Molecular assembly and subcellular distribution of ATP-sensitive potassium channel proteins in rat hearts

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Abstract Cardiac ATP-sensitive K^+ (K_{ATP}) channels are proposed to contribute to cardio-protection and ischemic preconditioning. Although mRNAs for all subunits of K_{ATP} channels (Kir6.0 and sulfonylurea receptors SURs) were detected in hearts, subcellular localization of their proteins and the subunit combination are not well elucidated. We address these questions in rat hearts, using anti-peptide antibodies raised against each subunit. By immunoblot analysis, all of the subunits were detected in microsomal fractions including sarcolemmal membranes, while they were not detected in mitochondrial fractions at all. Immunoprecipitation and sucrose gradient sedimentation of the digitonin-solubilized microsomes indicated that Kir6.2 exclusively assembled with SUR2A. The molecular mass of the Kir6.2–SUR2A complex estimated by sucrose sedimentation was 1150 kDa, significantly larger than the calculated value for $(Kir6.2)_4$–$(SUR2A)_4$, suggesting a potential formation of micellar complex with digitonin but no indication of hybrid channel formation under the conditions. These findings provide additional information on the structural and functional relationships of cardiac K_{ATP} channel proteins involving subcellular localization and roles for cardioprotection and ischemic preconditioning.

Key words: ATP-sensitive K^+; Sulfonylurea receptor; Inwardly rectifying potassium channel; Heart; Mitochondrion

1. Introduction

ATP-sensitive potassium (K_{ATP}) channels are found in diverse cell types, including pancreatic β cells, brain, heart and skeletal and smooth muscle cells, where they are thought to couple metabolism with membrane electrical activity [1,2]. K_{ATP} channels are abundantly expressed in cardiac tissue. Cardiac cells are considered to contain two distinct K_{ATP} channels: the classical one in the sarcolemma [3] and the other in the mitochondrial inner membrane [4]. Sarcolemmal K_{ATP} ($sarcK_{ATP}$) channels were first proposed to play an important role in the cardioprotective effect, because potassium channel openers (KCOs) mimicked the cardioprotection and the K_{ATP} channel blocker glibenclamide abolished the ischemic preconditioning (IPC) [5,6]. However, it has been postulated lately that mitochondrial K_{ATP} ($mitoK_{ATP}$) channels rather than the $sarcK_{ATP}$ channels are important as end effectors and/or triggers of IPC [7,8].

$SarcK_{ATP}$ channels are hetero-octameric complexes consisting of four pore-forming subunits Kir6.0 (inwardly rectifying potassium channel), and four sulfonylurea receptor subunits (SURs) belonging to the ATP-binding cassette superfamily [1,2]. Two Kir subunits (Kir6.2 and Kir6.1) and three major types of SURs (SUR1, SUR2A, and SUR2B) have been cloned to date [9–13]. It is reported that all subunits of a K_{ATP} channel are expressed in heart at mRNA levels [9,13–15]. Putative association of Kir6.2 with SUR2A was proposed for $sarcK_{ATP}$ channels, based upon electrophysiological and pharmacological characterization of channel activity following co-expression in heterologous expression systems [12,16,17] and Kir6.2 knockout studies in mice [18], but their features of subcellular distribution and molecular assembly at the protein level in hearts are not clear yet. Although Kir6.1 was proposed to be a component of the $mitoK_{ATP}$ channels in skeletal muscle and liver [19], it is controversial [14,20,21] and the molecular entity of the $mitoK_{ATP}$ channels has not been determined.

We investigated subcellular distribution and molecular composition of K_{ATP} channels in rat hearts by immunoblot and immunoprecipitation using several subunit-specific antibodies and by sucrose density gradient sedimentation of the solubilized channel proteins. We have found that all K_{ATP} subunits are located in microsomal fractions but not in mitochondria, and SUR2A forms a heteromultimer with Kir6.2 but not with Kir6.1.

2. Materials and methods

2.1. Antibodies

Rabbit polyclonal antibodies were generated against synthetic peptides corresponding to particular sequences of the rat K_{ATP} channel subunits [9,11–13]: anti-Kir6.1 (near C-terminus), NNSSLMVPKVQFMTPEGNQCG; anti-Kir6.2 (near N-terminus), RLAEDPTEPRYRTRERRAC; anti-SUR2A (near C-terminus), CNLLQHKNGLFSTLVMTNK; and anti-SUR2B (near C-terminus), CSLLAQEDGVFASFVRADM. Due to the high sequence homology of the SUR2B peptide to that of SUR1 (KLLSQKDSVFASFVRADK), anti-SUR2B was confirmed to recognize SUR1 peptide by enzyme-linked immunosorbent assay. The antibodies were purified using protein A–Sepharose columns. Monoclonal antibodies against rabbit cardiac L-type Ca^{2+} channel α1 subunit (4D6) and ryanodine receptor 2 (R29) were previously prepared and characterized in our laboratory. Anti-cytochrome *c* oxidase subunit IV antibody was obtained from Molecular Probes Inc. (Eugene, OR, USA).

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Abbreviations: IPC, ischemic preconditioning; K_{ATP}, ATP-sensitive potassium channel; Kir, inwardly rectifying potassium channel; SUR, sulfonylurea receptor

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2.2. Cell culture and transfection

Plasmid DNAs containing the mouse (Kir6.2+SUR2A) or (Kir6.1+SUR2B) cDNA were transfected into COS-7 cells using LipofectAMINE 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

2.3. Membrane preparations

Cardiac microsomes [22] and mitochondrial fractions [23] from Wistar rats (6 weeks old, male) were prepared as the procedures in the references indicated above in the presence of five protease inhibitors (0.1 mM phenylmethyl sulfonylfluoride, 1 μg/ml leupeptin, 1 μg/ml pepstatin A, 10 μg/ml soybean trypsin inhibitor, and 0.5 mM iodoacetamide).

2.4. Immunoblot analysis

Proteins were separated on 8.5% SDS–polyacrylamide gel, and electrically transferred to polyvinylidene difluoride (PVDF) membranes. The transferred blots were blocked with 2% skim milk in Tris-buffered saline (TBS, 150 mM NaCl, 20 mM Tris–HCl, pH 7.4) and incubated for 6 h at 4°C with the antibodies in TBS, 0.1% Tween 20. After washing, the blots were reacted with peroxidase-conjugated secondary antibodies (Zymed Laboratories, South San Francisco, CA, USA) for 60 min at room temperature and developed using enhanced chemiluminescent substrates (Pierce, Rockford, IL, USA).

2.5. Immunoprecipitation

Rat cardiac microsomes (2 mg/ml) were solubilized by 1% digitonin in TBS containing five protease inhibitors and clarified by centrifugation at 12 000×*g* at 4°C. The digitonin extracts were diluted with a four-fold volume of TBS, followed by incubation with 10 μg of the antibodies. After the antigen–antibody complex was adsorbed on protein A–Sepharose gel (20 μl), the immunocomplex was separated on 8.5% SDS–polyacrylamide gels, and subjected to immunoblot analysis as described above.

2.6. Sucrose density gradient sedimentation

Digitonin extracts from rat cardiac microsomes were layered onto 5–20% linear sucrose gradients in 0.1% digitonin, TBS, and protease inhibitors. The gradients were centrifuged at 4°C in a Beckman SW28 rotor at 122 000×*g* for 9 h. Gradients were fractionated from top to bottom using automatic liquid charger (Advantec, Tokyo, Japan). Aliquots were subjected to SDS–polyacrylamide gels and immunoblot analysis. Protein markers used were pentameric IgM (950 kDa), thyroglobulin (690 kDa), and catalase (240 kDa). Ryanodine receptor 2 is a homotetramer of 400 kDa subunit (1600 kDa) [24].

3. Results

3.1. Specificity of antibodies

We generated antibodies against peptides corresponding to regions in the N or C terminus of rat K_{ATP} subunits; Kir6.2, Kir6.1, SUR2A, and SUR2B. We first tested whether these antibodies can specifically recognize each subunit using COS-7 cells that transiently express Kir6.2+SUR2A, Kir6.1+SUR2B, and vector alone. Fig. 1 shows that all antibodies recognize K_{ATP} subunit proteins of the predicted molecular weight in immunoblots. Using anti-Kir6.2 antibody, only the 38 kDa band of Kir6.2 was detected in the crude membranes from COS-7 cells expressing Kir6.2+SUR2A (Fig. 1A, lane 1). Anti-Kir6.1 antibody specifically recognized the Kir6.1 protein with apparent molecular mass of 43 kDa (Fig. 1B, lane 2). Anti-SUR2A antibody recognized the SUR2A polypeptide of 140 kDa (Fig. 1C, lane 1), but not SUR2B. Anti-SUR2B antibody recognized 150 kDa polypeptides of SUR2B and possible proteolytic fragments of 100 and 55 kDa (Fig. 1D, lane 2). These results demonstrate that all antibodies are subunit-specific, and can be used for further immunochemical studies.

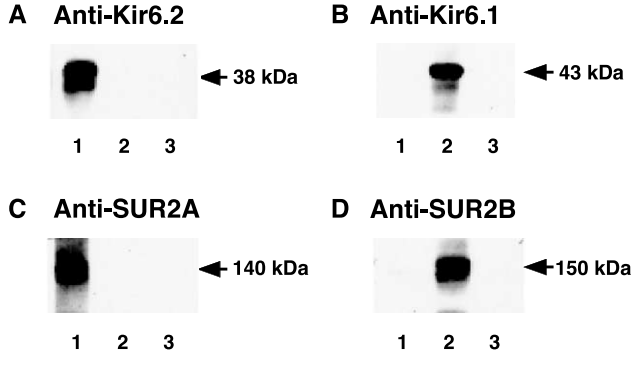


Fig. 1. Specificity of the generated antibodies by immunoblot analysis. Crude membrane fractions (3.0 μg) of COS-7 cells transfected with SUR2A+Kir6.2 (lane 1), SUR2B+Kir6.1 (lane 2), and vector alone (lane 3) were separated on 8.5% SDS–polyacrylamide gel and immunoblotted with each antibody. Protein bands were visualized using enhanced chemiluminescent substrates. The position of each subunit is indicated by an arrow.

3.2. Subcellular distribution of K_{ATP} subunits in native rat hearts

To determine subcellular localization of K_{ATP} subunits, immunoblot analysis was carried out for the microsomes and mitochondrial fractions prepared from rat adult hearts. We first evaluated the purity of both fractions using antibodies against marker proteins: cardiac L-type Ca^{2+} channel α1 subunit for sarcolemmal membranes and cytochrome *c* oxidase subunit IV for mitochondria. Fig. 2A clearly shows that both preparations were well-separated and no cross-contamination was observed. Using these preparations, Kir6.2 was detected in microsomes as 40 kDa protein, the same size as Kir6.2 expressed in COS-7 cells, while it was not detected in mitochondria (Fig. 2B). Kir6.1 was also identified as a 43 kDa band in microsomes, but not in mitochondrial fractions (Fig. 2C). Anti-SUR2A antibody recognized the 150 kDa polypeptide in microsomes. The faint 50 kDa band that was observed both in microsomal and mitochondrial fractions by the antibody (lanes 1 and 2 in Fig. 2D), was obviously nonspecific staining, because it was stained even in the presence of competitive antigen peptide (lanes 4 and 5 in Fig. 2D). Anti-SUR2B detected three polypeptides (150, 100, and 55 kDa) in microsomes, but not in mitochondrial fractions at all (Fig. 2E). The 100 and 55 kDa peptides appear to be proteolytic fragments produced from 150 kDa during membrane preparation. These bands are assigned as SUR2B but can also be assigned as SUR1, since the sequences of antigen peptide of SUR2B are highly homologous to the corresponding region of SUR1. These results indicate that all subunits of K_{ATP} cloned to date are located in microsomal fractions, but none of them exist in mitochondria.

3.3. Immunoprecipitation experiments

To address the questions whether and what type of Kir and SUR assemble, we performed immunoprecipitation experiments of solubilized microsomes with the subunit-specific antibodies (Fig. 3). Anti-SUR2A antibody co-immunoprecipitated SUR2A and Kir6.2. Inversely, anti-Kir6.2 antibody immunoprecipitated Kir6.2 and SUR2A, which are the same subunits as those by anti-SUR2A antibody. By contrast, anti-Kir6.1 antibody only precipitated Kir6.1 itself, and no co-immunoprecipitation with either of SUR2A, SUR2B, and Kir6.2 was observed. These results indicate that Kir6.2 and

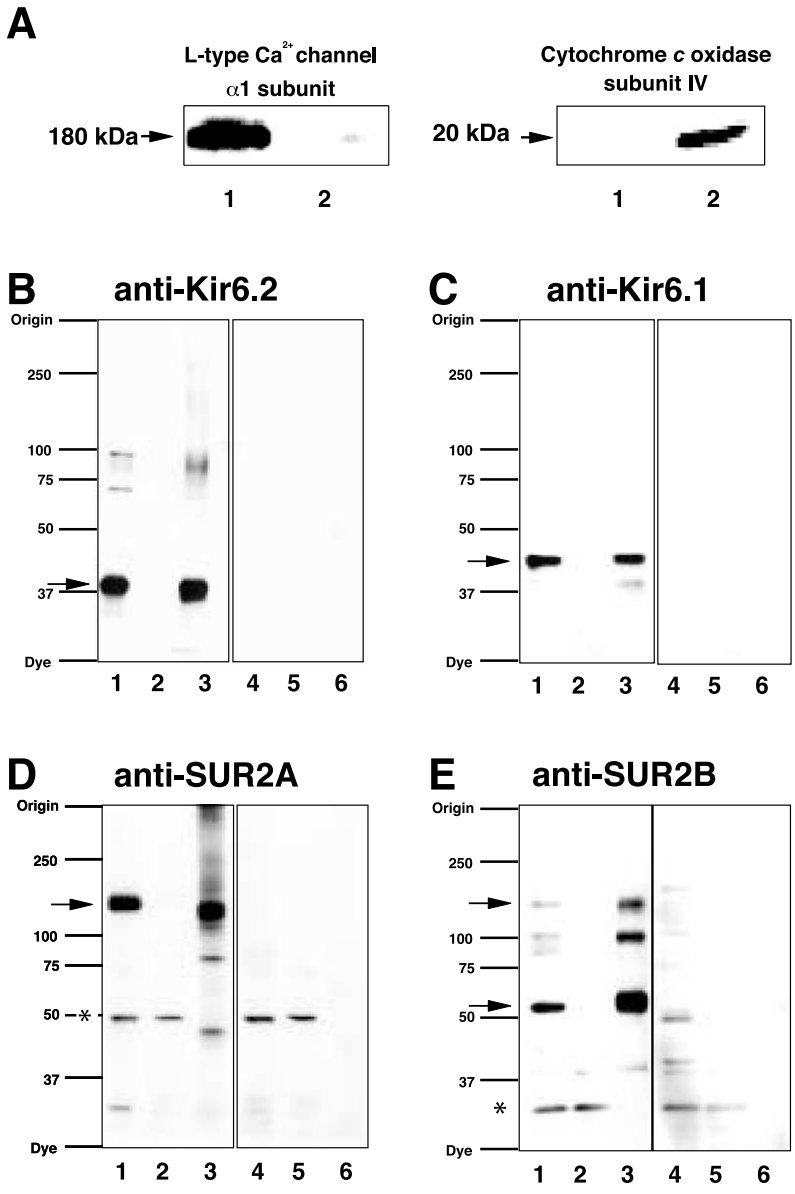


Fig. 2. Immunoblot analysis of K_{ATP} channel subunits in rat hearts. Microsomal (lanes 1 and 4) and mitochondrial fractions (lanes 2 and 5) of rat hearts, and crude membrane fractions of COS-7 cells transfected with SUR2A+Kir6.2 (lanes 3 and 6 in panels B and D) or SUR2B+Kir6.1 (lanes 3 and 6 in panels C and E) were prepared. Samples (15 μg each for rat hearts and 3 μg each for COS-7 cells) were run on 8.5% or 15% SDS–polyacrylamide gels. Electrophoresed proteins were blotted onto PVDF membranes, and probed with antibodies; anti-cardiac Ca^{2+} channel a1 subunit (panel A, left) and anti-cytochrome *c* subunit IV (panel A, right), anti-Kir6.2 (panel B), anti-Kir6.1 (panel C), anti-SUR2A (panel D), and anti-SUR2B (panel E). Specifically blotted bands are indicated by arrows, and non-specific ones that are confirmed by competition in the presence of antigen peptide (lanes 4–6 in panels B–E) are indicated by an asterisk. Molecular weight (kDa) markers are also shown.

Kir6.1 are not present in the same channel complex under these conditions.

3.4. Sucrose density gradient sedimentation

To inspect the subunit assembly in a different method, we performed sucrose density gradient sedimentation analysis of the digitonin-solubilized cardiac microsomes using 5–20% sucrose density gradients. As shown in Fig. 4A, SUR2A exhibited two peaks: one peak at fractions 3 and 4, and the other peak at fractions between 8 and 10. However, Kir6.2 was sedimented as a single peak at fractions between 8 and 10, where SUR2A and Kir6.2 were co-migrated. In sharp contrast to Kir6.2, Kir6.1 migrated at fractions near the top of the gradient (fractions 3 and 4). SUR2B and/or SUR1 were also detected in the same fractions, suggesting that the SUR2B and/or SUR1 do not assemble with Kir6.2 under these conditions. These results indicate that all Kir6.2 co-assemble with SUR2A. The molecular mass of the SUR2A–Kir6.2 complex is estimated at 1150 kDa by comparison with standard proteins (Fig. 4B).

4. Discussion

We have investigated the subcellular distribution of K_{ATP} channels and their subunit assembly in rat hearts using subunit-specific antibodies and revealed the following: (1) Kir6.1, Kir6.2, and SUR2A proteins were abundantly expressed, but SUR2B and/or SUR1 were detected mainly as proteolytic

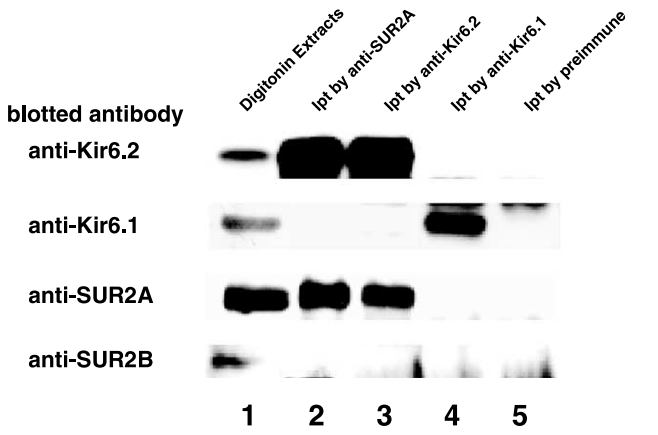
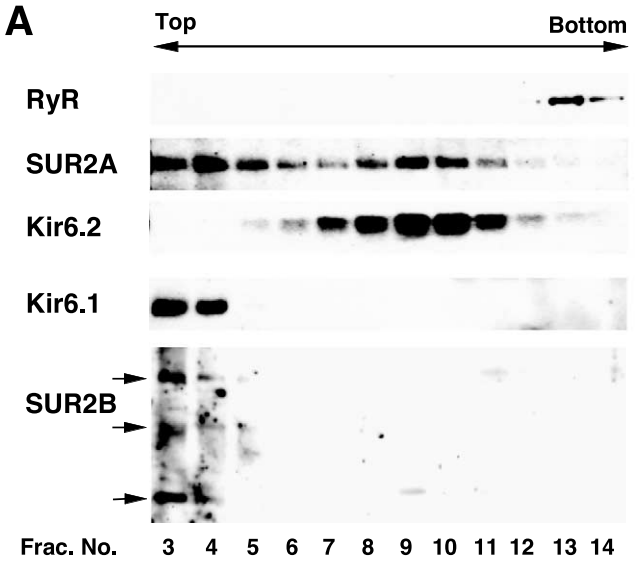


Fig. 3. Immunoprecipitation of the cardiac K_{ATP} channel complex. Immunoprecipitants were separated on 8.5% SDS–polyacrylamide gel and transferred to PVDF membranes. Digitonin extracts from microsomes (lane 1) were used as positive controls. Immunoprecipitated samples from the digitonin extracts by anti-SUR2A (lane 2), anti-Kir6.2 (lane 3), anti-Kir6.1 (lane 4), and control antibody (lane 5) were analyzed. The blots were probed with the indicated antibodies and visualized by enhanced chemiluminescence.



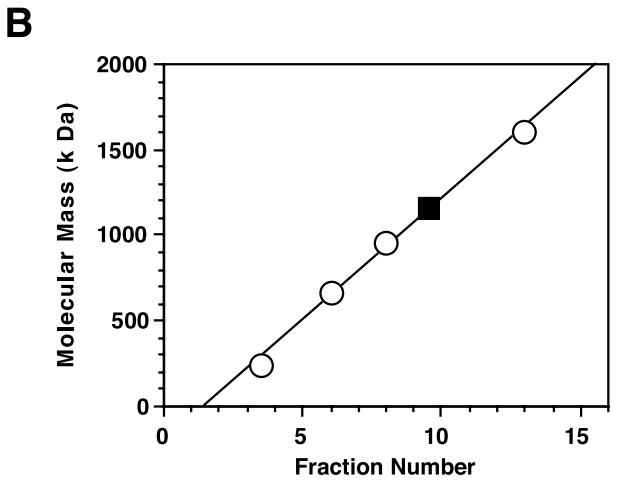


Fig. 4. Sucrose density gradient sedimentation of the K_{ATP} channel complex from rat hearts. A: Digitonin extracts were sedimented on 5–20% sucrose density gradients. Fractionated proteins were analyzed on 8.5% SDS–polyacrylamide gels, followed by immunoblotting. In blots of SUR2B, three polypeptide bands (150, 100, and 55 kDa, indicated by arrows) were detected similarly as in Fig. 2E. B: Molecular mass of the SUR2A–Kir6.2 complex (filled square) was estimated by comparing with standard protein markers (open circle); pentameric IgM (950 kDa), thyroglobulin (690 kDa), catalase (240 kDa), and ryanodine receptor 2 (1600 kDa of homotetramer [24]).

fragments. (2) All of the subunits were exclusively localized in the microsomal fractions, but not detected in the mitochondrial ones at all. (3) SUR2A tightly associated with Kir6.2, but not with Kir6.1. Immunoblot data clearly showed that all of the K_{ATP} subunits tested were located in microsome fractions containing sarcolemmal membranes. In contrast, there was no indication that K_{ATP} channels exist in the highly purified mitochondrial fractions. Several precedent studies indicated that mitochondrial K_{ATP} channels (mitoK_{ATP} channels), rather than sarcolemmal K_{ATP} channels, play important roles for ischemic preconditioning and pharmacological cardioprotection as the target for K^+ channel openers (KCOs). Garlid et al. reported that the K_{ATP} channel from bovine mitochondrial inner membranes was purified and reconstituted [8], although its primary structure has not been reported yet. Previous immunohistochemical studies also suggested that Kir6.1 might be a component of the mitoK_{ATP} channels in skeletal muscle [19], but it remained controversial [14,20,21]. Our present data have brought a conclusion in this issue that none of the K_{ATP} channel subunits cloned so far confer mitoK_{ATP} channels in rat hearts.

The conclusion raises a new question, namely what the target molecule for KCOs is. Based on our data, the mitoK_{ATP} channel, if it exists, might be composed differently from the K_{ATP} channel subunits cloned so far. Alternatively, it is more likely that the target molecule is an enzyme related to mitochondrial energy metabolism, such as succinate dehydrogenase that is inhibited by KCOs [25]. Recent reports that diazoxide directly regulates the mitochondrial oxidation–reduction [26] and reduces mitochondrial oxidant stress at the reoxygenation process [27], supports this possibility.

In the present study of immunoprecipitation and sucrose sedimentation, we demonstrated that Kir6.2 is tightly and exclusively associated with SUR2A, even after digitonin solubilization. Therefore, the cardiac K_{ATP} channel is composed of Kir6.2 and SUR2A proteins at least, but no combination of Kir6.2 and SUR2B/1 exists. The finding was consistent with the results obtained by dominant negative gene transfer [28] and Kir6.2 knockout mice [14,18]. Kir6.2 and SUR2A form a complex of apparent M_r 1150 kDa estimated by the sucrose sedimentation. It is of note that the estimated value of M_r is much larger than the calculated one (760 kDa) for the octameric complex of $(SUR2A)_4$–$(Kir6.1)_4$. The difference may be explained by the possibility that the solubilized channel forms a large micellar complex with detergent digitonin. Another possibility, that the octameric complex forms a higher ordered complex with lactose dehydrogenase like a sarcolemmal K_{ATP} channel reported recently [29], is not excluded.

In addition to the complex form of Kir6.2–SUR2A, we observed unassembled SUR2A, Kir6.1 and SUR2B at the

top of the sucrose gradients. Expression of Kir6.1 protein in chick heart was reported [30], but which type of SUR subunit is coupled with Kir6.1 remains unrevealed. The possibility of octameric complex formation between Kir6.1 and SUR2A in rat hearts can be excluded by the results of recombinant subunits expression [16,17,31] and more recent results using Kir6.2 knockout mice [18]. In this context, the unassembled SUR2A is likely a 'spare subunit' that is stored in the endoplasmic reticulum (ER) until co-trafficking with newly synthesized Kir6.2 to the plasma membrane where they form a functional K_{ATP} channel [33], since ER was not eliminated from the microsomal fractions used here. Stability of the complex between Kir6.0 and SUR seems sensitive to detergent, because we could not detect the Kir6.2–SUR2A complex when Triton X-100 was used (data not shown). Therefore, potential formation of hybrid channels between SUR2A and Kir6.2/6.1 [34–37] might not be excluded by the present results, only if the Kir6.1 dissociates from the hybrid channel during digitonin extraction. It is rather likely that Kir6.1 and SUR2B (or SUR1) dissociate from their complex during digitonin extraction (see Fig. 4A). Even in that case, the possibility that the complex confers a functional cardiac K_{ATP} channel may be negligible, based on the previous reports by co-expression of the recombinant components [32] and Kir6.1 knockout mice [14]. These reports also indicated that Kir6.1 and SUR2B comprise the vascular type K_{ATP} channel. Therefore, the possibility that the SUR2B and Kir6.1 we observed may originate from vascular tissues in our microsomal fractions is not excluded. Unfortunately, our antibodies have no ability to immunostain the concerned tissues; this issue remains to be solved. Compared with the stable complex between Kir6.2 and SUR2A after digitonin solubilization, unassembled subunits are still needed to investigate whether and what counterparts they assemble with.

In summary, we propose that the observed complex of Kir6.2 with SUR2A, which is possibly octameric and exclusively located in microsomal fractions, is a strong candidate for the cardiac K_{ATP} channel. Further study is required to clarify the role of Kir6.1 and SUR2B/1 as to what extent.

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References

[1] Aguilar-Bryan, L., Clement IV, J.P., González, G., Kunjilwar, K., Babenko, A. and Bryan, J. (1998) Physiol. Rev. 78, 227–245.
[2] Seino, S. (1999) Annu. Rev. Physiol. 61, 337–362.
[3] Noma, A. (1983) Nature 305, 147–148.
[4] Inoue, I., Nagase, H., Kishi, K. and Higuti, T. (1991) Nature 352, 244–247.
[5] Gross, G.J. and Auchampach, J.A. (1992) Circ. Res. 70, 223–233.
[6] Mizunuma, T., Nithipatikom, K. and Gross, G.J. (1995) Circulation 92, 1236–1245.
[7] Sato, T., Sasaki, N., Seharaseyon, J., O'Rourke, B. and Marbán, E. (2000) Circulation 101, 2418–2423.
[8] Garlid, K.D., Paucek, P., Yarov-Yarovoy, V., Murray, H.N., Dabenzio, R.B., D'Alonzo, A.J., Smith, M.A. and Grover, G.J. (1997) Circ. Res. 81, 1072–1082.
[9] Inagaki, N., Tsuura, Y., Namba, N., Masuda, K., Gonoi, T., Horie, M., Seino, Y., Mizuta, M. and Seino, S. (1995) J. Biol. Chem. 270, 5691–5694.
[10] Aguilar-Bryan, L., Nichols, C.G., Wechsler, S.W., Clement IV, J.P., Boyd III, A.E., González, G., Herra-Sosa, H., Nguy, K., Bryan, J. and Nelson, D.A. (1995) Science 268, 423–426.
[11] Inagaki, N., Gonoi, T., Clement IV, J.P., Namba, N., Inazawa, J., González, G., Aguilar-Bryan, L., Seino, S. and Bryan, J. (1995) Science 270, 1166–1170.
[12] Inagaki, N., Gonoi, T., Clement IV, J.P., Wang, C.Z., Aguilar-Bryan, L., Bryan, J. and Seino, S. (1996) Neuron 16, 1011–1017.
[13] Isomoto, S., Kondo, C., Yamada, M., Matsumoto, S., Higashiguchi, O., Horio, Y., Matsuzawa, Y. and Kurachi, Y. (1996) J. Biol. Chem. 271, 24321–24324.
[14] Miki, T., Suzuki, M., Shibasaki, T., Uemura, H., Sato, T., Yamaguchi, K., Koseki, H., Iwanaga, T., Nakaya, H. and Seino, S. (2002) Nat. Med. 8, 466–472.
[15] Akao, M., Otani, H., Horie, M., Takano, M., Kuniyasu, A., Nakayama, H., Kouchi, I., Murakami, T. and Sasayama, S. (1997) J. Clin. Invest. 100, 3053–3059.
[16] Babenko, A.P., González, G., Aguilar-Bryan, L. and Bryan, J. (1998) Circ. Res. 83, 1132–1143.
[17] Lorenz, E. and Terzic, A. (1999) J. Mol. Cell Cardiol. 31, 425–434.
[18] Suzuki, M., Li, R.A., Miki, T., Uemura, H., Sakamoto, N., Ohmoto-Sekine, Y., Tamagawa, M., Ogura, T., Seino, S., Marbán, E. and Nakaya, H. (2001) Circ. Res. 88, 570–577.
[19] Suzuki, M., Kotake, K., Fujikura, K., Inagaki, N., Suzuki, T., Gonoi, T., Seino, S. and Takata, K. (1997) Biochem. Biophys. Res. Commun. 24, 1693–1697.
[20] Seharaseyon, J., Ohler, A., Sasaki, N., Fraser, H., Sato, T., Johns, D.C., O'Rourke, B. and Marbán, E. (2000) J. Mol. Cell Cardiol. 32, 1923–1930.
[21] Thomzig, A., Wenzel, M., Karschin, C., Eaton, M.J., Skatchkov, S.N., Karschin, A. and Veh, R.W. (2001) Mol. Cell. Neurosci. 18, 671–690.
[22] Jones, L.R., Besch Jr., H.R., Fleming, J.W., McConnaughey, M.M. and Watanabe, A.M. (1979) J. Biol. Chem. 254, 530–539.
[23] Holmuhamedov, E.L., Jovanovic, S., Dzeja, P.P., Jovanovic, A. and Terzic, A. (1998) Am. J. Physiol. 275, H1567–1576.
[24] Anderson, K., Lai, F.A., Liu, Q.Y., Rousseau, E., Erickson, H.P. and Meissner, G. (1989) J. Biol. Chem. 264, 1329–1335.
[25] Schafer, G., Portenhauser, R. and Trolp, R. (1971) Biochem. Pharmacol. 20, 1271–1280.
[26] Ozcan, C., Bienengraeber, M., Dzeja, P.P. and Terzic, A. (2002) Am. J. Physiol. Heart Circ. Physiol. 282, H531–539.
[27] Pain, T., Yang, X.M., Critz, S.D., Yue, Y., Nakano, A., Liu, G.S., Heusch, G., Cohen, M.V. and Downey, J.M. (2000) Circ. Res. 87, 460–466.
[28] Seharaseyon, J., Sasaki, N., Ohler, A., Sato, T., Fraser, H., Johns, D.C., O'Rourke, B. and Marbán, E. (2000) J. Biol. Chem. 275, 17561–17565.
[29] Crawford, R.M., Budas, G.R., Jovanovic, S., Ranki, H.J., Wilson, T.J., Davies, A.M. and Jovanovic, A. (2002) EMBO J. 21, 3936–3948.
[30] Lu, C. and Halvorsen, S.W. (1997) FEBS Lett. 412, 121–125.
[31] Okuyama, Y., Yamada, M., Kondo, C., Satoh, E., Isomoto, S., Shindo, T., Horio, Y., Kitakaze, M., Hori, M. and Kurachi, Y. (1998) Pfluegers Arch. 435, 595–603.
[32] Russ, U., Hambrock, A., Artunc, F., Loffler-Walz, C., Horio, Y., Kurachi, Y. and Quast, U. (1999) Mol. Pharmacol. 56, 955–961.
[33] Zerangue, N., Schwappach, B., Jan, Y.N. and Jan, L.Y. (1999) Neuron 22, 537–548.
[34] Kono, Y., Horie, M., Takano, M., Otani, H., Xie, L.H., Akao, M., Tsuji, K. and Sasayama, S. (2000) Pfluegers Arch. 440, 692–698.
[35] Pountney, D.J., Sun, Z.Q., Porter, L.M., Nitabach, M.N., Nakamura, T.Y., Holmes, D., Rosner, E., Kaneko, M., Manaris, T., Holmes, T.C. and Coetzee, W.A. (2001) J. Mol. Cell Cardiol. 33, 1541–1546.
[36] Babenko, A.P. and Bryan, J. (2001) J. Biol. Chem. 276, 49083–49092.
[37] Cui, Y., Giblin, J.P., Clapp, L.H. and Tinker, A. (2002) Proc. Natl. Acad. Sci. USA 98, 729–734.