Scorpion venom inhibits selectively Ca^{2+}-activated K^+ channels in situ

Estelle Leneveu* and Michel Simonneau†

Laboratoire de Neurobiologie Cellulaire et Moléculaire, Centre National de la Recherche Scientifique, 91190 Gif-sur-Yvette, France

Received 14 October 1986

Using patch-clamp techniques, a study was made of the component of *Leiurus quinquestriatus* scorpion venom which caused a blockade of one class of membrane potassium channels, the calcium activated potassium (BK) channels. This blockade was obtained on channels in their native lipidic environment and was specific for this class of channels as other types of potassium channels were not affected by this venom.

(Leiurus quinquestriatus) *Ion channel* *Ca^{2+} activation* *K^+ channel* *Patch clamp*

1. INTRODUCTION

Calcium activated potassium channels have been found in a variety of different cell types [1,2]. In many cases, the increase in intracellular calcium which opens these channels correlates with the activation of a membrane receptor through a second messenger (see, for instance, muscarinic activation of BK channels [3]). For this important class of membrane channels, no selective ligands have been available so far that permit an experimental manipulation of these channels. Recently, Miller et al. [4] found that a partly purified toxin from the venom of *Leiurus quinquestriatus* scorpion blocked Ca^{2+}-activated K^+ channels isolated from sarcoplasmic reticulum and reincorporated in lipid bilayers.

To qualify this toxin as a pharmacological tool, two questions needed to be answered: (i) does the toxin act on in situ channels and (ii) does this toxin selectively block BK channels? We took advantage of multipotential embryonal carcinoma cells which present a limited repertoire of K channels [5] to address these two points.

2. MATERIALS AND METHODS

2.1. *Cell culture*

All experiments were done on the 1003 EC cell line cultured as described in [6].

2.2. *Electrophysiological recordings*

Patch-clamp techniques were applied [7] using the outside-out configuration which allows the extracellular face of the channel to be in contact with the extracellular medium, thus making it accessible to the toxin. Acquisition and analysis of data using a PDP 11-23 microcomputer were performed as in [7]. Distribution of open times was made as described in [8].

2.3. *Scorpion venoms*

Lyophilized samples of scorpion venom were the generous gifts of Drs M.F. Martin and H. Rochat or were obtained from Latoxan, Rosans, France. These samples were solubilized in distilled water, centrifuged to remove mucus and stored at −20°C.

† To whom correspondence should be addressed
* Present address: Laboratoire d'Endocrinologie, UA CNRS 650, Université de Rouen, 76130 Mont Saint-Aignan, France

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

3. RESULTS

3.1. *Ca^{2+}-activated K^+ channels*

As for many other preparations, calcium-activated potassium channels were identified in outside-out patches of 1003 EC cells using different specific characteristics: (i) their large unit conductance (in the range of 200 pS), (ii) a high selectivity for potassium and (iii) dependence on both the voltage and the intracellular calcium concentration. Furthermore, the BK channels present in 1003 EC patches have properties similar to those described for other preparations [1,2,8].

3.2. *Blockade of Ca^{2+}-activated K^+ channels by scorpion venom*

Fig.1 illustrates the effect of 250 $\mu g \cdot ml^{-1}$ of *Leirus quinquestriatus hebraeus* venom (LQV) on a BK channel present in an outside-out patch with 1 mM Ca present inside the pipette. For this concentration at the cytoplasmic face of the BK channel, its active state probability was always larger than 0.14 for V_h = 0 mV. This probability, p_o, decreased to 0 after bath application of LQV. The blockade was reversed by washing out the venom. The delay before obtaining a complete recovery depended on the duration of LQV application. For doses lower than 250 $\mu g \cdot ml^{-1}$, no clear blockade effect was found.

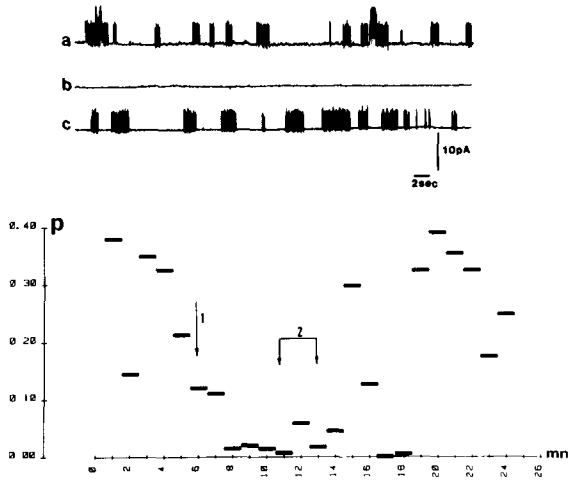

Fig.1. Effect of LQV on BK channel mean open state probability. Blockade of Ca^{2+}-activated K^+ channel by 250 $\mu g \cdot ml^{-1}$ LQV. LQV was applied at 1 and washed out during 2. Samples of recordings before (a), during LQV application (b) and after wash-out (c) are shown in insets. Mean open state probability (p) was determined from the proportion of time a channel current spent above a pre-selected threshold. Note that two channels were present in the patch. Outside-out patch configuration was used. The unit conductance of the channel was 210 pS, calculated from its permeability value. Note the reversibility of the blockade. Pipette solution, in mM: KCl, 140; $MgCl_2$, 2; Tes buffer, 2; $CaCl_2$, 1. Extracellular medium: NaCl, 140; KCl, 4; $CaCl_2$, 2.5; $MgCl_2$, 1; Hepes, 10.

3.3. *BK toxin appears to be found only in* Leiurus quinquestriatus hebraeus *venom*

The component acting on BK channels appeared to be present only in the venom of *Leiurus quinquestriatus* from a specific geographic area (Israel) as LQV from Sudan and Mali were without clear effect on BK channels. Venoms of *Androctonus* and *Buthus* were not active for doses of 250 $\mu g \cdot ml^{-1}$ and upwards.

3.4. *Effect of LQV on BK channel kinetics*

In situ BK channels present a complex kinetic behavior with at least one open state, three closed states and an inactivation by intracellular calcium (Distasi et al., in preparation; see also [9]). In the cases where the number of openings after LQV was

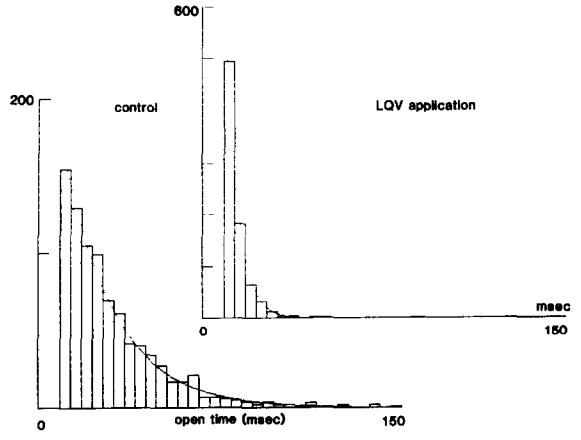

Fig.2. Effect of LQV on BK channel kinetics. Distribution of open times before (control) and after (LQV application) scorpion venom application. The single channel recordings were filtered at 1 kHz and only one component of mean open time was found (20 ms during the control and 4 ms during LQV application). 862 openings were plotted in the control histogram and 800 in the LQV histogram.

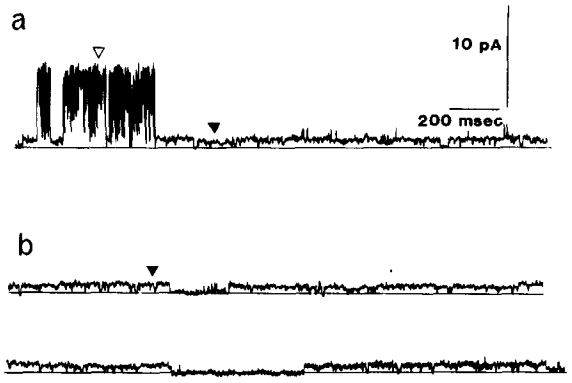

Fig.3. Selectivity of action of LQV. (a) Control. Two different types of K^+ channels were present in the patch: a BK channel (▽) and a delayed rectifier channel (▼). (b) After application of 100 $\mu g \cdot ml^{-1}$ LQV the BK channel was blocked but not the delayed rectifier. Outside-out patch with 4 mM KCl outside; 140 mM KCl, 10^{-3} M $[Ca]_i$ inside; V_h = 0 mV.

sufficient, kinetic analysis showed that the mean open time was decreased during the toxin application (fig.2). This change in kinetics appears different from that reported by Miller et al. [4] but which concerned reincorporated BK channels in lipid bilayers. In their system, they showed that the toxin induced a shortening of active bursts and a change in the mean time of inactive states, but without any change in the kinetics inside the burst being observed. This discrepancy may be due to a different mode of action of the toxin on reincorporated channels and on in situ channels.

3.5. *Selectivity of action: absence of effect of the toxin on other types of K channels*

We next examined the selectivity of LQV on different K^+ channels found in the outside-out patches of 1003 EC cells. Ca^{2+}-activated cationic channels with properties similar to those described in fully differentiated cells [10] were not affected by the toxin. Delayed rectifier K channels were also found in this preparation but were unaffected by the toxin (fig.3). These results point to a selective action of LQV on BK channels.

4. DISCUSSION

We report here that LQV selectively inhibits BK channels in their native lipidic environment. Miller et al. [4] reported that BK channels isolated from rat muscle sarcoplasmic reticulum and reconstituted into artificial bilayers were blocked by a toxin from LQV. These results suggest that BK channels from different preparations may present a similar site for a toxin from *Leiurus quinquestriatus*. This site seems not to be directly linked with lipids as reincorporated channels can be blocked by LQV. As for other polypeptides found in scorpion venoms, the relative quantity of the toxin acting on BK channels appears to vary from one species to another. The kinetics of blockade appear to be more complex than those reported for reincorporated channels. Furthermore, our experimental system permits us to test directly the selectivity of action of LQV on K^+ channels, using patches presenting more than one type of channel. In all cases tested, LQV appeared to modify only the activity of BK channels.

Preliminary results using a partly purified toxin (Morel and Simonneau, unpublished) from LQV, prepared according to the Miller et al. [4] procedure, gave a similar inhibition of BK channels to that illustrated in fig.1a. All these experiments suggest that the polypeptide partially purified by Miller et al. [4] represents a potential pharmacological tool which can bind specifically to BK channels from different preparations. Its selectivity may permit its use as a ligand to isolate and purify this type of membrane channel.

ACKNOWLEDGEMENTS

This work was supported in part by an MRT 'Dynamique du Neurone' 84C-1317, DRET, Action Valorisation (CNRS) and Fondation pour la Recherche Médicale grants (to M.S.) and an ARC fellowship (to E.L.). The authors thank Dr M.F. Martin and H. Rochat for their gift of venoms, Dr C. Miller for advice on the peptide isolation procedure and Dr S. O'Regan for correcting the manuscript.

REFERENCES

[1] Marty, A. (1981) Nature 291, 497–500.
[2] Petersen, O.H. and Maruyama, Y. (1984) Nature 307, 693–696.
[3] Trautmann, A. and Marty, A. (1984) Proc. Natl. Acad. Sci. USA 81, 611–615.

[4] Miller, C., Moczydlowski, E., Latorre, R. and Phillips, M. (1985) Nature 313, 316–318.
[5] Simonneau, M., Edde, B., Nicolas, J.F. and Jakob, H. (1985) Cell Diff. 17, 21–28.
[6] Simonneau, M., Distasi, C., Tauc, L. and Poujeol, C. (1985) J. Physiol. (Paris) 80, 312–320.
[7] Hamill, I.P., Marty, A., Neher, E., Sakmann, B. and Sigworth (1981) Pflügers Arch. 391, 85–100.
[8] Barrett, J.N., Magleby, K.L. and Pallotta, B.S. (1982) J. Physiol. 331, 211–230.
[9] Findlay, I., Dunne, M.J. and Petersen, O.H. (1985) J. Membrane Biol. 83, 169–175.
[10] Yellen, G. (1982) Nature 296, 411.