Supplemental Material

TRPM2 is modulated by cellular acidification

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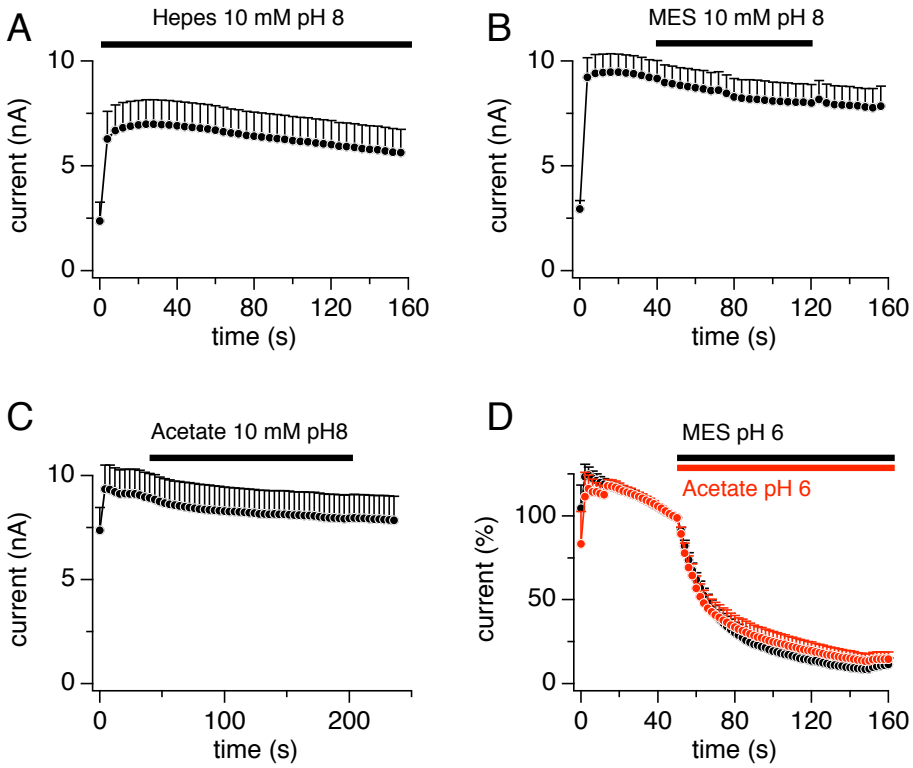


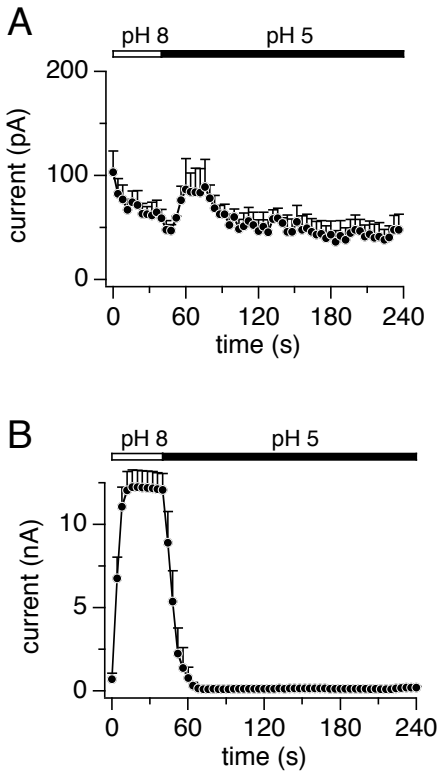
Supplemental Figure S1: pH buffers do not inhibit TRPM2. Development of TRPM2 currents expressed in HEK293-TRPM2 cells kept in $NMDG^+$-based external solution at pH 8 (see methods). Internal solution was standard K^+-glutamate solution (see methods). Application of the respective buffer is indicated by the bars. In the case of MES and Acetate, HEPES buffer was applied before and after their application. Data were acquired as in Fig. 1A and analyzed as in Fig. 5A.

(A) HEPES buffer with a pH range of 6.8 – 8.2 showed no effects on TRPM2, n = 9.

(B) MES buffer with a pH range of 5.5 – 6.7, was primarily used for the pH 6 experiments and demonstrated no effects on the TRPM2, n = 5.

(C) Acetate buffer with a pH range of 3.7 – 5.6, was primarily used for pH 5 experiments and demonstrated no effects on TRPM2, n = 7.

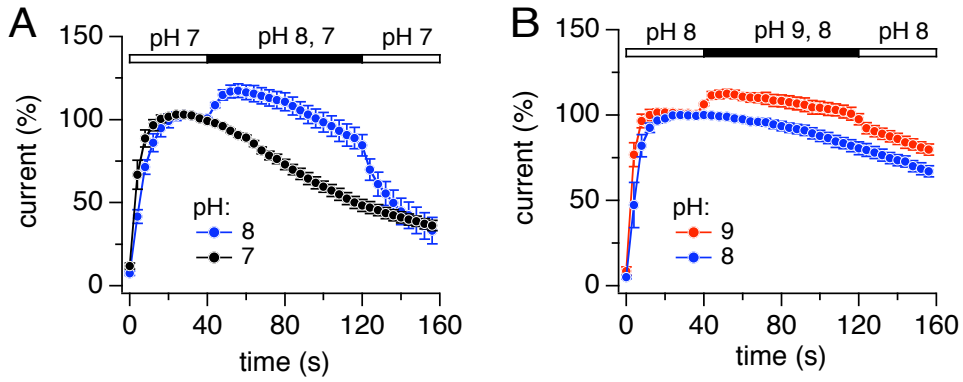
(D) Comparison of MES and acetate buffers at pH 6, demonstrating the specificity of inactivation by pH independent of the buffer species used.



Supplemental Figure S2: Extracellular acidification does not induce significant acid-sensitive chloride currents in HEK29-TRPM2 cells.

(A) Average current response to extracellular acidification in HEK293-TRPM2 cells kept in NMDG+-based solution supplemented with 1 mM Ca^{2+} (n = 4). Internal solution was K^+-glutamate based solution without any ADPR and in the absence of the Ca^{2+} buffer BAPTA (unbuffered conditions). Neither I_{ADPR} nor I_{Cl} develop in these conditions.

(B) Average current response measured in HEK293-TRPM2 cells in the presence of internal ADPR and 800 nM $[Ca^{2+}]i$. Cells were kept in $NMDG^+$ solution with 1 mM Ca^{2+} and superfused with pH 5 as indicated by the thick black bar (n = 5). While TRPM2 currents activate and are inhibited in these conditions, no significant additional current was observed during pH 5 application.



Supplemental Figure S3: Extracellular alkalinization facilitates TRPM2 currents.

(A) Average normalized currents measured in HEK293-TRPM2 cells kept in pH 7 (black circles, n =11) or exposed to pH 8 (blue circles, n = 8) as indicated by the thick black bar.

(B) Average normalized TRPM2 currents acquired in cells kept in pH 8 (blue circles, n = 8) or exposed to pH 9 (red circles, n = 9) as indicated by the black bar. External solutions were $NMDG^+$ in nominally divalent-free conditions; internal solution was standard K^+-glutamate supplemented with 1 mM ADPR and 800 nM $[Ca^{2+}]i$.