Intracellular Regulation of Ion Channels

Edited by

Martin Morad

and

Zalman Agus

University of Pennsylvania
School of Medicine
Dept. of Physiology
Philadelphia, PA 19104-6085
USA

Springer-Verlag
Berlin Heidelberg New York London Paris Tokyo
Hong Kong Barcelona Budapest
Published in cooperation with NATO Scientific Affairs Division

TABLE OF CONTENTS

PART I: Regulation of K^+ Channels	Page
K^+ channel diversity O. Pongs	3
Regulation of voltage-activated K^+ channels A. M. Brown	9
Gating mechanisms of *Shaker* potassium channels R. Aldrich	15
Vaccinia virus: A novel expression system for receptors and ion channels A. Karschin	21
Delayed rectification in the heart: Regulation and physiology W.M. Kwok, L.C. Freemen, J. Anumonwo, and R.S. Kass	27
Receptor-dependent modulation of G protein-gated muscarinic K^+ channel by arachidonic acid metabolites Y. Kurachi	43
The Na^+-activated K^+ channel in cardiac cells E. Carmeliet and H.-N. Luk	53
Regulation of the ATP-dependent K^+ channel of muscle by ATP and pH N.B. Standen, N.W. Davies, A. Pettit, and P.R. Stanfield	61
Regulation of cardiac ATP-sensitive K^+ channels J.N. Weiss, N. Venkatesh, and N.A. Deutsch	71
ATP-sensitive K^+ channels: Molecular pharmacology, regulation and role in diseased states M. Lazdunski, M. Fosset, J. De Weille, E. Honoré, C. Mourre	83
PART II: Regulation of Ca^{2+} Channels	
Tissue distribution and possible function of the subunits of the L-type calcium channels F. Hofmann, M. Biel, R. Hullin, E. Bosse, V. Flockerzi	89

Phosphorylation and regulation of calcium channels by multiple protein kinases 99
M.M. Hosey, R.M. Brawley, C.F. Chang, L.M. Gutierrez, C. Mundina-Weilenmann, J. Ma, and E. Rios

Action of the GTP-binding protein Gs on cardiac Ca^{2+} channels 107
A. Cavalié, T.J.A. Allen, W. Trautwein

Membrane-delimited stimulation of heart cell calcium current by ß-adrenergic signal-transducing Gs protein 113
S. Pelzer, Y.M. Shuba, L. Birnbaumer, T.F. McDonald, D.J. Pelzer

Participation of a fast G-protein pathway in the ß-adrenergic regulation of cardiac calcium channels: Neither proven nor needed 129
R. Fischmeister, P.-F. Méry, H.C. Hartzell, G. Szabo

Kinetics of G-protein mediated action on neuronal Ca^{2+} channels 141
H.-D. Lux, N. Tokutomi and F. Grassi

Identification of G-proteins involved in the inhibition of Ca^{2+} currents in neuronal and endocrine cells 153
J. Hescheler, C. Kleuss, C. Ewel, B. Wittig, W. Rosenthal, G. Schultz

Voltage-dependent α-adrenergic modulation of Ca^{2+} channels in peripheral neurons and insulin-secreting cells 161
E. Carbone, A. Pollo, M. Lovallo, G. Aicardi, and E. Sher

Voltage-dependent modal gating in cardiac and neuronal L-type calcium channels 173
D. Pietrobon, L. Forti, and P. Hess

T-type calcium channels in cardiac muscle: News in kinetics and modulation 181
B. Nilius

Calcium regulation of ion channels in neurons 191
R.S. Zucker

Role of calpastatin in calcium channel regulation 203
M. Kameyama, A. Kameyama, E. Takano, K. Yazawa, K. Yasui, T. Murachi

PART III: Regulation of Na^+, Cl^-, and I_f Channels

Structure and modulation of voltage-gated sodium channels 209
W.A. Catterall, T. Scheuer, R. Numann, M. Li, J. West, B. Murphy, and S. Rossie

Regulation of the cAMP-dependent chloride current in cardiac ventricular myocytes 221
R.D. Harvey, J.A. Jurevicius, J.R. Hume

Magnesium as an intracellular modulator of calcium, potassium and chloride channels 231
Z.S. Agus, E. Kelepouris, I. Dukes, R. Kasama and M. Morad

Cyclic AMP regulation of pacemaker (I_f) current in heart 241
D. DiFrancesco

Subject Index 243

Tissue distribution and possible function of the subunits of the L-type calcium channels

F. Hofmann, M. Biel, R. Hullin, E. Bosse, V. Flockerzi

Institut für Pharmakologie und Toxikologie der Technischen Universität, Biedersteinerstr. 29, D-8000 München 40, Germany

Introduction

L-type calcium channels are present in many tissues and are the major pathway for voltage activated calcium entry in heart and smooth muscle and are essential for EC-coupling in skeletal muscle. The transverse tubular membrane of skeletal muscle contains a high density of the calcium channel protein, the so called receptor for calcium channel blockers (CaCB-receptor) [1]. The charge moved during excitation-contraction coupling (EC-coupling) is located in the CaCB-receptor and can be blocked by dihydropyridines and phenylalkylamines [2]. It is likely that in skeletal muscle the CaCB-receptor functions only as voltage sensor and not as calcium conducting channel since EC-coupling does not require calcium entry in skeletal muscle.

Component of the skeletal muscle CaCB receptor/calcium channel

The rabbit skeletal muscle CaCB-receptor has been purified to homogeneity, and contains five proteins with apparent molecular weights of 165,000 (α_1), 55,000 (β), 32,000 (y) and a disulfide-linked dimer of 135,000 (α_2) and 28,000 (δ). The primary sequence of the subunits has been deduced by cloning of the corresponding mRNAs from rabbit skeletal muscle [3-7] (Fig.1).

The α_1 subunit contains the structural components of a voltage dependent ion channel (3,4), and the high affinity binding sites for dihydropyridines, phenylalkylamines and benzodiazepine [see 1 for

NATO ASI Series, Vol. H 60
Intracellular Regulation of Ion Channels
Edited by M. Morad and Z. Agus

references]. High affinity binding to the dihydropyridine sites requires the presence of μmolar calcium [8]. Phenylalkylamine binding depence also on the presence of μmolar calcium, although binding is inhibited by millimolar concentrations of calcium [8]. The dihydropyridine site has been identified by photoaffinity labelling of the purified receptor with azidopine and nifedipine [9]. Both compounds label peptides which follow directly the last putative transmembrane helix (S6) of repeat IV. The phenylalkylamines bind apparently to the same region of the α_1 subunit. An antibody raised against aa 1349-1391 of the α_1 subunit - this sequence contains the transmembrane helix IV S6 - identified a tryptic peptide which contained covalently bound [N-methyl-^{3}H]LU449888, a photoaffinity analogue of devapamil [10].
The deduced sequence of the α_1 subunit contains seven consensus sequences for cAMP-dependent phosphorylation [3]. One of these sites, Ser-687, is rapidly phosphorylated in vitro by cAMP kinase [11]. A second serine, Ser-1617, is phosphorylated slowly in vitro by cAMP kinase. Phosphorylation of these sites may be significant, since the open probability of the reconstituted skeletal muscle CaCB-receptor/calcium channel is increased several fold by cAMP dependent phosphorylation [12-14]. Furthermore, the α_1 subunit of isolated rat myocytes is phosphorylated at least at two sites in response to isoproterenol in vivo [5]. One phosphorylation site could be identical with Ser-687 suggesting that phosphorylation of this residue may influence the open probability of the skeletal muscle calcium channel.
<u>The α_2/δ subunit</u> contains three putative transmembrane segments [4]. In agreement with its behaviour as a glycoprotein several consensus sequences for glycosylation are present in a large extracellular domain. The δ subunit is identical with the carboxy terminal part of the cloned α_2 protein starting at aa 935 of the predicted sequence [16]. Presumably, the mature α_2- and δ-protein are the product of the same gene and arise by posttranslational processing. The mature α_2-protein has two transmembrane domains and is linked by disulfide bridges to the δ-protein which contains the third putative transmembrane helix.
<u>The β-subunit</u> is compatible with that of a peripheral membrane protein of 57,868 Da [5]. It contains four α-helical domains,

three of which contain a heptad repeat structure. Heptad repeats have been found in cytoskeletal proteins. This suggests that the β subunit may be a cytoskeletal protein which anchors the α_1 subunit to the cytoskeleton. The deduced amino acid sequence contains several potential phosphorylation sites. Two of these sites, Ser-182, and Thr-205 are phosphorylated in vitro by cAMP kinase. Further sites may be phosphorylated by cGMP kinase, casein kinase II and protein kinase C, which rapidly modify the β subunit in vitro and phosphorylate specific peptides [17].

The y-subunit is an integral membrane protein, of 25,058 Da [6,7] containing four putative transmembrane domains and two glycosylation sites which are located extracellular. The purified y-protein is glycosylated, supporting a model, which locates the N- and C-terminus of the protein on the cytosolic site of the membrane (Fig.1).

Tissue distribution of the CaCB-receptor subunits

Complete cDNAs for the α_1 subunit of the CaCB receptor have been isolated from rabbit heart [18] and rabbit smooth muscle [19] (Fig.2). Both, the cardiac and smooth muscle cDNAs encode large polypeptides (2171 and 2166 amino acids in length, respectively) showing an overall homology of 66% (cardiac) and 65% (smooth muscle) to the skeletal muscle CaCB-receptor. The amino acid sequence of the smooth muscle CaCB receptor differs from the rabbit heart receptor at four sites comprising the aminoterminus, segments IS6 and IVS3 and an intervening sequence between repeat I and II [19]. Both channel proteins are differentially expressed. The mRNA of the cardiac channel is exclusively expressed in heart whereas the mRNA of the smooth muscle channel is present in airway and vascular smooth muscle cells which exist in lung, trachea, heart, aorta and brain [20]

The skeletal muscle and the cardiac/smooth muscles channels are the product of two different genes. Analysis of partial clones obtained by PCR suggests that both genes give rise to several differently splices variants of the skeletal and cardiac muscle [20,21]. One variable region has been located to the transmembrane helix S3 and the extracellular connecting loop between S3 and S4 of repeat IV. This location is interesting since the S4 segment

has been implicated as voltage sensor of the channel. Variation in this area may, therefore, affect the gating kinetics of the channel. Further analysis yielded evidence for the excistence of 3 additional calcium channel genes [22].
Northern blot analysis shows [20], that a probe of the skeletal α_2/δ subunit hybridized to an 8.0 kb transcript in poly $(A)^+$RNA from skeletal muscle, brain, heart, aorta, trachea and lung. In addition, a weakly hybridizing α_2 7.0 kb transcript was observed in these tissues. The β subunit derived probe hybridized to three poly $(A)^+$RNA species of skeletal muscle with estimated sizes of 1.6, 1.9 and 3.0 kb. The size of the 1.9 kb species is consistent with the size of the cloned cDNA (1835 nt) [5]. mRNA species of similar size as the 3.0 kb species appear to be present in brain and aorta. A less abundant mRNA population of about 4.8 kb, which was detected only as a faint band after long autoradiographic exposure, is present in heart. The y-subunit probe hybridized to a 1.3 kb poly $(A)^+$RNA species of skeletal muscle [6,7]. Weakly hybridizing y-transcripts of 1.0 kb (heart) and 1.3 and 1.8 kb (aorta) were observed. In brain a 1.5 kb signal was clearly visible after 5 day autoradiographic exposure but not after 16 hours.

Regulation of the expressed α_1 subunit by the other subunits

Adult cardiac ventricular and smooth muscle myocytes express mainly high voltage activated calcium channels with slow inactivating properties. These are sensitive to calcium channel blockers. In agreement with this, microinjected synthetic RNA derived from the cloned cardiac and smooth muscle CaCB receptor α_1 subunit cDNA directs the synthesis of similar channels in Xenopus oocytes [18,19]. These results indicate that the cardiac and smooth muscle α_1 subunit of the CaCB receptors alone is sufficient to induce calcium channel current. The current density approximately doubles when the cRNA for one α_1-subunit is coinjected with the cRNA for the α_2/δ-subunit. However, a dramatic about 100fold increase in current density is observed when in addition to the cRNA for the α_1 subunit the cRNA of the skeletal muscle β-subunit and the α_2/δ subunit are coinjected [23,24]. Coexpression of the cardiac α_1 subunit with the other skeletal muscle subunits effects also quite significantly the electrophysiological properties of

the channel [24]. The α_2/δ subunit doubles the speed of channel activation and all three subunits (α_2/δ,β and y) increase inactivation of the channel at the end of a 200 msec depolarizing pulse. The coexpression of the α_2/δ and β-subunit with the α_1 subunit shifts the voltage dependence of activation of Ba-currents by about 15mV to the left. The y subunit shifts the voltage dependence of the steady state inactivation by 40mV to the left. The results [24] show that coexpression of a cardiac α_1- subunit with the α_2, β and y-subunit of skeletal muscle has quite dramatic effects on the electrical properties of the ion channel.

Conclusion

These studies suggest that the α_1-subunit of the cardiac or smooth muscle calcium channel is the calcium pore forming protein. However, the properties of this channel depend largely on the coexpression with other subunits. With the exception of the α_2-subunit, it is unlikely that cardiac or smooth muscle contains identical subunits as the skeletal muscle. The northern blot analysies [20] suggests that the expression of these smaller subunits occurs in a tissue specific manner. The tissue specific expression provides the basis for a tissue specific regulation of L-type calcium channels. F. ex., the β-adrenergic receptor stimulates L-type calcium current in cardiac [25] and in tracheal smooth muscle [26]. Both tissues containe almost identical α_1-subunits [19]. However, cAMP-dependent phosphorylation is only demonstrable in cardiac [25], but not in tracheal smooth muscle cells [26]. It is likely that this differential regulation is caused by the expression of distinct small subunits in each tissue. It will be very important, therefore, to identify these subunits in each tissue.

Acknowledgment: We thank Mrs. Schatz for typing the manuscript. The experimental work of the authors was supported by grants from Fonds der chemischen Industrie and Deutsche Forschungsgemeinschaft.

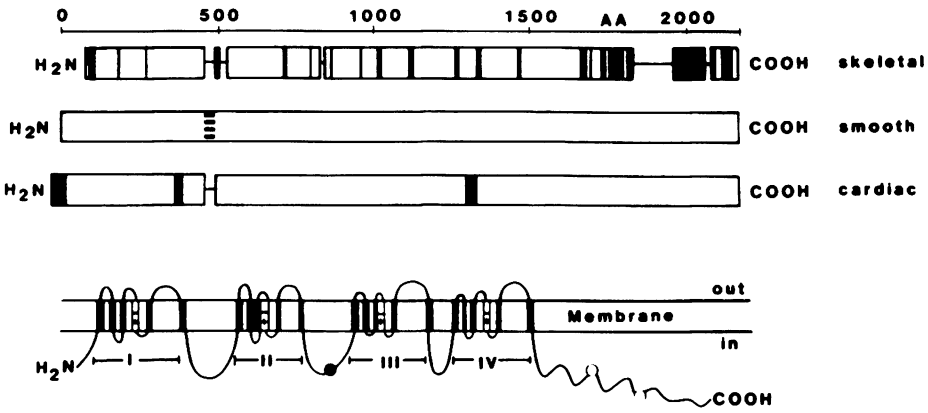

Fig. 1

Oligomeric structure of the skeletal muscle calcium channel. The molecular structures of the α_1, $\alpha_{2/\delta}$, β and y subunits were constructed from the primary sequences given in Refs. 3-7. The upper and lower horizontal lines indicate the extra- and intracellular surface of the plasma membrane, respectively. Rods between these lines represent transmembrane helices. The S4 segment is indicated by a +. In vitro phosphorylation is indicated by a P. Note, that the β subunit is localized intracellularly.

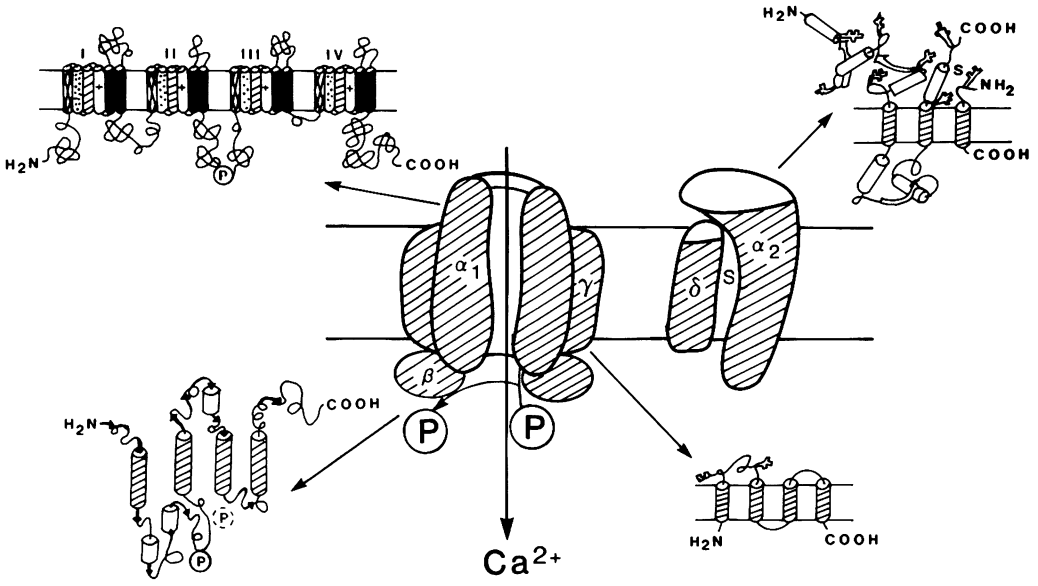

Fig. 2

Comparison of the primary sequence of the skeletal, cardiac and smooth muscle α_1-subunit of the calcium channel. The sequence is shown as block. Significant differences between the smooth muscle and the skeletal muscle or cardiac muscle are indicated by black. A line between blocks indicates a lack of the sequence in the respective clone. The putative transmembrane topology is shown at the bottom. The location of the repeats I-IV, of the phosphorylated Ser-687 of skeletal muscle and of the conserved, putative cAMP-kinase phosphorylation sites are indicated by roman numericals, a closed circle and two open circles.

References

1. F. Hofmann, V. Flockerzi, W. Nastainczyk, P. Ruth, T. Schneider, Current Topics in Cell. Regulation 31, 223-239 (1990).

2. E. Rios, G. Brum, Nature 325, 717-720 (1987).

3. T. Tanabe, H. Takeshima, A. Mikami, V. Flockerzi, H.Takahashi, K. Kangawa, M. Kojima, H. Matsuo, T. Hirose, S. Numa, Nature 328, 313-318 (1987).

4. S. B. Ellis, M. E. Williams, N. R. Ways, R. Brenner, A. H. Sharp, A. T. Leung, K. P. Campbell, E. McKenna, W. J. Koch, A. Hui, A. Schwartz, M. M. Harpold, Science 241, 1661-16 (1988).

5. P. Ruth, A. Röhrkasten, M. Biel, E. Bosse, S. Regulla, H. E. Meyer, V. Flockerzi, F. Hofmann, Science 245, 1115-1118 (1989).

6. E. Bosse, S. Regulla, M. Biel, P. Ruth, H. E. Meyer, V. Flockerzi, F. Hofmann, FEBS Letters 267, 153-156 (1990).

7. S. D. Jay, A. B. Ellis, A. F. McCue, M. E. Williams, T S. Vedvick, M. M. Harpold, K. P. Campbell, Science 248, 490-492 (1990).

8. T. Schneider, S. Regulla, F. Hofmann (1991), E.J.Biochem. in press

9. S. Regulla, T. Schneider, W. Nastainczyk, H. F. Meyer, F. Hofmann (1991) Embo J. 10, 45-49

10. J. Striessnig, H. Glossmann, W. A. Catterall, Proc.Natl.Acad.Sci.USA 87, 9108-9112 (1990).

11. A. Röhrkasten, H. Meyer, W. Nastainczyk, M. Sieber, F. Hofmann, J.Biol.Chem. 263, 15325-15329 (1988).

12. V. Flockerzi, J. Oeken, F. Hofmann, D. Pelzer, A. Cavalié, W. Trautwein, Nature 323, 66-68 (1986).

13. L. Hymel, J. Striessnig, H. Glossmann, H. Schindler, Proc. Natl.Acad.Sci.USA 85, 4290-4294 (1988).

14. K. S. De Jongh, D. K. Merrick, W. A. Catterall, Proc.Natl. Acad.Sci.USA 86, 8585-8589 (1989).

15. Y. Lai, M. J. Seagar, M. Takahashi, W. A. Catterall, J.Biol.Chem. 265, 20839-20848 (1990).

16. K. S. De Jongh, C. Warner, W. A. Catterall, J.Biol.Chem. 265, 14738-14741 (1990).

17. H. Jahn, W. Nastainczyk, A. Röhrkasten, T. Schneider, F. Hofmann, Eur.J.Biochem. 178, 535-542 (1988).

18. A. Mikami, K. Imoto, T. Tanabe, T. Niidome, Y. Mori, H. Takeshima, S. Narumiya, S. Numa, Nature 340, 230-233 (1989).

19. M. Biel, P. Ruth, E. Bosse, R. Hullin, W. Stühmer, V Flockerzi, F. Hofmann, FEBS Letters 269, 409-412 (1990).

20. M. Biel, R. Hullin, St. Freundner, D. Singer, N. Dascal, V. Flockerzi, F. Hofmann (1991) submitted

21. E. Perez-Reyers, X. Wei, A. Castellano, L. Birnbaumer, J.Biol Chem. 265, 20430-20436 (1990).

22. T. P. Snutch, J. P. Leonard, M. M. Gilbert, H. A. Lester, N. Davidson, Proc.Natl.Acad.Sci.USA 87, 3391-3395 (1990).

23. Y. Mori, Th. Friedrich, M-S. Kim, A. Mikami, J. Nakai, P. Ruth, E. Bosse, F. Hofmann, V. Flockerzi, T. Furuichi, K. Mikoshiba, K. Imoto, T. Tanabe, S. Numa (1991) Nature 350, 398-402

24. D. Singer, M. Biel, I. Lotan, V. Flockerzi, F. Hofmann, N. Dascal (1991) submitted

25. M. Kameyama, J. Hescheler, F. Hofmann, W. Trautwein, Pflügers Arch. 407, 123-128 (1986).

26. A. Welling, J. Felbel, K. Peper, F. Hofmann (1991) Blood Vessels 28, 154-158