Structure analysis of K_{ATP} channel

ATP mediated trapping of polyamine analogs in Kir6.2 channels

Ion channel: K_{ATP}

Cell type: COS7

Chip type: DF-16

Data courtesy of C. Nichols & H. T. Kurata, Dep. of Cell Biology & Physiology, Washington University, St. Louis, MO

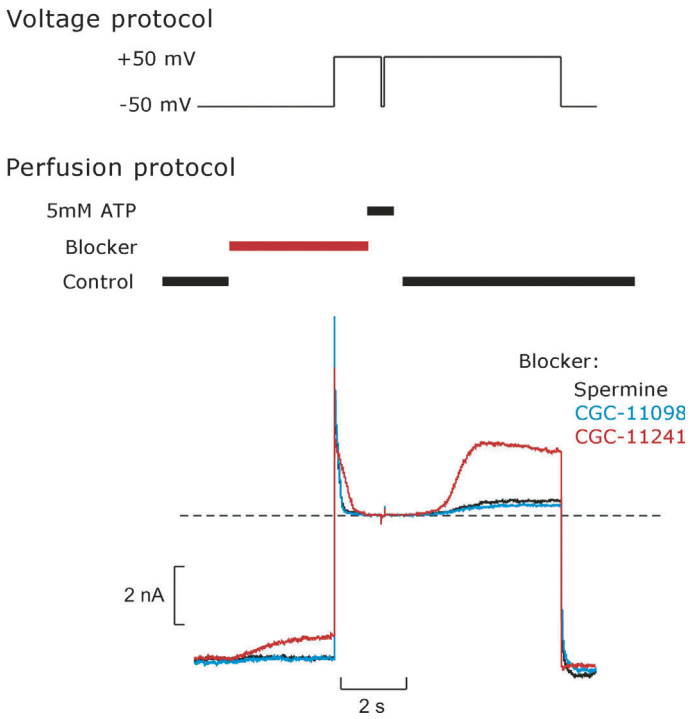
Methods

Binding of ATP to the pore-forming subunits of the K_{ATP} channel ($K_{ir}6.2$) induces a conformational change that results in channel closure. To show that ATP-dependent channel closure can trap polyamines in the channel pore $K_{ir}6.2$ subunits have been modified to exhibit potent block by polyamines. The purpose of this study was to use spermine and various polyamine analogues of different length as molecular calipers to measure the distance between the spermine binding site and the ATP-operated gate. By combining voltage and perfusion protocols it was possible to test the hypothesis that progressively longer polyamines will eventually impede closure of the ATP-operated gate in K_{ATP} channels and thereby abolish any ATP-dependent trapping of the polyamine.

Voltage and compound application protocol

Inside-out patches were excised from COS7 cells transiently transfected with $K_{ir}6.2$ [N160E] and SUR1. The voltage and perfusion protocols are outlined in **Figure 1**. Cells were initially exposed to polyamine-free intracellular buffer at a holding potential of -50 mV. A polyamine blocker was then applied to the cytoplasmic side of the patch. A voltage step to +50 mV was then applied to initiate the rapid onset of a voltage-dependent block of channels by the polyamine. While held at +50 mV, patches were exposed to a bathing solution containing 5 mM ATP (polyamine-free) to close the ATP-operated gate of the $K_{ir}6.2$ channel. While in the 5 mM ATP solution, a 300 ms voltage pulse to –50 mV was applied to dislodge polyamines, followed by a return to +50 mV and control buffer.

Figure 1



Localization of ATP binding site

The current magnitude at the final stage of the protocol reflects the extent of polyamine unblock during the pulse to –50 mV. Persisting polyamine block (small current) in this step suggests that the applied polyamine is not dislodged by the -50 mV voltage pulse, and demonstrates that the polyamine is trapped by ATP-mediated closure of the channel pore. Alternatively, a large current in this step suggests that ATP-mediated channel closure can not trapp the polyamine.

In the data presented in Fig 1, three different polyamine blockers were applied to the patch; Spermine, CGC-11241 and CGC-11098. Spermine and CGC-11098 are shorter polyamines (blue and black traces) and are not dislodged by the voltage pulses to –50 mV and are thereby assumed to be trapped in the pore by the ATP-mediated closure. In contrast, block by the longer polyamine analogue CGC-11241 is significantly relieved by the voltage pulse to –50 mV, demonstrating that the ATP-operated gate is unable to trap this blocker in the channel pore.

The Dynaflow system and DF-16 chips were used for this study and resulted in a significant increase in experimental efficiency and throughput. Precision timing of solution exchange events allowed for reliable analysis of the gating kinetics and the design of the chip allowed for the analysis of several polyamines in the same experimental set-up.

References

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