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A large number of novel Ergtoxin-like genes and ERG K$^+$-channels blocking peptides from scorpions of the genus *Centruroides*

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Abstract **Twenty-three novel sequences similar to Ergtoxin (ErgTx) were obtained by direct sequencing of peptides or deduced from gene cloned using cDNAs of venomous glands of *Centruroides* (*C.*) *elegans*, *C. exilicauda*, *C. gracilis*, *C. limpidus limpidus*, *C. noxius* and *C. sculpturatus*. These peptides have from 42 to 47 amino acid residues cross-linked by four disulfide bridges. They share sequence similarities (60–98% compared with ErgTx1) and were shown to block ERG K$^+$-channels of F-11 clone (N18TG-2×rat DRG) cultured cells. An unrooted phylogenetic tree analysis of these peptides showed that they conform at least five different subfamilies, of which three are novel subfamilies.**

Key words: *Centruroides*; ERG-channel; Ergtoxin; K$^+$-channel; Nomenclature; Phylogeny; Scorpion toxin

1. Introduction

The number of novel scorpion toxins specific for K$^+$-channels described has increased significantly in recent years, adding new subfamilies to the original classification proposed by a panel of scientists working in the field [1]. In the original paper by Tytgat and co-workers [1], 49 different peptides were analyzed and three main family names were proposed for their classification: the α-KTx subfamilies 1 to 12, now expanded up to subfamily 17 (see [2]), the β-KTx subfamily from the work done with tityustoxin Kβ (synonymous to the TsTx-Kβ) [3], now expanded with two additional peptides BmTxKβ* and BmTxKβ 2* [2], and the γ-KTx, based on a new type of scorpion toxin, whose first example was Ergtoxin (abbreviated ErgTx) isolated from *Centruroides (C.) noxius*. This is a 42 amino acid residue peptide containing four disulfide bridges [4], shown to affect specifically K$^+$-channels of the *ether-a-go-go*-related family of genes (*eag*) [5]. The ErgTx and ErgTx-like peptides described thus far are represented by four different peptides: (i) the original ErgTx [4,5]; (ii) ErgTx2, a 43 amino acid long peptide, also from the venom of the Mexican scorpion *C. noxius* shown to affect a long-lasting *eag*-related gene-type K$^+$-current in MMQ lactotrops [6], (iii) BeKm-1, a 36 amino acid long peptide with only three disulfide bridges, isolated from the scorpion *Buthus eupeus*, capable of blocking ERG-channels [7], and finally (iv) CsE-Kerg1, a 43 amino acid residue long peptide containing 4 disulfide bridges, isolated from the scorpion *C. sculpturatus* Ewing and shown to affect ERG-currents of NG108-15 cells [8]. Thus, different members of the ERG-type K$^+$-channel family seem to be recognized by several different peptides isolated from different species of scorpions. The *eag* superfamily of genes encoding potassium channels encodes proteins with six transmembrane domains, having a conserved P region that conforms the ion pore when clustered in the tetrameric arrangement of the channel protein [9]. Mutations in the human-ERG-channel (HERG) give rise to inherited, type 2 long-QT syndrome [10], caused by a loss of the cardiac repolarizing current I_{Kr} [11,12]. Currents arising from expression of HERG or ERG, as the homologs in other species are known, also play a roll in spike-frequency adaptation in neurons [13] and in human pancreatic β-cells [14]. The HERG-channels were earlier known to be the target of a group of potent drugs, including antiarrhythmics, antihistaminics and antibiotics [15,16], that block K$^+$-currents, causing acquired long-QT syndrome as a side effect. The discovery of naturally occurring substances that can specifically and reversibly block or recognize these types of channels are fundamental for the search and development of new putative drugs to treat some of the diseases and malfunctions associated with HERG-channels. ErgTx was used successfully to examine the binding site on the HERG-channels [17] and to verify by site-directed mutagenesis of HERG genes the involvement of critical residues at the vestibule of the channel, where ErgTx is supposed to bind [18].

In this communication we report 23 novel primary structures directly determined and/or deduced from cDNA cloned genes of scorpions of the genus *Centruroides*. The presence of the corresponding peptides in the venom of the six species of scorpions studied was demonstrated by electrophysiological recordings, using the mouse neuroblastoma cell line F-11 (N18TG-2×DRG cells). They all contain *bona fide* blockers of the ERG-type K$^+$-currents, supporting the assumption of their specificity towards this type of ion-channels. The sequences obtained allowed the construction of a phylogenetic tree, where these peptides were grouped in at least five distinct subfamilies. Except for the two already known [19], all the other subfamilies constitute original data of this communica-

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tion, and were listed as three novel subfamilies of the family-type named γ-KTx.

2. Materials and methods

2.1. Toxin purification and primary structure determination

ErgTx1 and ErgTx2 from *C. noxius* venom were purified by chromatographic procedures as earlier described [4,5]. The primary structure of ErgTx2 from *C. noxius* and ErgTx1 and ErgTx3 from *C.l. limpidus* are shown for the first time in this communication and were obtained by direct Edman degradation and mass spectrometry analysis, as will be discussed in details elsewhere (forthcoming). Since they are all homologous peptides from different species of scorpions, and in order to differentiate the nomenclature, we will precede the toxin name with two or three letters derived from the abbreviation of the genus, species and sub-species, depending on the scorpion. Thus ErgTx1 of *C. noxius* is called CnErgTx1, whereas that of *C.l. limpidus* is CllErgTx1. All these trivial names are now defined in a systematic manner (numbering system), as will be presented and discussed below. The amino acid sequence of BeKm-1 and CsEKerg1 were obtained from [7] and [8], respectively. The remaining sequences were determined from gene cloning as described in the next section.

2.2. Gene cloning

RNA extraction from the venomous glands of six species of scorpion of the genus *Centruroides*, the object of this communication, was performed by the method of Chirgwin et al. [20], using three to five individuals of each species. Total RNA (approx. 500 ng) was used for cDNA synthesis by means of a poliT22NN oligonucleotide, that is, a 22mer of Ts, followed at the end by two degenerate nucleotides, as earlier described by our group on the cloning of genes of the scorpion *C. sculpturatus* [21]. The oligonucleotides used for PCR amplification were GATAGAGATAGCTGTGTTGATAAATCA and the poliT22NN mentioned above. The procedure utilized thereafter is the same as earlier described by Corona et al. [21]. Plasmid DNA was sequenced from both strands, using fluorescent nucleotides in an automatic Perkin Elmer Applied Biosystems apparatus (Foster City, CA, USA) as described by the manufacturer. All the nucleotide sequences are deposited at the GenBank, accession numbers AY159334–AY159355. For the 5′-RACE of the gene encoding for ErgTx1, the FirstChoice™ RLM-race kit of Ambion (Austin, USA) was used. The complete nucleotide sequence was obtained using the oligonucleotides 5′-TTGAAGAGACAATTTAAGACG-3′ and 5′-TTTGAAATTTC-CGGAAATT-3′.

2.3. Electrophysiological recordings

2.3.1. Cell culture. Cells of the F-11 clone (mouse neuroblastoma N18TG-2×rat DRG) [22] were routinely cultured in Dulbecco's modified Eagle's medium (DMEM) containing 4.5 g/l of glucose and 10% fetal calf serum (FCS). The cells were incubated at 37°C in a humidified atmosphere with 5% CO_2.

2.3.2. Solutions. The standard extracellular solution contained (in mM): NaCl 130, KCl 5, $CaCl_2$ 2, $MgCl_2$ 2, HEPES–NaOH buffer 10, D-glucose 5, pH 7.40. In the high K^+ external solution ($[K^+]_o = 40$ mM), NaCl was replaced by an equimolar amount of KCl. The standard pipet solution at $[Ca^{2+}]_i = 10^{-7}$ M (pCa 7) contained (in mM): K^+-Aspartate 130, NaCl 10, $MgCl_2$ 2, $CaCl_2$ 1.3, EGTA–KOH 10, HEPES–KOH buffer 10, ATP (Mg^{2+} salt) 1, pH 7.30. The pipet solution contained (in mM) K^+-aspartate 110, KCl 23, $CaCl_2$ 0.4 (pCa 7), $MgCl_2$ 3, HEPES–KOH buffer 5, GTP (Na^+ salt) 0.4, ATP (Na^+ salt) 5, creatine phosphate 5, pH 7.3. Venom was added to the extracellular solutions from a stock solution in distilled water.

2.3.3. Patch-clamp recordings. The currents were recorded at room temperature as previously described [6]. Pipet resistance (1–2 MΩ), cell capacitance and series resistance errors were carefully compensated (85–95%) before each voltage-clamp protocol run. The extracellular solutions were delivered through a nine-hole (0.6 mm) remote-controlled linear positioner placed near the cell under study, which has an average response time of 5–8 s. Currents were recorded by means of an MC700A patch-clamp amplifier (Axon Instruments, Foster City, CA, USA). Protocol pulses were delivered every 12 s and holding potential was set at −60 mV. During data acquisition and analysis, pClamp 8.2 (Axon Instruments) and Origin 4.1 (Microcal, USA) software were routinely used.

2.4. Sequence analysis and construction of a phylogenic tree

Multiple alignment of the amino acid sequences was performed by means of the program CLUSTALX [23]. Based on this initial alignment, a re-sample of 1000 bootstrapped data set was processed, using the SEQBOOT program of J. Felsenstein's PHYLIP phylogeny inference package program [24]. Genetic distances of these alignments were calculated using the Dayhoff PAM matrix with the PROTDIST program [24]. Subsequently, the trees were constructed by successive clustering of lineages using the neighbor-joining algorithm [25] as implemented in the NEIGHBOR program [24]. The strict consensus tree was obtained using the CONSENSUS program [24] and the unrooted tree diagram was generated with the DRAWTREE program [24]. Finally, the artwork was done using the drawing program MacDraw Pro.

3. Results and discussion

3.1. New subfamilies of the γ-KTx scorpion toxins

The full primary structures of three out of four scorpion toxin peptides that modify the function of HERG, ERG or ERG-like K^+-channels are known, as mentioned in the introductory section [5–8]. Since these are peptides with a significantly different structure and specificity than those of the α-KTx toxins, it was suggested they be given a new family name (γ-KTx) [1]. The earlier reported Ergtoxin [5], an isoform containing three disulfide bridges, was later corrected to the actual sequence [4]. Indeed, a very similar sequence with four disulfides, was finally named Ergtoxin-1 (now abbreviated here as CnErgTx1). At almost the same time that CnErgTx1 was reported, another ERG K^+-channel toxin (BeKm-1) was isolated [7], whose primary structure is quite different. More recently, another peptide Ergtoxin-2 (CnErgTx2), for which only the three most N-terminal-situated residues were reported, was shown to have a different specificity as that of CnErgTx1 [6]. On these grounds, three subfamilies were already surmised to exist: γ-KTx 1.1, represented by CnErgTx1; γ-KTx 2.1, represented by BeKm-1 and possibly γ-KTx 3.1, represented by CnErgTx2. Toxin CsEKerg1 [8] was not known at that time.

The present discovery of a large number of such peptides allowed the arrangement of all the information available in a manner similar to that proposed earlier for the α-KTx family [1]. Here we have organized the 26 known sequences in five subfamilies as shown in Fig. 1. The criteria used for this proposal is based on similar arguments already presented in [1]: size of peptides, cysteine positions, disulfide bridges, their chronological appearance, toxicity to animals and ion-channel specificity. At this stage, due to the limited information available concerning toxicity and ion-channel specificity of the newly described sequences, we decided to analyze the primary structure using programs available for the construction of phylogenetic trees, which will be discussed in the next section.

Fig. 1 shows the amino acid sequence of the 25 ErgTx-like peptides isolated from scorpions of the genus *Centruroides* and one from the genus *Buthus*. For the trivial subfamily names we have used the abbreviations of genus, species and sub-species (as mentioned in Section 2) before the abbreviation ErgTx, meaning toxins that recognize ERG-like K^+-channels. For the case of the *Buthus* species and the toxin purified from *C. sculpturatus*, we have respected the original names proposed by the authors [7,8], respectively. The percentage identity was calculated by pairwise comparison with CnErgTx1, in the first column labeled identity in Fig. 1, and

Subfamily		Sequence	γ-KTx	Identity(%)		Ref.
		1 10 20 30 40				
1	CnErg1	DRDSCVDKSRCAKYGYYQECQDCCKNAGHNGGTCMFFKCKCA	1.1	100	98	[5]
	CeErg1	DRDSCVDKSRCAKYGYYQECTDCCKKYGHNGGTCMFFKCKCA	1.2	93	95	This work
	CgErg1	DRDSCVDKSRCAKYGHYQECTDCCKKYGHNGGTCMFFKCKCA	1.3	90	93	This work
	CsErg1	DRDSCVDKSRCAKYGYYQECQDCCKKAGHNGGTCMFFKCKCA	1.4	98	100	This work
	CllErg1	DRDSCVDKSRCSKYGYYQECQDCCKKAGHNGGTCMFFKCKCA	1.5	95	98	This work
	CexErg1	DRDSCVDKSRCAKYGYYQECQDCCKKAGHSGGTCMFFKCKCA	1.6	95	98	This work
	Consensus	DRDSCVDKSRCAKYGYYQECQDCCKKAGHNGGTCMFFKCKCA				
2	BeKm-1	-RPTDI---KCSES--Y-QCFPVCKSRFGKTNGRCVNGFCDCF	2.1	11		[7]
3	CnErg2	GRDSCVNKSRCAKYGYYSQCEVCCKKAGHKGGTCDFFKCKCKV	3.1	74	93	This work
	CeErg2	DRDSCVDKSRCAKYGYYQQCEICCKKAGHRGGTCEFFKCKCKV	3.2	81	98	This work
	CsErg2	DRDSCVDKSRCAKYGYYGQCEVCCKKAGHRGGTCDFFKCKCKV	3.3	79	100	This work
	CgErg2	DRDSCVDKSRCQKYGNYAQCTACCKKAGHNKGTCDFFKCKCT-	3.4	76	81	This work
	Consensus	DRDSCVDKSRCAKYGYY.QCEVCCKKAGHRGGTCDFFKCKCKV				
4	CllErg2	DRDSCVDKSKCSKYGYYGQCDECCKKAGDRAGNCVYFKCKCNP	4.1	65	93	This work
	CnErg5	DRDSCVDKSKCGKYGYYQECQDCCKNAGHNGGTCVYYKCKCNP	4.2	84	81	This work
	CexErg2	DRDSCVDKSKCGKYGYYGQCDECCKKAGDRAGICEYYKCKCNP	4.3	63	95	This work
	CexErg3	DRDSCVDKSKCAKYGYYYQCDECCKKAGDRAGTCEYFKCKCNP	4.4	70	91	This work
	CexErg4	DRDSCVDKSQCAKYGYYYQCDECCKKAGDRAGTCEYFKCKCNP	4.5	70	88	This work
	CllErg3	DRDSCVDKSKCSKYGYYGQCDKCCKKAGDRAGNCVYFKCKCNQ	4.6	65	88	This work
	CllErg4	DRDSCVDKSKCAKYGYYGQCDECCKKAGDRAGNCVYLKCKCNQ	4.7	65	91	This work
	CeErg3	DRDSCVDKSKCGKYGYYHQCDECCKKAGDRAGNCVYYKCKCNP	4.8	63	95	This work
	CsErg3	DRDSCVDKSRCGKYGYYGQCDDCCKKAGDRAGTCVYYKCKCNP	4.9	70	98	This work
	CsErg4	DRDSCVDKSRCGKYGYYGQCDECCKKAGDRAGTCVYYKCKCNP	4.10	67	98	This work
	CnErg4	DRDSCVDKSQCGKYGYYGQCDECCKKAGERVGTCVYYKCKCNP	4.11	65	93	This work
	CsEKerg1	ERDSCVEKSKCGKYGYYGQCDECCKKAGDRAGTCVYYKCKCNP	4.12	60	95	[8]
	CnErg3	DRDSCVDKSKCGKYGYYGQCDECCKKAGDRAGTCVYYKCKCNP	4.13	65	100	This work
	Consensus	DRDSCVDKSKCGKYGYYGQCDECCKKAGDRAGTCVYYKCKCNP				
5	CsErg5	DRDSCVDKSRCAKYGYYGQCEVCCKKAGHNGGTCMFFKCMCVNSKMN	5.1	74		This work
	CgErg3	DRDSCVDKSRCQKYGPYGQCTDCCKKAGHTGGTCIYFKCKCGAESGR	5.2	68		This work

Fig. 1. The subfamily members of the γ-KTx scorpion toxins. This figure shows the five subfamilies of peptides proposed in this work (numbers in first column), followed by the trivial names (abbreviated from the genus and species of scorpions). The third column gives the full amino acid sequences, either directly determined or deduced from cDNAs. The fourth column gives the systematic name (γ-KTx) and numbers proposed for the various toxins. The fifth column gives the identity score based on pairwise comparison against CnErg1 (γ-Tx 1.1) and against the consensus sequence for each subfamily (second set of percentages). The last column provides the references. The amino acid sequences of CnErg1 (γ-KTx 1.1) [5], CllErg1 (γ-KTx 1.5) and CllErg2 (γ-KTx 4.1) were all obtained by direct peptide sequence and also confirmed from the deduced nucleotide sequence obtained from cDNA cloning. The amino acid sequence of toxin CnErg2 (γ-KTx 3.1) was obtained only from direct peptide sequencing. All the others were deduced from the nucleotide sequences of cDNA clones, except for peptides BeKm-1 (γ-KTx 2.1) and CsEKerg1 (γ-KTx 4.12) obtained from the literature [7,8], respectively. The cysteine residues are highlighted and variable amino acids within each subfamily are in bold.

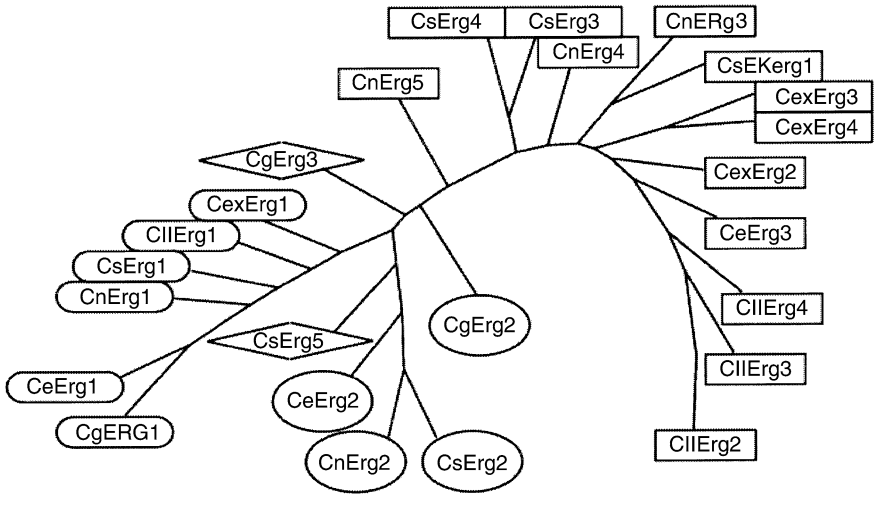

Fig. 2. Unrooted phylogenetic tree for the γ-KTx peptides of *Centruroides* scorpions. An unrooted phylogenetic tree was constructed for the 25 peptides belonging to scorpions of the genus *Centruroides* as described in Section 2. Three separated branches are clearly shown, corresponding to subfamilies 1 (flattened circles), 3 (elliptic circles) and 4 (rectangles). Subfamily 2 was not used for the phylogenetic studies due to its very different similarity (only 11% identical to CnErg1, γ-KTx 1.1). Subfamily 5 (rhombus) is represented by two sequences placed in short distance of each other, one shown in the upper part and the other in the lower part of the left segment of the tree. Instead of the systematic names, we used the trivial names of Fig. 1, since they give a better indication of the scorpions from which the peptides come from.

compared pairwise with the sequence most closely related to the consensus sequence, shown in the second column labeled identity, for each of the subfamilies. Exceptions to these calculations are BeKm-1 and the long-chain peptides, because these subfamilies are formed by only one and two sequences (subfamily 5), respectively. In total, five subfamilies are proposed. The first member of the first four subfamilies is occupied by a peptide, whose sequence was the first to be discovered and for which the sequence was directly determined. Subfamily 1 has six members, all containing 42 amino acids and identical segments of the N-terminal (11 first amino acids) and 12 most C-terminally situated residues. Subfamily 2.1 with only one sequence is the most diverse one, presenting only 11% identity to CnErgTx1 and three disulfide bridges. Several gaps were introduced (see Fig. 1) in order to calculate identity.

Subfamily 3 has four members, most of them with 43 amino acid residues, presenting 74 to 81% identity to CnErgTx1. The first member (CnErg2) was shown to have a different specificity than that of CnErg1 [6]. The fourth subfamily is the larger one with 13 members, all having 43 amino acid residues and an extra charged residue (arginine) in position 30, except for the peptide 4.2, but all of them with a lesser identity to CnErg1 (approx. 60–70%). It is worth observing that when the percentage identity is calculated within the subfamily against the consensus sequence (second column) the similarities are much better within that same subfamily. This fact supports in part the positioning of these sequences in the same subfamily. Finally, subfamily 5 has only two representative examples, but neither of them was obtained as a peptide nor has the function been directly determined yet. They are longer peptides (47 amino acid residues) whose N-terminal sequence is quite similar to the members of the subfamily 3.

3.2. Phylogenetic tree

The unrooted phylogenetic tree of Fig. 2 shows the clear presence of three different branches, corresponding to subfamilies 1 (flattened circles), 3 (elliptic circles) and 4 (rectangles) of Fig. 1. It is worth noting that most peptides of these subfamilies have identity values close to 90% or even higher when compared to their consensus sequence (second column Fig. 1). The lowest identity values obtained are for CgErg2 and CnErg5, which are located more distantly from their corresponding branch as indicated in Fig. 2. A fourth branch (rhombus in Fig. 2) is proposed for subfamily 5, not too far apart from each other, but represented for clarity purposes, one in the upper section of the tree, and the other in the lower segment. Subfamily 2, represented by the sole example (BeKm-1), could not be included for the generation of the phylogenetic tree, due to its highly different structure (only 11% identity to CnErgTx1), as already mentioned earlier. In this way, the five subfamilies proposed in Fig. 1 have an acceptable closely situated position in the phylogenetic tree, again giving support to the proposed nomenclature.

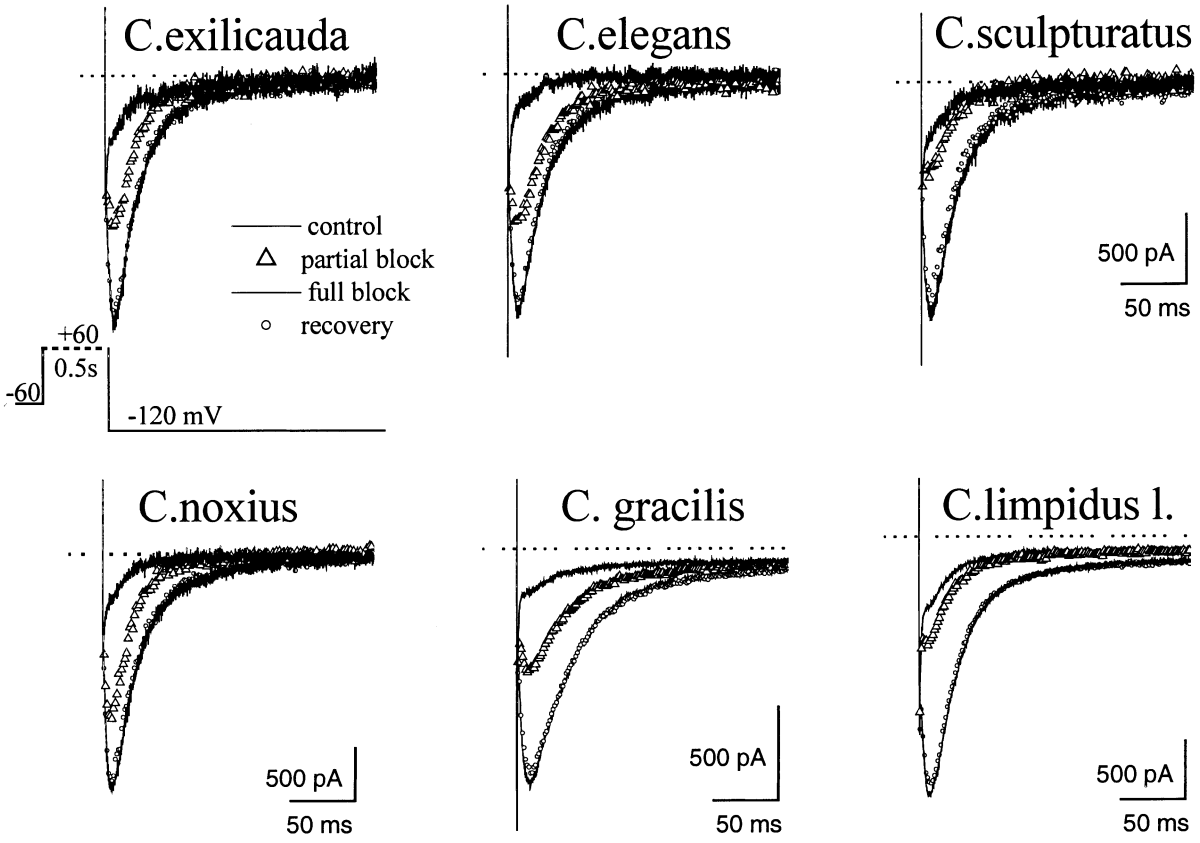

Fig. 3. ERG-currents blocked by the venoms of the indicated scorpions. Recordings show superimposed traces elicited according to the protocol shown in first panel (see Section 2), before the venom application, during the venom (180 μg/ml) perfusion, at the maximum block and after washout (see different symbols). Usually the complete block was attained in 45–60 s and the complete washout lasted 2–3 min. The leak correction procedure was not applied in the last two panels (*C. gracilis* and *C. limpidus limpidus*). The traces during the venom perfusion show a variable amplitude because protocols and the beginning of perfusion were not linked. $[K^+]_o = 40$ mM. Cell clone F-11 was used.

3.3. Physiological effects

The first member of subfamily 1 has the only peptide γ-KTx 1.1 (CnErgTx1) from the scorpion *C. noxius*, extensively studied [5,17,18,26]. Subfamily 2, represented by the *Buthus eupeus* BeKm-1 toxin (γ-KTx 2.1), is also well studied [7], whereas toxin γ-KTx 3.1, the first example of subfamily 3, has been used for electrophysiological studies [6], but its amino acid sequence is reported for the first time in this communication. Toxin γ-KTx 4.1 (CllErgTx1), the first member of subfamily 4, isolated from *C. l. limpidus* scorpion venom, has been shown to block ERG K^+-channels of F-11 clone, but neither this result nor its amino acid sequence has been published thus far. However, in this same subfamily 4 is toxin CsEKerg1 (γ-KTx 4.12) from *C. sculpturatus*, whose primary structure and physiological actions were demonstrated by Nastainczyk et al. [8].

Thus, from all the subfamilies the only one not directly tested on HERG, ERG or ERG-type K^+-channels are those from subfamily 5. However, there are peptides corresponding to genes cloned from venomous glands of the scorpions of the species *C. elegans*, *C. exilicauda* and *C. gracilis* that were also not tested.

In order to verify that the *Centruroides* identified genes indeed codify for peptides capable of blocking ERG-currents (I_{ERG}), we performed an assay using the venoms of the scorpions reported here. All the tested venoms (180 μg/ml) were able to rapidly and reversibly block I_{ERG}, as shown in Fig. 3 (see legend for Fig. 3 for more details). These results simply demonstrate that at least one ERG-selective peptide is present in each venom, but we cannot exclude that more ERG-specific peptides contribute to the effects shown in Fig. 3. At least for the case of the venom from *C. noxius*, more than one peptide was isolated and shown to affect the ERG-type K^+-currents [5,6].

Finally, concerning the known genes coding for proteins selective for potassium (ion-channels), three different ERG genes were cloned. They encode α-subunits responsible for very similar currents and were shown to be affected in a similar manner by the antiarrhythmics [27]. Thus, we putatively hypothesize that at least some of the novel sequences could codify either for peptides selective for the ERG1, ERG2 and/or ERG3-currents or for the other members of the *eag* superfamily, namely ELK and EAG-channels [28], work now in progress in our laboratories. Thus far, we know that CnErg1 (γ-KTx1.1) is quite specific for ERG1-channels [5], and CnErg2 (γ-KTx3.1) recognizes preferentially an *eag*-related gene-type K^+-channel [6]. Most of the other peptides, whose sequences were deduced from cloned genes, still need to be purified or expressed by DNA-recombinant techniques and assayed.

3.4. Complete nucleotide sequence of CnErg1 (γ-KTx1.1)

The complete nucleotide sequence for the first toxin (γ-KTx1.1) was obtained from cDNA using 5′-RACE protocols (GenBank accession number AY164271). A 287 nucleotides long sequence was found, in which the positions occupied by the signal peptide and the sequence corresponding to the mature peptide are clearly evident. A signal peptide sequence coding for a 20 amino acid long segment of the gene is present. Contrary to other scorpion toxins it does not seem to involve additional processing of a propeptide-type precursor sequence. A putative polyadenylation site was identified at the most 3′-end of the sequence, 51 nucleotides after the stop codon. This is the first time that a complete nucleotide sequence of an Ergtoxin-like gene is reported and should help in finding the equivalent information for the other peptide described here.

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