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Application Note

Channel: CFTR

Cells: BHK

Tools: Patchliner©

Effect of internal F^- on activation of Cystic Fibrosis Transmembrane Conductance (CFTR) regulator by forskolin

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Summary

The aim of this study was to investigate the effect of internal F^- on the activation of the cystic fibrosis transmembrane conductance regulator (CFTR) by forskolin in the whole cell patch clamp configuration. Experiments were conducted with Nanion's fully automated patch clamp device, the Patchliner©.

The CFTR is a phosphorylation regulated Cl^- channel. In order for the channel to be active it needs to be phosphorylated. Subsequent binding of ATP to intracellular nucleotide binding sites controls the opening and closing of the channel (Zhou *et al.*, 2006).

Our standard internal solutions contain F^-. Regulation of many ion channels does not seem to be affected by the presence of F^- in the intracellular solution, however, that is not true for every ion channel. F^-, for example, has been shown to activate adenylate cyclase (Eckstein *et al.*, 1979, Insel and Ostrom, 2003). Forskolin – a commonly used CFTR activator – is known to activate CFTR through activation of adenylate cyclase. This leads to phosphorylation (and hence activation) of CFTR by protein kinase A (PKA). It would be interesting to see if F^- can be used as a substitute for forskolin in its role as a CFTR activator.

In this report we summarize some preliminary measurements of CFTR currents in the whole cell patch clamp configuration with the Patchliner© to obtain insight into the effects of F^- on CFTR currents.

Results

Characterization of untransfected BHK cells indicates that the cells express a voltage activated Na^+ channel (*Figure 1A & B*) and, if at all, only very small K^+ currents. To test for forskolin-activated currents data right after establishing the whole cell configuration (*control*), after a 5 min control period (*5 min*) and after 5 min exposure to 2 µM forskolin (*fsk*) were compared. There was no increase in current observed, neither in standard external Nanion solution (*Na/K*) nor in Na^+-free (TEA/Cs) extermal solution.

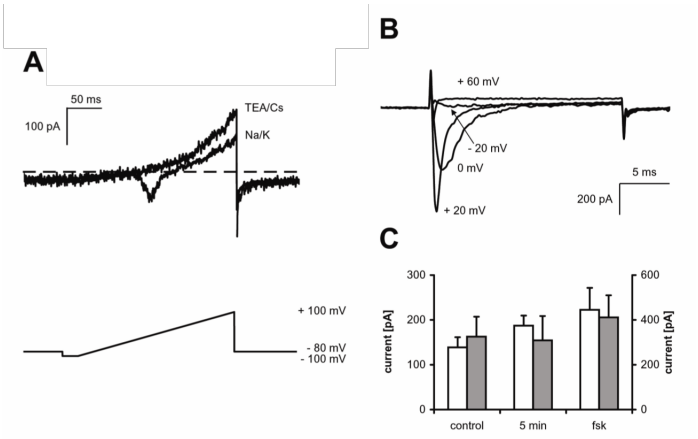

Figure 1: *Untransfected BHK cells have Na^+-currents but no forskolin regulated currents. (A) Current responses to a voltage ramp in standard Nanion solutions (Na/K) and in Na/K-free (TEA/Cs) solutions. (B) Na^+-IV of cell shown in (A, Na/K). (C) Mean currents at +95 mV for cells recorded under Na/K conditions (open bars, n = 4) and TEA/Cs conditions (grey bars, n = 4).*

Seal resistances of transiently transfected BHK cells (CFTR) determined right after establishing the whole cell configuration differed significanlty between cells recorded with internal F^- (150 MΩ ± 27 MΩ, n = 12) to cells recorded without F^- (650 MΩ ± 257 MΩ, n = 3). This difference is also reflected in the current responses to the very first voltage ramp (Figure 2A). Since this difference in seal resistance was not observed for untransfected cells (not shown) it seems unlikely to be a true difference in seal resistance but more likely due to increased activitly of the CFTR - a channel which is not voltage-activated.
F^- might be activating CFTR via the PKA pathway. If this is the case forskolin should not have any further effect on the F^--activated current since it might activate CFTR through the very same pathway.
As seen in Figure 2 currents in the absence of internal F^- (Cl^-) are stable during the 5 min control period. Exposure to 2 μM forskolin strongly activates the channels. In the presence of internal F^- currents increase significantly during the 5 min control period but 2 μM forskolin has no further activating effect.
Our results suggest that internal F^- activates the PKA pathway. This raises the possibility of replacing expensive activator forskolin with internal F^- for CFTR studies. To confirm this will require further investigation.

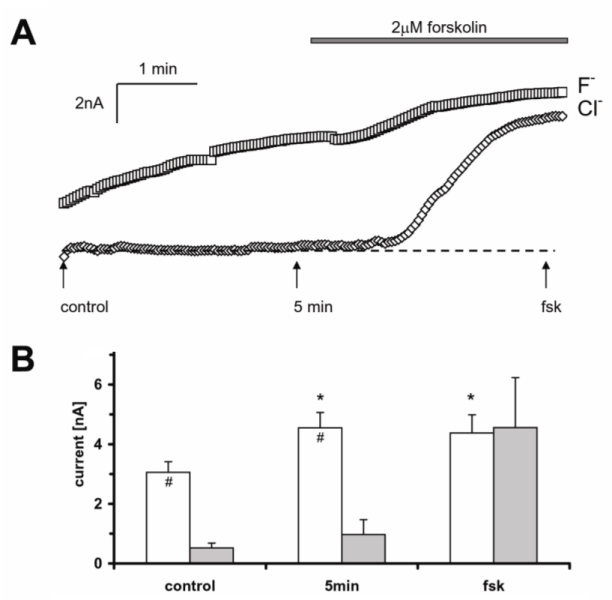

Figure 2: *Forskolin does not activate CFTR in the presence of internal F^-.* (A) Time course of currents with (F^-) and without (Cl^-) internal F^- during 5 min control period and 5 min exposure to 2 μM forskolin. (B) Average data taken at points in time indicated by arrows in (A). White bars F^-; Gray bars Cl^-.

References

1. Zhou, Z., Wang, X., Liu, H., Zou, X., Li, M. Hwang, T. 2006. The two ATP binding sites of cystic fibrosis transmembrane conductance regulator (CFTR) play distinct roles in the gating kinetics and energetics. *J. Gen. Physiol.* 128(4): 413-422.

2. Ecksteine, F., Cassel, D., Levkovitz, H., Lowe, M., Selinger, Z. 1979. Guanosine 5'-O-(2-Thiodiphosphate) An inhibitor of adenylated cyclase stimulation by guanine nucleotides and fluoride ions. *J. Biol. Chem.* 254(10): 9829-1834.

3. Insel, P.A. and Ostrom, R.S. 2003. Forskolin as a Tool for Examining Adenylyl Cyclase Expression, Regulation, and G Protein. *Signaling Cellular and Molecular Neurobiology*. 23(3): 305-314.

Methods

Cells
Baby hamster kidney cells (BHK) expressing *wt* CFTR or untransfected BHK.

Cell culture
Cells were culture and harvested according to our standard cell culture protocol.

Electrophysiology
Whole cell patch clamp recordings were conducted according to Nanion's standard procedure with the 2- or 4- channel Patchliner©. Solutions used for the recordings were either Nanion's standard solutions (internal solution with and without F^-) or a *Na^+/K^+ free* external/internal set of solutions (in mM: 145 TEACl, 10 $CaCl_2$, 10 HEPES, pH 7.4 (external); 70 CsF, 70 CsAsp, 5 $MgCl_2$, 10 CsEGTA, 10 HEPES, pH 7.4 (internal)).
In all experiments cells were kept at a holding potential of -80 mV. Every 5 s a voltage ramp (100 mV to +100 mV) was applied and the current response to these voltage ramps was recorded. For comparison data were determined in the 95 mV ± 5mV inteval.

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