Vol. 201, No. 1, 1994 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 30, 1994 Pages 24-29

RELEASE OF TEA BLOCKADE OF MAXI-K+ CHANNELS BY ISOPROTERENOL

Shih-fang Fan[1]*, Shuya Wang[2] and C.Y. Kao[2]

[1]Department of Physiology and Biophysics, Health Sciences Center, State University of New York at Stony Brook, Stony Brook, NY 11794
[2]Department of Pharmacology, State University of New York, Health Sciences Center at Brooklyn, Brooklyn, NY 11023

Received March 28, 1994

The outward K^+ current induced by step depolarization of freshly dispersed myocytes of guinea-pig taenia coli decreased about 80% upon treatment with 3 mM tetraethylammonium chloride (TEA). Isoproterenol (ISO, 2-5 μM) restored it to a large extent. This restoration did not occur in the presence of propranolol (2 μM). In single-channel recordings from cell-attached patches, the activity of maxi-K^+ channel is dominant. When 3 mM TEA is incorporated in the pipette solution, the dominant channel-openings observed had much smaller unitary conductance. On the addition of ISO (2 μM) to the bath solution, but not to the pipette solution, K^+-channel openings with unitary conductance similar to that without TEA treatment appeared. Cyclic AMP incorporated into the cytoplasm through the pipette was ineffective. These results indicate that ISO release TEA decrease of maxi-K^+ channel conductance through some intracellular second messenger system other than adenylyl cyclase-protein kinase A system.

Tetraethylammonium (TEA) produces flickery block of big Ca^{2+}-activated K^+ channel in various cells including smooth muscle cells (e.g., arterial and intestinal myocytes [1, 2]). Isoproterenol (ISO) increases the opening of big Ca^{2+}-activated K^+ channel (maxi-K^+ channel) in these same cells (e.g. taenia coli myocytes [3]). TEA blockade acts directly on the channel by binding to a specific region of the channel [4]. The effect of ISO acts indirectly through *beta*-adrenergic receptor-G_s-adenylyl cyclase-protein kinase A transduction system [3]. However we found that ISO could increase the conductance of Ca^{2+}-activated K^+ channel that had been decreased by TEA.

MATERIALS AND METHODS: Freshly dispersed myocytes from guinea pig taenia coli were used. The methods of cell preparation, recording and analyzing of data can be found in Fan *et al.* [3]. Records were taken under symmetrical K^+ (140 mM) conditions. For whole

*Corresponding author. Fax: (516) 444-3432.

0006-291X/94 $5.00

cell recording the bath solution contained in mM: KCl or K-gluconate 140; $CaCl_2$ 0.1; EGTA, 0.6 HEPES 10, pH adjusted to 7.2 with KOH. The pipette solution used was made by the addition of 2 mM ATP and 0.5 mM $MgCl_2$ into the bath solution. Experiments were done at room temperature (20 - 22°C). All drugs used were from Sigma Chemical Co. (St. Louis, MO).

RESULTS: Our findings are illustrated in Fig. 1 and 2. Fig. 1 shows the results obtained with whole-cell current recording. The outward whole cell K^+ currents were elicited by step depolarization from 0 to 70 mV in eight steps. Upon application of 3 mM TEA, the outward current elicited by depolarization to 70 mV was decreased to 20% of the control (Fig. 1B). Upon the addition of 2 μM ISO into the bath solution, it recovered to about 80% of the control (Fig. 1C). Fig. 1D shows the corresponding I-V curve of Fig. 1 A-C. Fig. 1E shows the time course of change of the current elicited by depolarization to 70 mV upon the addition of

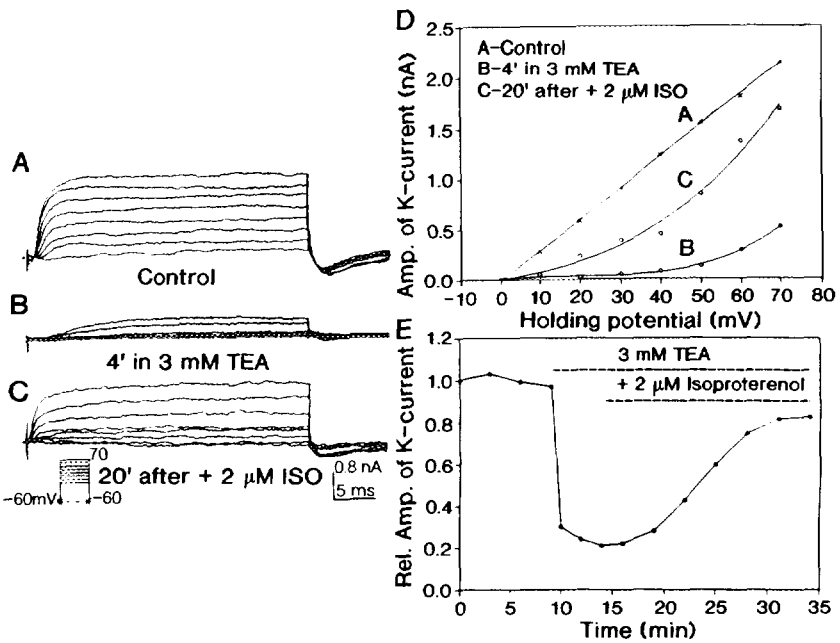

Fig. 1. Showing that the suppression of outward K^+ current during step depolarization by TEA can be counteracted by ISO. Records were taken from taenia coli myocyte under symmetrical K^+ (140 mM) condition.

A-C. Outward current elicited by depolarization from 0 to 70 mV in eight steps. A. Control. B. 4 min in 3 mM TEA. C. 20 min after the addition of 2 μM ISO. Holding potential was - 60 mV.

D. I-V curves of the control (curve A), 4 min in 3 mM TEA (curve B) and 20 min after the addition of 2 μM ISO (curve C).

E. Time course of change of the current elicited by depolarization to 70 mV upon the addition of 3 mM TEA and 2 μM ISO.

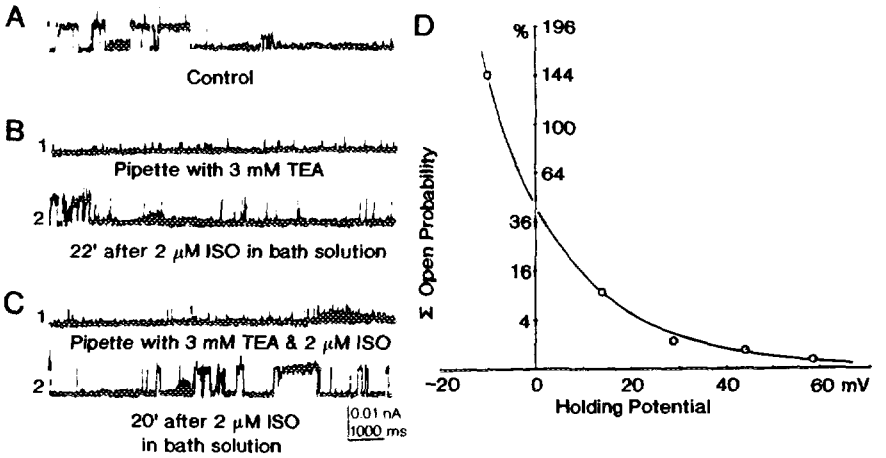

Fig. 2. Showing that in cell-attached single channel recording mode ISO incorporated into the pipette solution has no effect on the TEA decrease of the conductance of maxi-K^+ channel. Only when incorporated into the bath solution ISO can increase the conductance that had been decreased by TEA.

A. Control.

B. Showing that the unitary conductance of maxi-K^+ channel is decreased by 3 mM TEA with 2 μM ISO added in the pipette solution (B-1). The conductance increased to the control value after the additional application of 2 μM ISO into the bath solution (B-2).

C. Showing that the unitary conductance of maxi-K^+ channel decreased by TEA (C-1) is increased by bath application of 2 μM ISO (C-2).

Records A-C were taken from the same cell with 3 pipettes filled with 3 different solutions. Holding potential was - 30 mV.

D. An example of open probability vs. holding potential curve of maxi-K^+ channels recorded from a cell-attached patch of a taenia coli myocyte bathed in solution containing 3 mM TEA. At low holding potentials, several channels were open. Σopen probability is the sum of open probabilities of the several active channels. Unlike cells bathed in TEA-free solution, the open probability in 3 out of 8 cells tested was decreased with increase of holding potential.

3mM TEA and 2 μM ISO. Same results were obtained from 7 cells. The outward current elicited by depolarization to 70 mV was decreased to (17 $\pm$ 7)% (mean $\pm$ standard error) of the control value by 3 mM TEA and later recovered to (78 $\pm$ 12)% of the control value. The effect of ISO was suppressed completely by prior application of *beta*-adrenoceptor antagonist, propranolol (2 μM, n = 5). It has been reported that isoproterenol can activate Cl^- current in rabbit ventricular myocytes [5]. Following results showed that the increase of outward currents observed after ISO treatment was not due to the activation of Cl^- current: (1) Similar results were obtained in 4 cells with Cl^- in both bath and pipette solutions substituted by gluconate ions; (2) ISO effect was not affected by the addition of 1 mM $ZnCl_2$ into the bath solution. In single-channel recordings from cell-attached patches, the predominant activity is from the maxi-K^+ channel [6]. The unitary conductance of the maxi-K^+ channel was decreased by

TEA. Fig. 2 shows channel activities of three different cell-attached patches taken from one cell with 3 different pipettes. The first with a pipette filled with regular KCl solution (Fig. 2A, taken as the control). The second filled with KCl solution plus 3 mM TEA and the third filled with KCl solution plus 3 mM TEA and 2 μM ISO (Fig. 2B-1 and 2C-1). Fig. 2B-1 and 2C-1 were taken, respectively, at 20 and 22 min after making the high resistance seals between the pipette tips and the membrane. The amplitudes of unitary current recorded with the second and third pipettes were about the same and were much smaller than that showed in Fig. 2A. This indicates that ISO did not have substantial effect as long as it was only presented in the pipette. Upon the further addition of 2 μM ISO into the bath solution, the amplitudes of unitary current as measured with the second and third pipettes increased to the control value (Fig. 2B-2 and 2C-2). In cells untreated with ISO and TEA the open probability of the Ca^{2+}-activated K^+ channels increased with increase of negative holding potential (the cell interior became more positive). However, in 3 out of 8 cells tested, the opening of the Ca^{2+}-activated K^+ channel decreased with increase in negative holding potential. Among them, two were recorded with pipettes filled with KCl solution and the third was recorded with pipette filled with K-gluconate solution. Fig. 2D shows one example taken with pipette filled with KCl solution.

In 4 cell-attached patch recordings we incorporated 2 mM cyclic AMP with 3 mM TEA into the pipette solution. In 3 cell-attached patch recordings, with TEA incorporated into the pipette solution and 1 mM membrane permeable cyclic AMP analog, dibutyryl cyclic AMP added into the bath solution. The unitary conductance of the K^+ channels decreased to the same extent as no cyclic AMP was used. Cyclic AMP has no effect on the TEA blockade of K^+-channel.

DISCUSSION: As stated in the Introduction, TEA blockade acts directly on the channel by binding to a specific region. That ISO incorporated in the pipette had no effect indicates that (1) it does not affect the channel directly, instead it must act through diffusible intracellular second messenger system; (2) the effect needs a certain number of receptors be activated.

The effect of ISO cannot be duplicated with intracellular application of cyclic AMP indicates that the increase of K^+-conductance by ISO that had been decreased by TEA did not act through the protein kinase A phosphorylation. Besides G_s - adenylyl cyclase - protein kinase

A system, ISO affects other messenger systems as well. For instance, activation of G_s also can activate kinase C can be activated and the intracellular stored calcium can be mobilized. It has been reported that some K^+ channels expressed from *Drosophila Shaker* mRNA in *Xenopus* oocytes are highly charybdotoxin sensitive and moderately TEA sensitive. Site-directed mutagenesis and comparison of natural sequence variants of *Shaker* showed that changing of only one residue at position 449 or 451 close to the S6 segment dramatically alters the effectiveness of TEA. K_{TEA} increases from 27 mM to > 200 mM as threonine (residue 449) is changed into lysine or decreases to 0.6 mM as it is changed into tyrosine [8]. These results indicate that minute changes in the channel protein may result in dramatic changes in the effect of TEA. Therefore changes in the protein constituents maxi-K^+ channel through other messenger system, even it is small, can have dramatic influence on the channel conductivity.

In 1988 Iwaki et al. [9] reported that mM concentrations of TEA induces contraction of strips of coronary artery of rabbit heart. They argued that contraction is induced by Ca^{2+}-spike of the myocytes. Ca^{2+}-spike is initiated by a TEA-induced decrease of K^+-conductance of the membrane. This TEA-induced contraction is abolished in Ca^{2+}-free solution and suppressed by nitroglycerin, nicorandil and ISO. They attributed the suppressive effect of all three agents to the blocking of Ca^{2+} channels. However, in various types of cell, including A7r5 smooth muscle cell, the L-type calcium current is, instead of decreasing, but increased by ISO [10]. Therefore the cause of suppression of TEA-induced contraction by isoproterenol cannot be due to block of Ca^{2+} channels. Most probably it was the result of increase of K^+ conductance by ISO

ACKNOWLEDGMENTS: This work is supported by NIH grants HD-00378 and DK-39731 to C.Y.K.

REFERENCES

1. Benham, C.D., Botton, T.B., Lang. R.J. and Takewaki T. (1985) Pflügers Arch. 403,120-127.
2. Langton, P.D., Nelson, M.T., Huang, Y. and Standen, N.B. (1991) Am. J. Physiol. 260,H927-H934.
3. Fan, S.F., Wang, C.Y. and Kao, C.Y. (1993) J. Gen. Physiol. 102,257-275.
4. Hille B. (1992) Ionic Channels of Excitable Membranes, 2nd ed. Chapter 16. Sinauer Asso. Inc. Publ., Sunderland, MA.

5. Harvey, R.D. and Hume, J.R. (1989) Am. J. Physiol. 257,C1177-C1181.
6. Hu, S.L., Yamamoto, Y. and Kao, C.Y. (1989) J. Gen. Physiol. 9,833-847.
7. Martis, T.F.J. (1991) Pharmacol. Ther. 49,329-345.
8. MacKinnon, R. and Yellen, G. (1990) Science 250,276-279.
9. Iwaki, M., Nekaya, Y., Kawano, K., Mizobuchi, S., Nakaya, S. and Mori, H. (1988) Heart Vessels 4,141-148.
10. Marks, T.N., Dubyak, G.R. and Jones S.W. (1990) Pflügers Arch. 417,433-439.