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Supplement to

A novel pan-negative-gating modulator of KCa2/3 channels, the fluoro-di-benzoate, RA-2, inhibits EDH-type relaxation in coronary artery and produces bradycardia *in vivo*.

(RA-2, a negative-gating modulator of KCa2/3)

by

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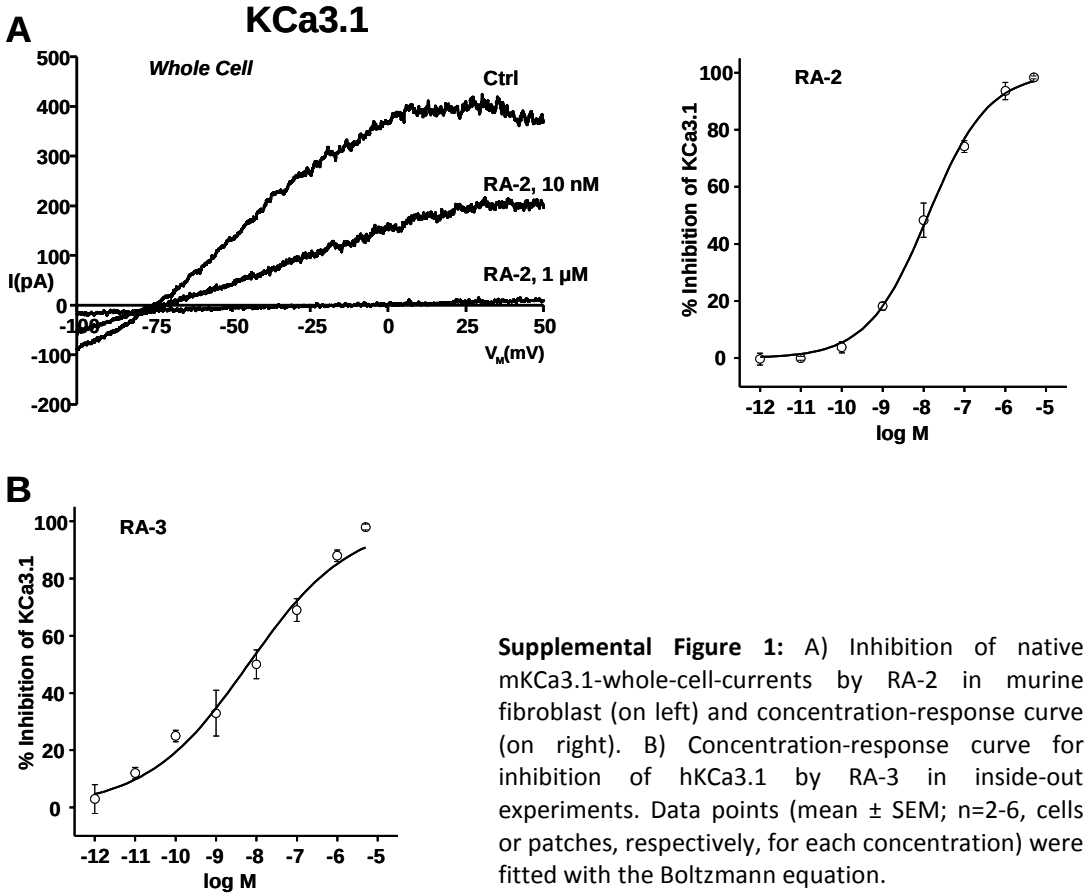
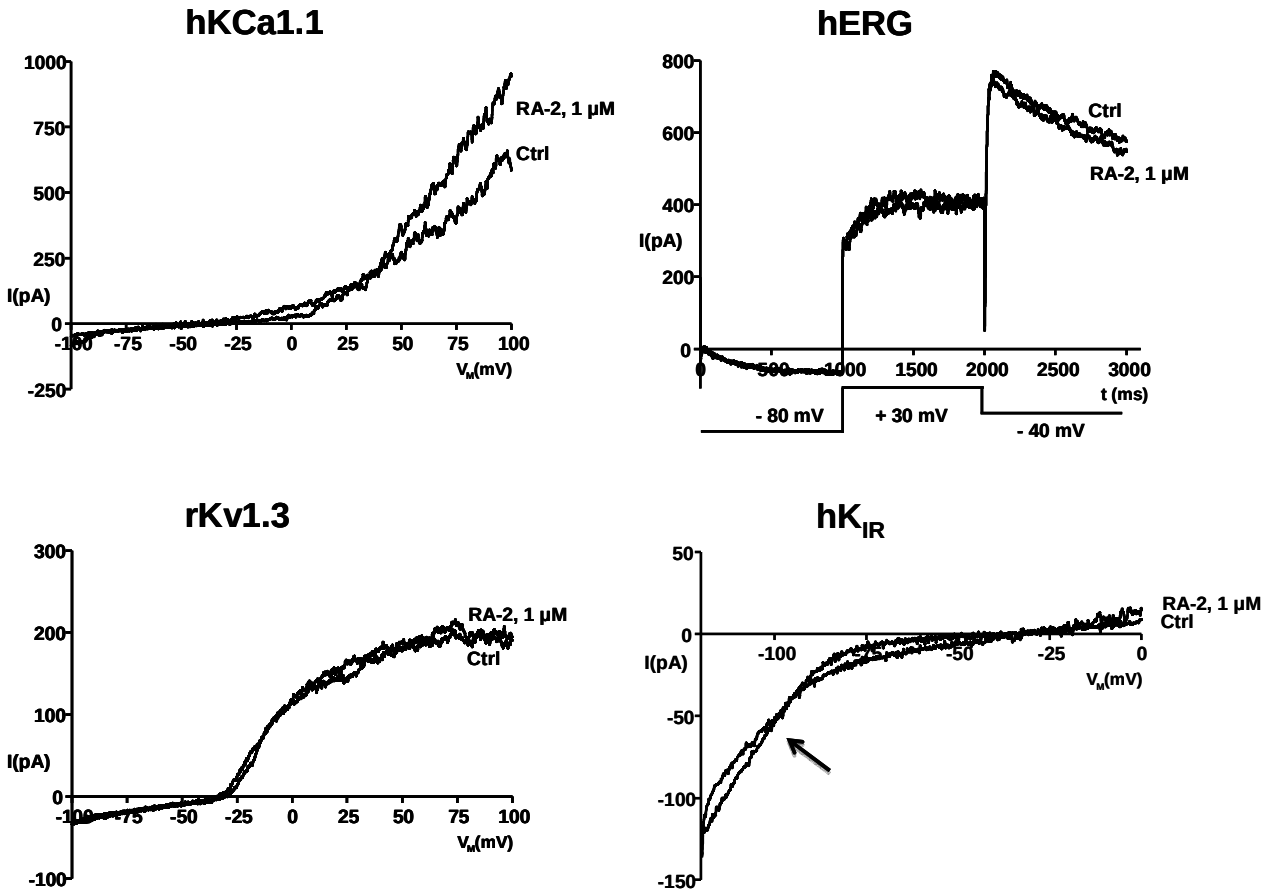
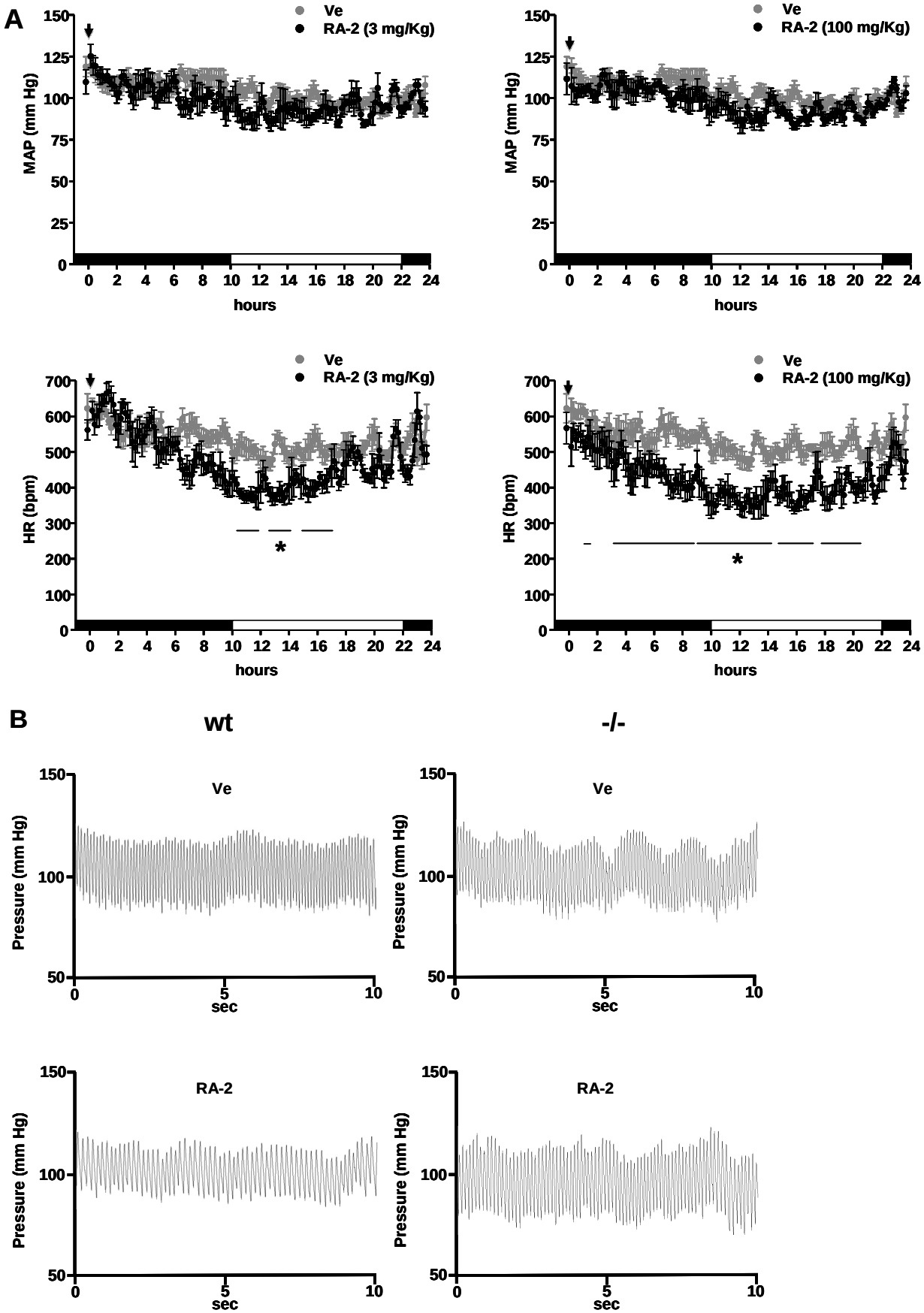


Supplemental Figure 1: A) Inhibition of native mKCa3.1-whole-cell-currents by RA-2 in murine fibroblast (on left) and concentration-response curve (on right). B) Concentration-response curve for inhibition of hKCa3.1 by RA-3 in inside-out experiments. Data points (mean ± SEM; n=2-6, cells or patches, respectively, for each concentration) were fitted with the Boltzmann equation.



Supplemental Figure 2: Representative whole-cell recordings showing that RA-2 did not inhibit hKCa1.1 in U251 glioblastoma cells, cloned hERG, rKv1.3, hK_{IR} in U251 cells (arrow in the lower panel on right indicates inward-rectifying hK_{IR} currents).



Supplemental Figure 3: A) Mean arterial blood pressure (MAP) and heart rate (HR) after intraperitoneal injections of 3 mg/kg (n=4, experiments) and 100 mg/kg (n=4, experiments) into 4 wt mice. Black and white parts of the y-axis indicate dark and light phases and arrow indicates time of injection (t=0). Data on vehicle have been re-plotted from Figure 4. B) Representative recordings of pulse waves after injection of 30 mg/kg RA-2 or vehicle (Ve) in wt (on left) and in KCa3.1-/- mice (on right). Data points are mean ± SEM; horizontal lines below data points indicate statistical significant difference from vehicle; * P<0.05; Student T test.

Supplemental Table 1: Effects of RA-2 on other K^+ channels

Compound	% of control at		
	100 nM	1 μM	5μM
hKCa2.3* (in COS7)			
RA-2	38 ± 2	21 ± 3	3 ± 1
RA-3	68 ± 4	36 ± 5	11 ± 1
RA-4	71 ± 5	45 ± 12	NT
hKCa2.1 (in HEK293)			
RA-2	61 ± 4	4 ± 2	NT
rKCa2.2 (in HEK293)			
RA-2	41 ± 6	10 ± 1	3 ± 1
hKCa1.1 (in U251)			
RA-2	NT	105 ± 10	NT
hKv1.2 (in B82)			
RA-2	NT	94 ± 4	NT
rKv1.3 (in L929)			
RA-2	NT	93 ± 14	NT
hKv7.4 (in HEK293)			
RA-2	NT	98 ± 7	NT
hERG (in HEK293)			
RA-2	NT	99 ± 2	NT
hK_{IR} (in U251)			
RA-2	NT	102 ± 10	NT

NT, not tested; data are given as mean ± SEM, n ≥ 3. * data from *inside-out* experimentation on cloned hKCa2.3, the other data derived from *whole-cell* experimentation on cloned or native channels in the respective cell line (name in brackets).