-Cys-Val-Phe-Phe-Cys-Asp-Gly-Leu-Cys-	
AEP	Asp-Gly-Tyr-Ile-Arg-Gly-Ser-Asp-Asn-Cys-Lys-Val-Ser-Cys-Leu-Leu-Gly-Asn-Glu-Gly-Cys-Asn-	
BmK III	Val-Arg-Asp-Ala-Tyr-Ile-Ala-Lys-Pro-Glu-Asn-Cys-Val-Tyr-Glu-Cys-Ala-Thr-Asn-Glu-Tyr-Cys-Asn-Lys-	
Be M10	Val-Arg-Asp-Ala-Tyr-Ile-Ala-Asp-Asp-Lys-Asp-Cys-Ala-Tyr-Phe-Cys-Gly-Arg-Asn-Ala-Tyr-Cys-Asp-Glu-	
Bot III	Val-Lys-Asp-Gly-Tyr-Ile-Val-Asp-Asp-Arg-Asn-Cys-Thr-Tyr-Phe-Cys-Gly-Arg-Asn-Ala-Tyr-Cys-Asn-Glu-	
AaH I	Lys-Lys-Asn-Gly-Gly-Ser-Ser-Gly-Ser-Ser-Cys-Phe-Leu-Val-Pro-Ser-Gly-Leu-Ala-Cys-Trp-Cys-Lys-Asp-	
AaH I'	Lys-Lys-Asn-Gly-Gly-Ser-Ser-Gly-Ser-Ser-Cys-Phe-Leu-Val-Pro-Ser-Gly-Leu-Ala-Cys-Trp-Cys-Lys-Asp-	
AaH II	Glu-Cys-Tyr-Lys-Leu-Lys-Gly-Glu-Ser-Gly-Tyr-Cys-Gln-Trp-Ala-Ser-Pro-Tyr-Gly-Asn-Ala-Cys-Tyr-Cys-	
AaH III	Lys-Lys-Asn-Gly-Ala-Lys-Ser-Gly-Ser-Cys-Gly-Phe-Lys-Ile-Pro-Ser-Gly-Leu-Ala-Cys-Trp-Cys-Val-Ala-	
AEP	Lys-Glu-Cys-Arg-Ala-Tyr-Gly-Ala-Ser-Tyr-Gly-Tyr-Cys-Trp-Thr-Val-Lys-Leu-Ala-Gln-Asp-Cys-Glu-Gly-	
BmK III	Leu-Cys-Thr-Asp-Asn-Gly-Ala-Glu-Ser-Gly-Tyr-Cys-Gln-Trp-Val-Gly-Arg-Tyr-Gly-Asn-Ala-Cys-Xaa-Cys-	
Be M10	Glu-Cys-Lys-Lys-Gly-Ala-Glu-Ser-Gly-Lys-Cys-Trp-Tyr-Ala-Gly-Gln-Tyr-Gly-Asn-Ala-Cys-Trp-Cys-Tyr-	
Bot III	Glu-Cys-Thr-Lys-Leu-Lys-Gly-Glu-Ser-Gly-Tyr-Cys-Gln-Trp-Ala-Ser-Pro-Tyr-Gly-Asn-Ala-Cys-Tyr-Cys-	
AaH I	Leu-Pro-Asp-Asn-Val-Phe-Ile-Arg-Asp-Thr-Ser-Arg-Lys-Cys-Thr	Rochat <i>et al.</i> (1970)
AaH I'	Leu-Pro-Asp-Asn-Val-Phe-Ile-Arg-Asp-Thr-Ser-Arg-Lys-Cys-Thr	Rochat <i>et al.</i> (1979)
AaH II	Tyr-Lys-Leu-Pro-Asp-His-Val-Arg-Thr-Lys-Gly-Phe-Gly-Arg-Cys-His	Rochat <i>et al.</i> (1972)
AaH III	Leu-Pro-Asp-Asn-Val-Pro-Ile-Lys-Asp-Pro-Ser-Tyr-Lys-Cys-His-Ser	Kopeyan <i>et al.</i> (1979)
AEP	Leu-Pro-Asp-Thr---	
BmK III	Ile-Lys-Leu-Pro-Asp-Arg-Val-Pro-Ile-Arg-Val-Trp-Gly-Lys-Cys-His-Gly	Liu & Lei (1987)
Be M10	Lys-Leu-Pro-Asp-Trp-Val-Pro-Ile-Lys-Gln-Lys-Val-Ser-Gly-Lys-Cys-His	Grishin <i>et al.</i> (1981)
Bot III	Tyr-Lys-Val-Pro-Asp-His-Val-Arg-Thr-Lys-Gly-Pro-Gly-Arg-Cys-Asn	Vargas <i>et al.</i> (1982)

Fig. 8. Comparison of N-terminal sequences of AEP and scorpion toxins

For experimental details see the text. Abbreviations according to the scientific names of the scorpions were used: AaH, *A. australis* Hector toxin; Be, *Buthus eupeus* toxin; BmK, *B. martensii* Karsch toxin; Bot, *B. occitanus tunetanus* toxin.

Table 5. Comparison between AEP and some toxins from the scorpion *Buthus martensii*

For details see the text.

	M_r	pI		M_r	pI
AEP	8300	8.52	Toxin I†	7567	—
Toxin I*	8980	7.58	Toxin 2†	7181	—
Toxin II*	8660	7.90	Toxin I§	6450	8.7
Toxin III†	8750	8.20	Toxin II§	5950	9.1
			Toxin III§	8930	9.1

* From Zhou (1983).
† From Zhou & Mao (1985a)
‡ From Ji *et al.* (1983).
§ From Wang & Lei (1985).

demonstrated that AEP strongly inhibited normal and epilepsy electroencephalograms by either intravenous or ventricular injection, which means that the AEP must easily penetrate the barrier to act on the brain.

The u.v.-absorption spectrum in our experiment indicated that the AEP has a strong absorption peak at 279 nm, and the fluorescence emission spectrum also indicated that the AEP has an excitation peak at 280 nm and a strong emission peak at 337 nm. From these results it was certain that the AEP contains at least one tryptophan residue, which fits the results of amino acid analysis.

Even though some properties of AEP are similar to those of scorpion toxins, such as M_r , pI and thermostability (Table 5), there is still a big difference between them. A toxic effect of AEP was not found in mice even after the dose reached 28 mg/kg, and furthermore there are hardly any effects on heart rate, electroencephalogram and breath rate, and only a little effect on blood pressure. Most scorpion toxins have LD₅₀ values in the range 0.5–1.5 mg/kg, and tachycardia, arrhythmia and blood pressure increase markedly (Possani, 1984). Evidently, this remarkable difference in functions depends on different structures, which should be evident in the amino acid sequences of AEP and scorpion toxins. It is a basal characteristic of the toxins that there are eight cysteine residues and no methionine (Possani, 1984). In AEP there are six cysteine residues and one methionine residue. However, some conserved fragments, such as Asp-Gly-Tyr-Ile, Asn-Cys and Leu-Pro-Asp, do exist in the sequences of AEP and scorpion mammalian toxins (Fig. 8), suggesting a certain homology between AEP and scorpion toxins. Three of the six cysteine residues of AEP are in the same positions as those of scorpion toxins. However, there are major differences between AEP and the toxins in the *N*-terminal partial sequence. First of all, the AEP molecule lacks two residues before the conservative sequence Asp-Gly-Tyr-Ile. Secondly, the positions of the other three of the six cysteine residues in the AEP sequence are different from those in toxins, which gives rise to structural diversity. Especially, the AEP molecule lacks a fragment of Cys-Trp/Tyr-Cys, which exists in all the toxins.

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