Biophysical Journal, Vol 99

Supporting Material

The Met268Pro mutation of mouse TRPA1 changes the effect of caffeine from activation to suppression

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Supplementary Table 1 Mean ± SEM values of the current amplitudes (μA) of the experiments in the Supplementary experiments.

A (current amplitude at +100 mV)

Suppl. Fig. 2A, B	mTRPA1 WT	hTRPA1 WT
20 mM sucrose	0.01 ± 0.01	0.05 ± 0.03
10 mM caffeine	2.95 ± 0.64	-
100μM AITC	-	22.5 ± 2.9
n value	4	4

B (current amplitude at +60 mV)

Cys site mutants	mTRPA1 WT	C622S
5 mM caffeine	1.02 ± 0.43	2.64 ± 1.34
n value	3	5

C (current amplitude at +100 mV)

Ca^{2+}_i site mutants	mTRPA1 WT	D469A
5 mM caffeine	1.62 ± 0.56	8.12 ± 1.40
n value	2	6

D (current amplitude at +60 mV)

Ca^{2+}_i site mutants	mTRPA1 WT	L475A
5 mM caffeine	1.54 ± 0.25	0.86 ± 0.14
n value	10	12

E (current amplitude at +60 mV)

Tyr site mutants	mTRPA1 WT	Y728A	Y788A
5 mM caffeine	1.99 ± 0.25	0.026 ± 0.003 (clearer resp after AITC)	0.38 ± 0.17

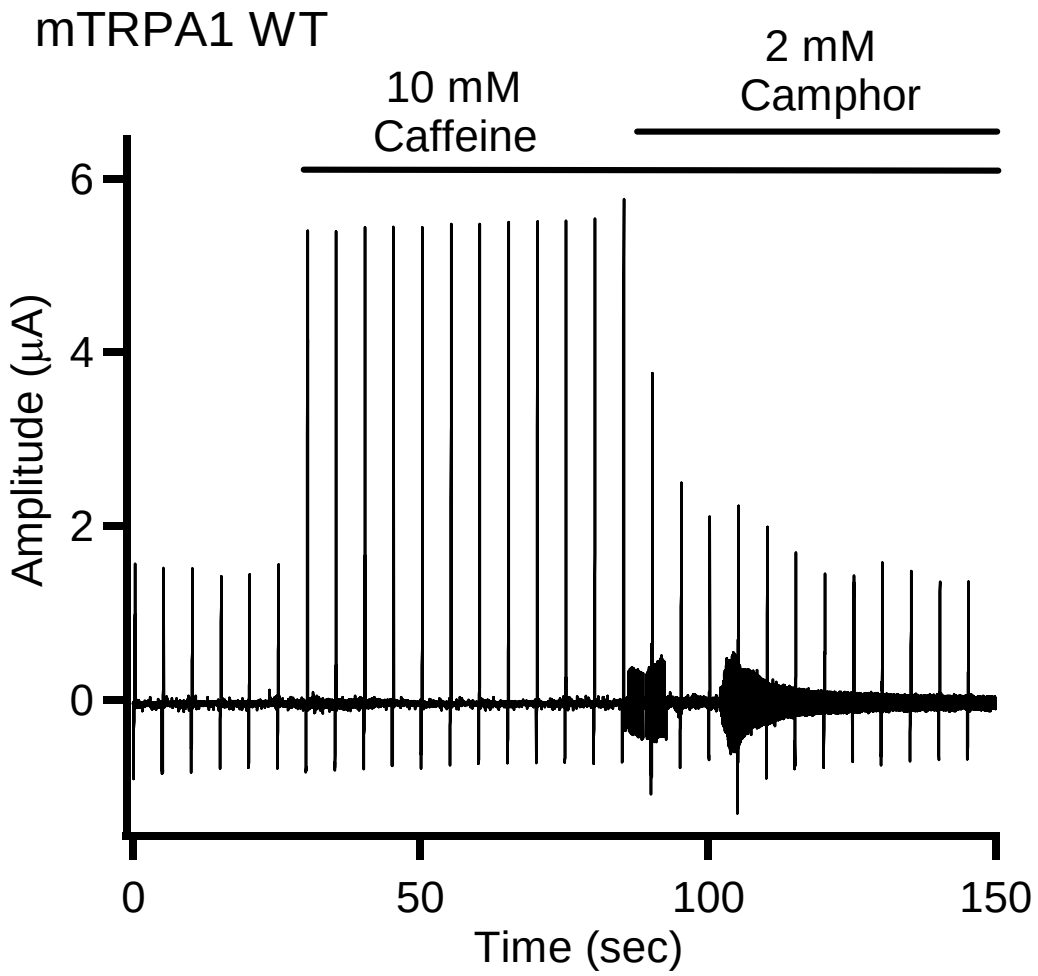
n value	3	2	3

F (current amplitude at +60 mV)

Tyr site mutants	mTRPA1 WT	Y815A	Y845A	Y852A
5 mM caffeine	0.96	0.15 ± 0.05	0.21 ± 0.03	0.32 ± 0.16
n value	1	2	2	2

G (current amplitude at +60 mV)

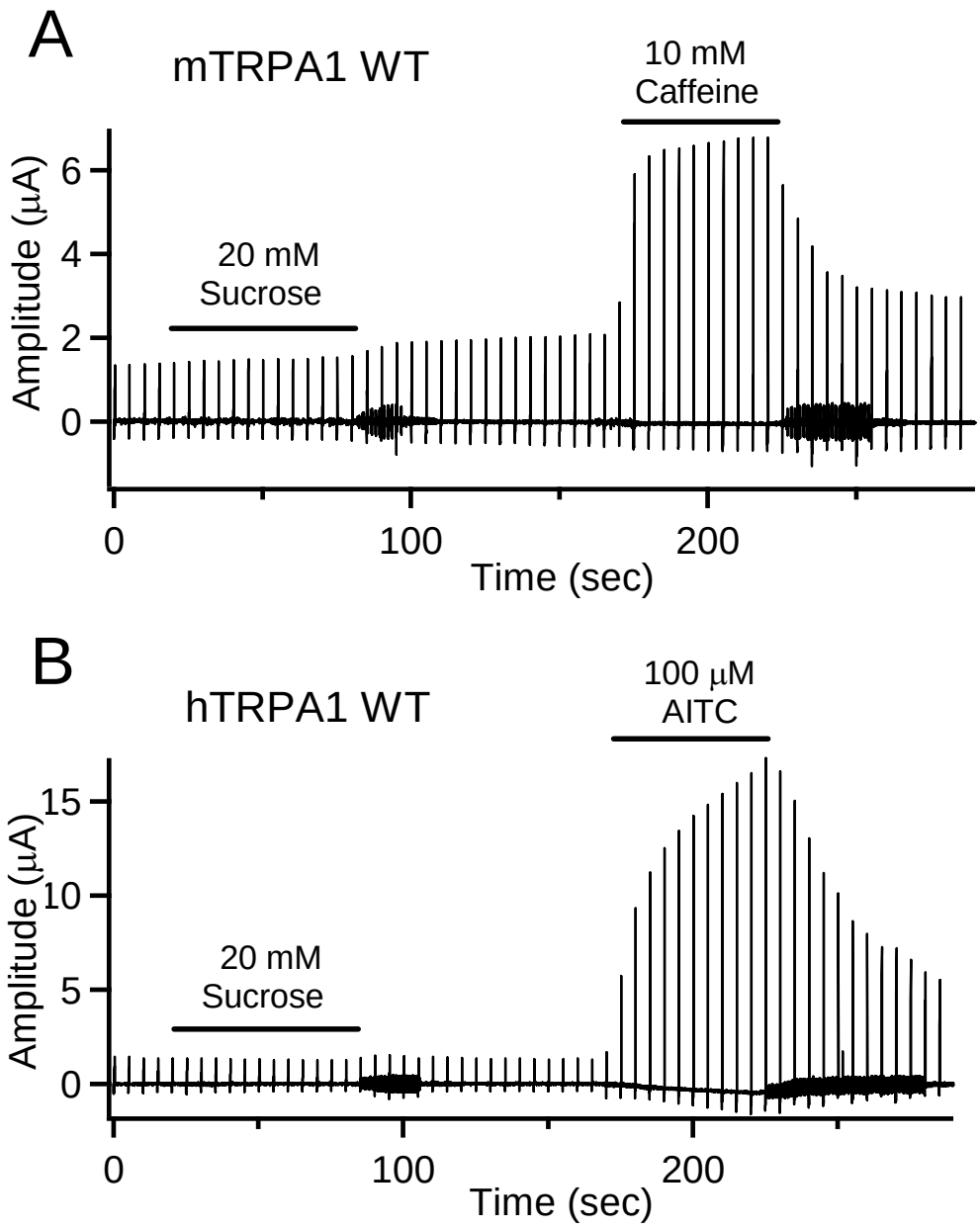
Suppl. Fig. 4B	mTRPA1 WT	Y1009A	Y1009L
5 mM caffeine	2.42 ± 0.45	12.1 ± 2.14	11.4 ± 2.70
100μM AITC	2.56 ± 1.12	8.11 ± 1.14	7.34 ± 2.09
n value	5	7	6



Supplementary Figure 1

Sensitivity of the caffeine-evoked current to the block by camphor, a TRPA1 channel blocker

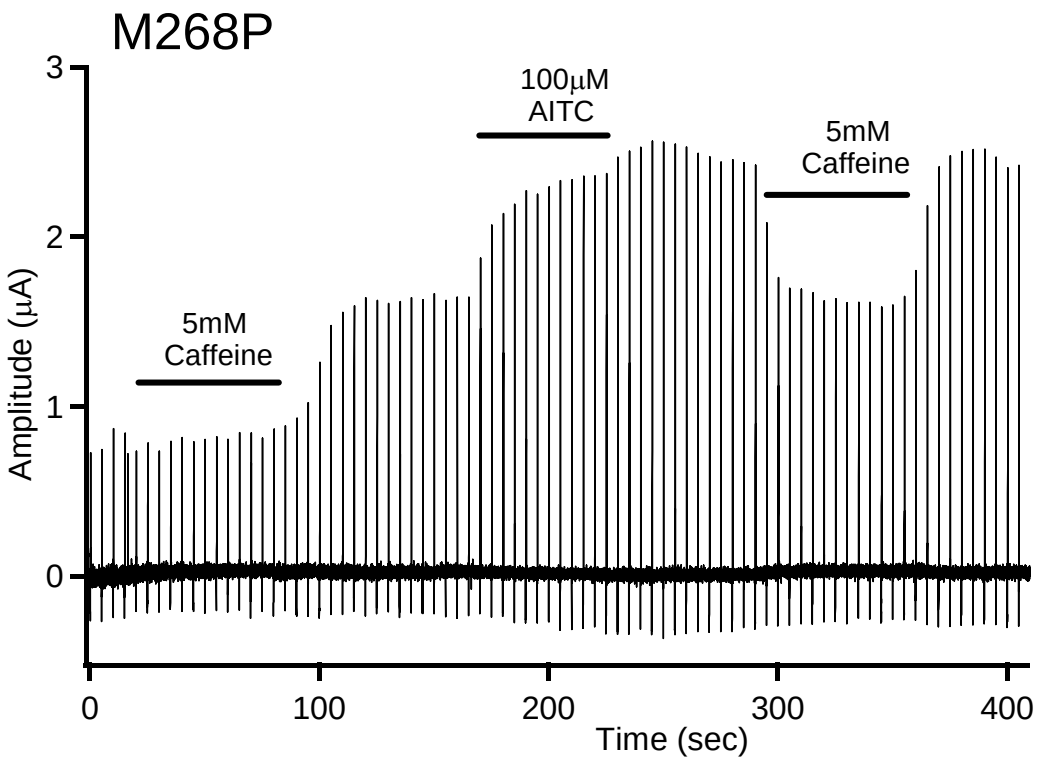
Details of the recordings are the same as in Figure 3. 10 mM caffeine was applied first by pipetting one-fifth volume (40 μl) of 5 times concentrated solution to the bath of 200 μl. Then 3 ml of bath solution containing 10 mM caffeine and 2 mM camphor was applied by pipetting with a continuous suction of the overflowed solution by a negative pressure to achieve complete solution exchange.



Supplementary Figure 2

Responses of mTRPA1 and hTRPA1 to 20 mM sucrose

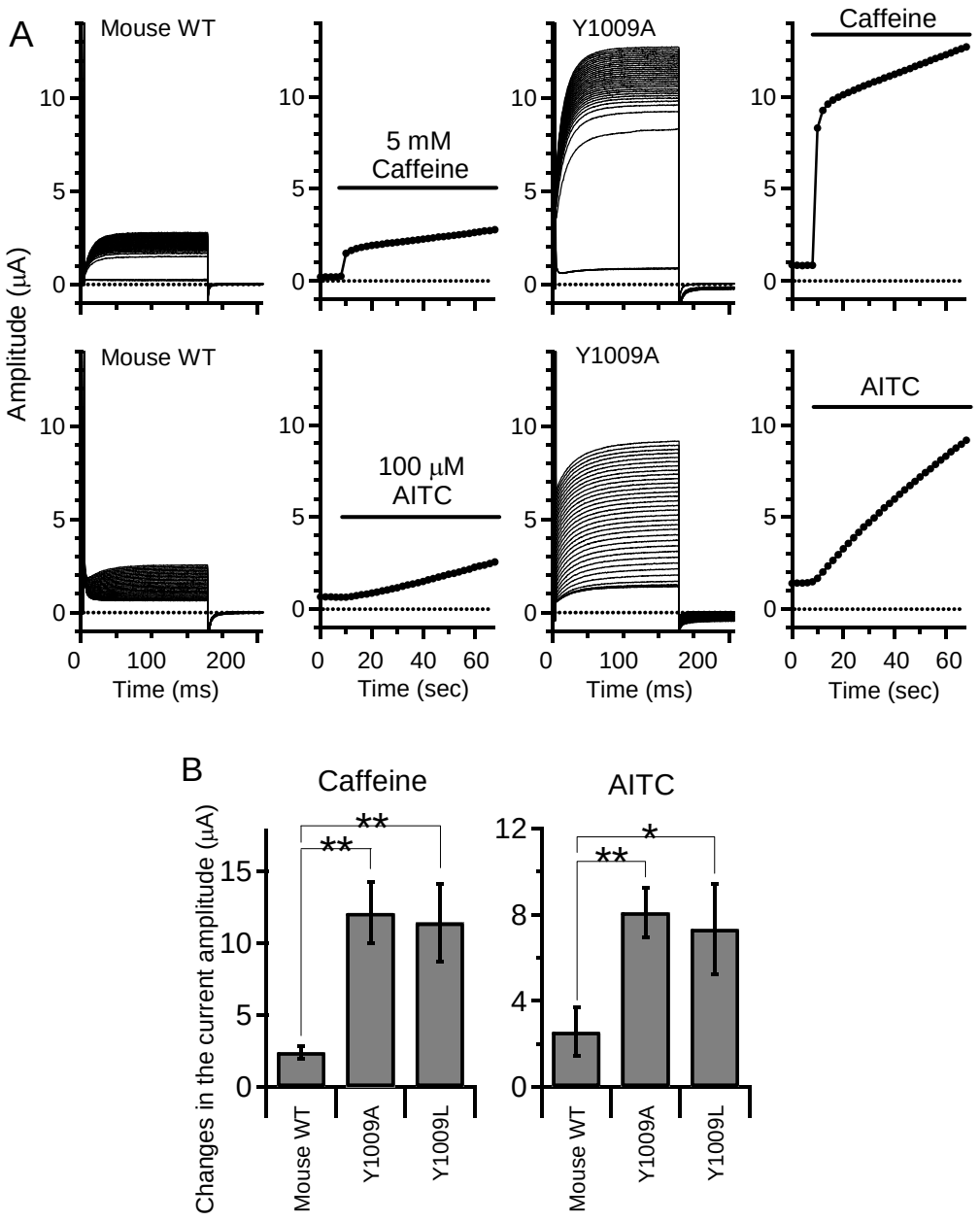
Details of the recordings are the same as in Figure 3. 20 mM sucrose was applied first by pipetting one-fifth volume (40 μl) of 5 times concentrated solution to the bath of 200 μl. After washing out the sucrose containing bath solution by perfusion, positive control stimuli (10 mM caffeine in A and 100 μM AITC in B) were applied. The values are shown in Supplementary Table 1A.



Supplementary Figure 3

Confirmation of AITC response of Met268Pro

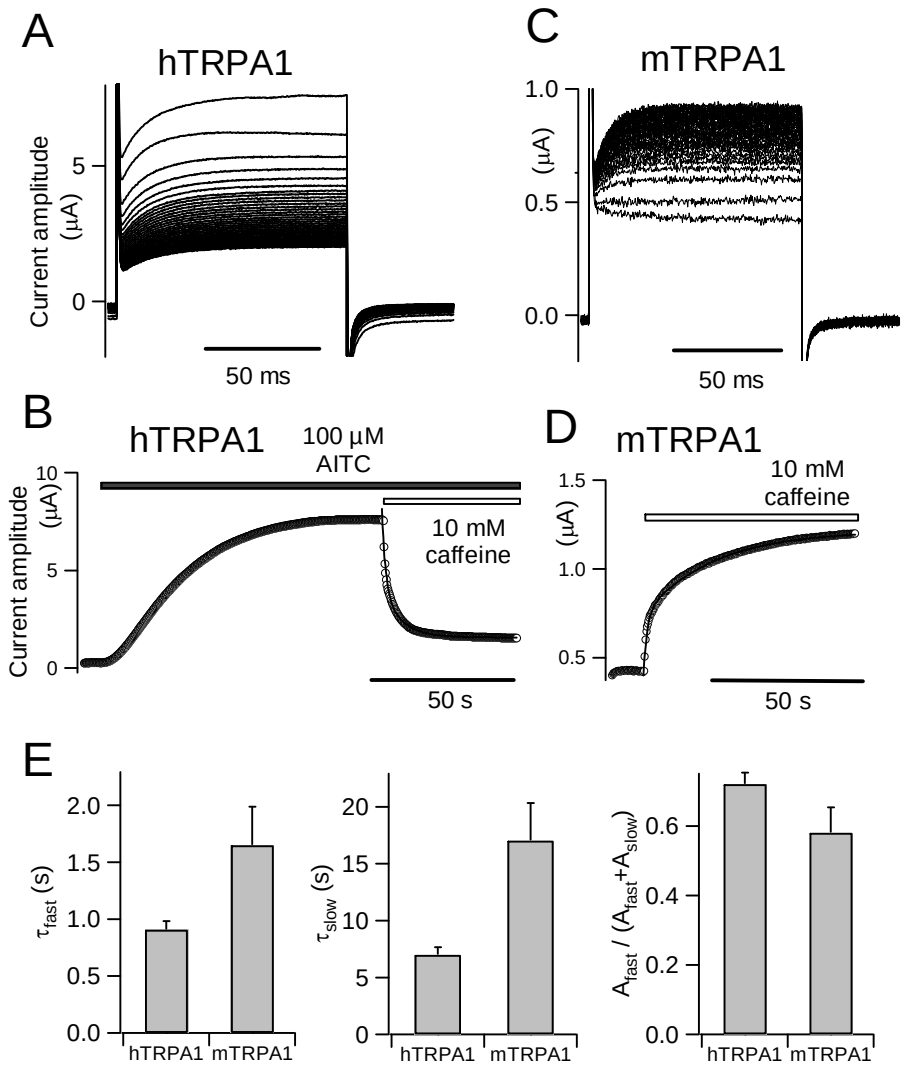
Details of the experiments are the same as in Figure 3.



Supplementary Figure 4

Responses of Tyr1009Ala and Tyr1009Leu mutants to caffeine and AITC

(A) Current recordings of mTRPA1 WT (left) and Tyr1009Ala (right) were obtained by applying depolarizing step pulses repeatedly every 2s before and after application of agonists. The time lapse changes of the peak current amplitude at the depolarized state were also shown. (B) Plots of mean and standard error of the changes in the current amplitude after 60s. The values are shown in Supplementary Table 1G.



Supplementary Figure 5

Comparison of the time course of inhibition of hTRPA1 current and activation of mTRPA1 current by caffeine

(A, C) Representative current traces of hTRPA1 during the course of inhibition by 10 mM caffeine (A) and mTRPA1 during the course of activation by 10 mM caffeine (C). Depolarizing pulses for 100 ms at +60 mV were applied to oocytes every 250ms from the holding potential of -20 mV. In hTRPA1, the most upper trace is the current activated by 100 μM AITC just before the caffeine application and the current gradually decreased after the caffeine application. In mTRPA1, the lowest current trace is the current just before the caffeine application and the current gradually increased after the caffeine application. (B, D) The time course of the change of current amplitude induced by caffeine. Data were taken from the same oocyte of (A, C). Caffeine-induced changes were fitted with a double exponential function (black curves). (E) Time constants of caffeine-induced changes deduced from the double exponential fit. Faster time constants (τ_{fast}, left), slower time constants (τ_{slow}, middle) and the fraction of faster component (A_{fast}/ (A_{fast} + A_{slow}), right). Bars indicate means ± SEM (n = 10 for each).