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Electrophysiological studies V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.v68e9hw

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EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Kusunoki M, Hayashi M, Shoji T, Uba T, Tanaka H, Sumi C, Matsuo Y, Hirota K, Propofol inhibits stromatoxin-1-sensitive voltage-dependent K channels in pancreatic β-cells and enhances insulin secretion. PeerJ doi: 10.7717/peerj.8157

BEFORE STARTING

- Extracellular bath solution

For patch-clamp experiments, extracellular solutions consisted of Krebs-Ringer bicarbonate HEPES (KRBH) buffer (140 mM NaCl, 3.6 mM KCl, 0.5 mM NaH_2PO_4, 0.5 mM $MgSO_4$, 1.5 mM $CaCl_2$, 2 mM $NaHCO_3$, 10 mM HEPES, and 0.1% bovine serum albumin) containing 2 mM glucose; pH was adjusted to 7.4 with NaOH.

- Gramicidin D

In gramicidin-perforated patch experiments, gramicidin D (Sigma-Aldrich) was dissolved in DMSO at 20 mg/ml and then diluted to a final concentration of 0.1 mg/ml in a standard KCl-rich pipette solution containing 150 mM KCl and 10 mM HEPES; pH was adjusted to 7.4 with KOH.

- Pipette solution

In whole-cell current recording, the standard K^+ pipette solution used contained 60 mM potassium aspartate, 65 mM KCl, 1 mM KH_2PO_4, 5 mM ethylenediaminetetraacetic acid, 3 mM K_2ATP, and 5 mM HEPES; pH was adjusted to 7.4 with KOH.

- Experimental condition

Experiments were conducted at 23–30 °C.

- References

Hayashi M. et al. 2016, Pflugers Arch 468:1171-81. doi: 10.1007/s00424-016-1806-9
Zhang Y. et al. 2016, Neurosci Lett 616:93-7. doi: 10.1016/j.neulet.2016.01.058
MacDonald PE. et al. 2002, J Biol Chem 277:44938-45. doi: 10.1074/jbc.M205532200

1 MIN6 cells were incubated in an extracellular bath solution containing 2 mM glucose for 30 min at 37 °C before patch-clamp experiments.

2 Membrane potential and whole-cell current were recorded using the EPC 800 patch-clamp amplifier (HEKA Elektronik Inc. Holliston, MA, USA).

- The amplifier was driven by Clampex 9 (Axon Instruments, Union City, CA, USA) to allow the delivery of a voltage-step protocol with concomitant digitization of the current. The capacitance transient current was compensated by the amplifier.
- Patch pipettes (G-1.5; Narishige, Tokyo, Japan) had a resistance of 5–7 MΩ when filled with the pipette solutions.
- The membrane potential was corrected for the liquid junction potential at the tip of the patch pipette in the KRBH buffer and for that at the tip of the indifferent reference electrode filled with KRBH buffer and placed in the bath.
- Whole-cell capacitance and series resistance (Rs) were 7.4 ± 1.5 pF and 13.3 ± 1.3 MΩ (n = 6), respectively.
- The Rs was not electronically compensated during the experiments, and the potentials reported here have not been corrected for the Rs.

3 The whole-cell current was filtered at 1 kHz with an internal four-pole Bessel filter, sampled at 2 kHz, and transferred to digital signals through Digidata 1322A (Axon Instruments).

4 A subsequent current analysis was performed using Clampfit 9 (Axon Instruments).