成熟心筋細胞 ATP 放出性マキシアニオンチャネルは T 管開口部に局在する

細胞器官研究系　機能協関研究部門

大型のポアを持つマキシアニオンチャネルは、細胞内から細胞外に ATP を放出する通路を与える。新生仔ラット心筋細胞は虚血条件下でこのチャネルが活性化されて、ATP を放出することを既に私達は報告している。ところが成熟ラット心筋細胞は、同様に虚血性 ATP 放出を示すにもかかわらず、マキシアニオンチャネルを発現していないと報告されて来た。今回私達は、走査イオンコンダクタンス顕微鏡法による微細構造観察下においてパッチクランプ単一チャネル記録を行うという「スマートパッチ法」を適用し、マキシアニオンチャネルがT管開口部とその付近の乙帯線上という大変狭い領域にのみ局在することをはじめて明らかにした。これによって長年にわたる矛盾が解決し、成熟心筋細胞の虚血性 ATP 放出にもマキシアニオンチャネルが関与することが明らかとなった。

Dutta AK, Korchev YE, Shevchuk AI, Hayashi S, Okada Y, Sabirov RZ.
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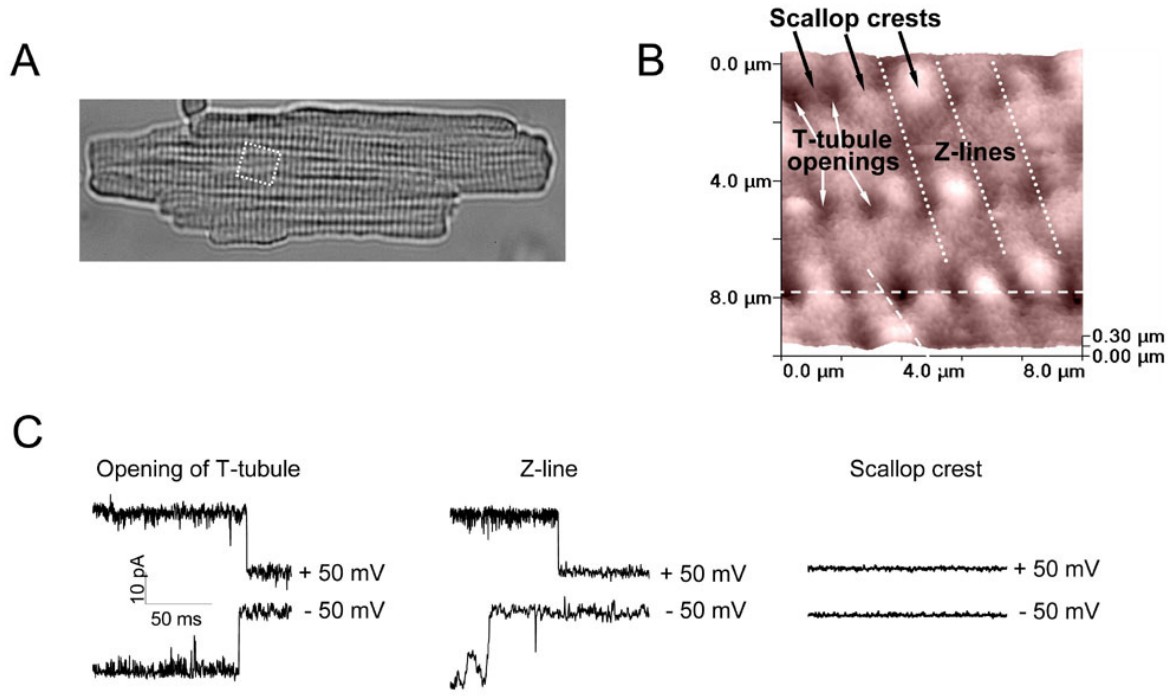

Cutting-edge Smart-patch technique reveals ATP-conductive maxi-anion channel in a hidden space of the T-system in cardiac myocytes.

We have recently determined that the maxi-anion channel is a nanoscopic pore (1) well suited to function as an ATP-releasing pathway (2, 3). Studying cardiac cells, we have demonstrated that the ATP release from neonatal rat cardiomyocytes is mainly mediated by activity of the maxi-anion channel under ischemic or hypotonic conditions (4). Paradoxically, these channels could not be observed in cardiomyocytes freshly isolated from adult hearts with commonly used patch-clamp conditions (4, 5). It was therefore suggested that maxi-anion channels are only

transiently expressed in neonatal cells, disappearing upon maturation (5). However, ATP release from mature cardiomyocytes and purinergic signalling in the normal and diseased heart are well-recognized phenomena. We thus hypothesized that the difference in maxi-anion channel activity between neonatal and adult cells could be related to different pattern of spatial distribution of the maxi-anion channels over the surface of sarcolemma. In the present study (6), the spatial distribution of maxi-anion channels and that of ATP release site were first studied in neonatal rat cardiomyocytes by using a recently developed "smart-patch" method and an ATP biosensor technique, respectively. Both distributions matched with each other providing further evidence for the "maxi-anion channel = ATP-conductive channel" concept. We then reexamined functional expression of maxi-anion channels in adult rat cardiomyocytes by the "smart-patch" technique using very fine-tipped patch pipettes. When fine-tipped 15-20 MΩ pipettes were targeted to only Z-line areas, we observed, for the first time, the maxi-anion events (Fig. 1). Smart-patching different regions of the cell surface, we found that the channel activity was maximal at the openings of T-tubules and along Z-lines, but was significantly decreased in the scallop crest area. Thus, it is concluded that maxi-anion channels are concentrated at the openings of T-tubules and along Z-lines in adult cardiomyocytes. Our study showed that the "smart-patch" technique provides a powerful method to detect a unitary event of channels which are localized at some specific site in the narrow region.

References

1. Sabirov, R.Z., and Y. Okada. 2004. Wide nanoscopic pore of Maxi-anion channel suits its function as an ATP-conductive pathway. *Biophys. J.* 87: 1672-1685.
2. Sabirov, R.Z., and Y. Okada. 2005. ATP release via anion Channels. *Purinergic Signalling* 1: 311-328.
3. Sabirov, R.Z. and Y. Okada. 2004. ATP-conductive maxi-anion channel: A new player in stress-sensory transduction. *Jpn. J. Physiol.* 54: 7-14.
4. Dutta, A.K., R.Z. Sabirov, H. Uramoto, and Y. Okada. 2004. Role of ATP-conductive anion channel in ATP release from neonatal rat cardiomyocytes in ischaemic or hypoxic conditions. *J. Physiol.* 559: 799-812.
5. Coulombe, A., and E. Coraboeuf. 1992. Large-conductance chloride channels of new-born rat cardiac myocytes are activated by hypotonic media. *Pflugers Arch.* 422: 143-150.
6. Dutta A.K., Y.E. Korchev, A.I. Shevchuk, S. Hayashi, Y. Okada and R.Z. Sabirov. 2008. Spatial Distribution of Maxi-anion Channel on Cardiomyocytes Detected by Smart-Patch Technique *Biophys. J.* 94: 1646-1655

Legend

Specifically localized maxi-anion channel activity in freshly isolated adult rat cardiomyocytes. (*A*) Optical image of freshly isolated adult rat cardiomyocytes. (*B*) Representative topographic image of a small area (white rectangle in *A*) on the surface of rat cardiomyocyte obtained using scanning ion conductance microscopy (SICM) with a fine nanopipette (90 MΩ, when filled with pipette solution). Z-grooves, T-tubule openings, and scallop crests are indicated. (C) Maxi-anion channel activity in patches excised from different zones of freshly isolated rat cardiomyocytes using the "smart-patch" technique. Representative single-channel events recorded at +50 mV (*upper traces*) and –50 mV (*lower traces*) in the opening of a T-tubule, in the Z-line, and on the scallop crest are shown.