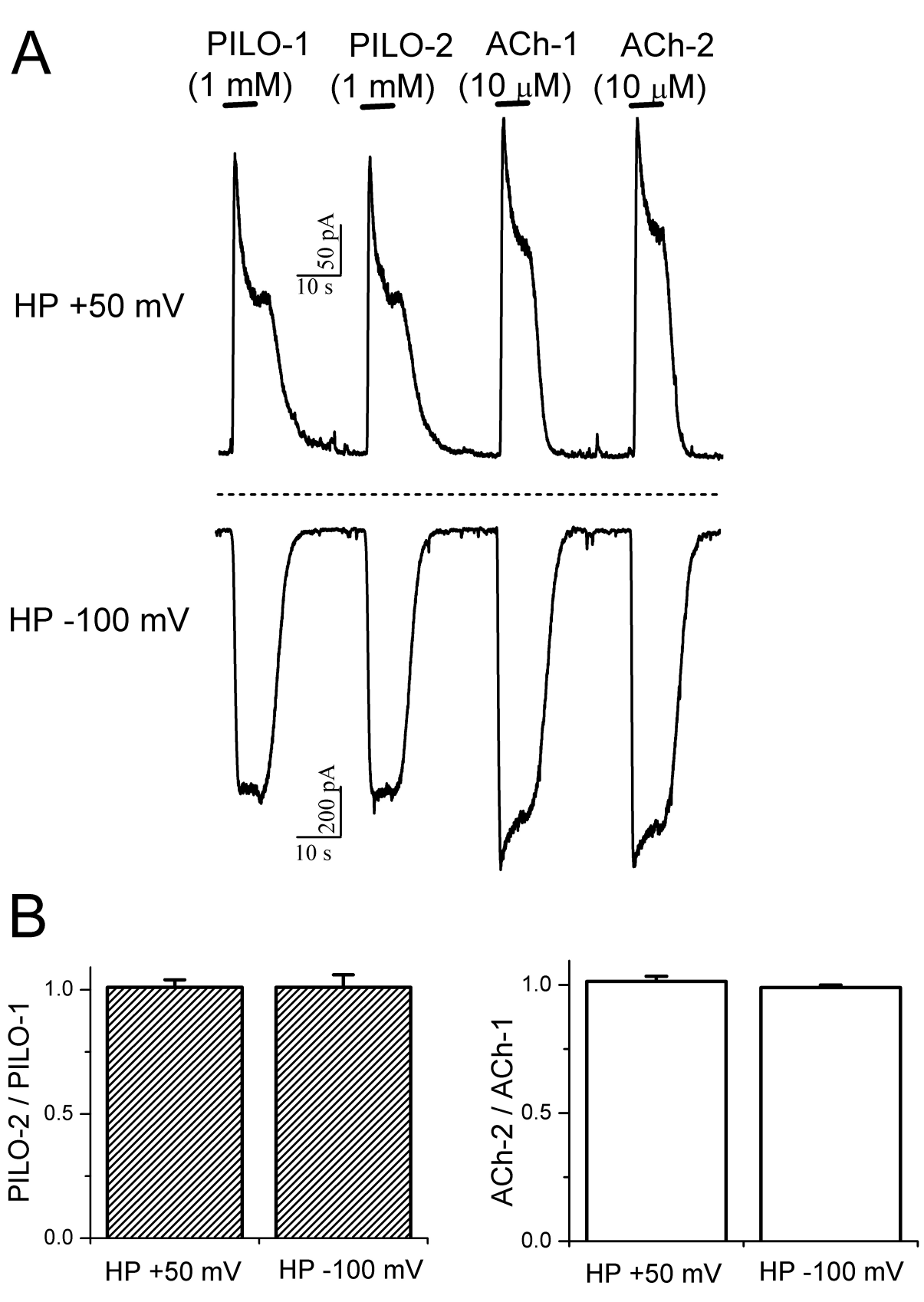
Suppl. Figure 1. The effects of repeated, brief application of agonists on atrial I_{KACh}.

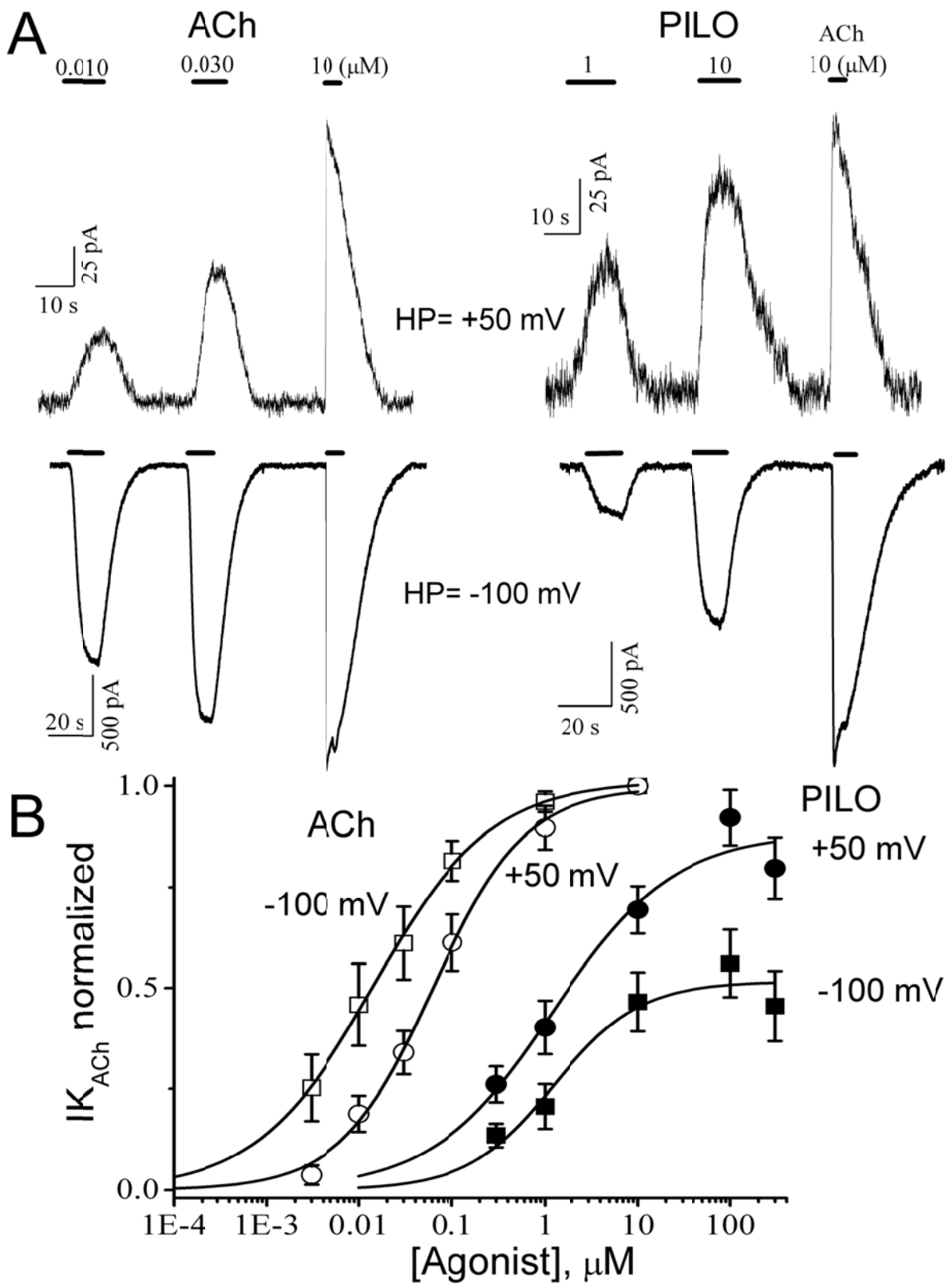
A, Examples of current elicited by consecutive applications of high concentrations of PILO (1 mM) and ACh (10 μM) at HP +50 mV and -100 mV. Horizontal bars indicate the duration of ligand application. Using a rapid switcher device, the cell was exposed to the agonist for 6 s, followed by a washout period of 25 s, before a second brief exposure to agonist. The peak current elicited by the second exposure of agonist was of similar magnitude to the initial exposure. The dashed line indicates the zero current level. *B*, Mean ± SEM of current elicited by second exposure (PILO-2 or ACh-2) divided by current elicited during initial exposure (PILO-1 or ACh-1). n = 4 cells.

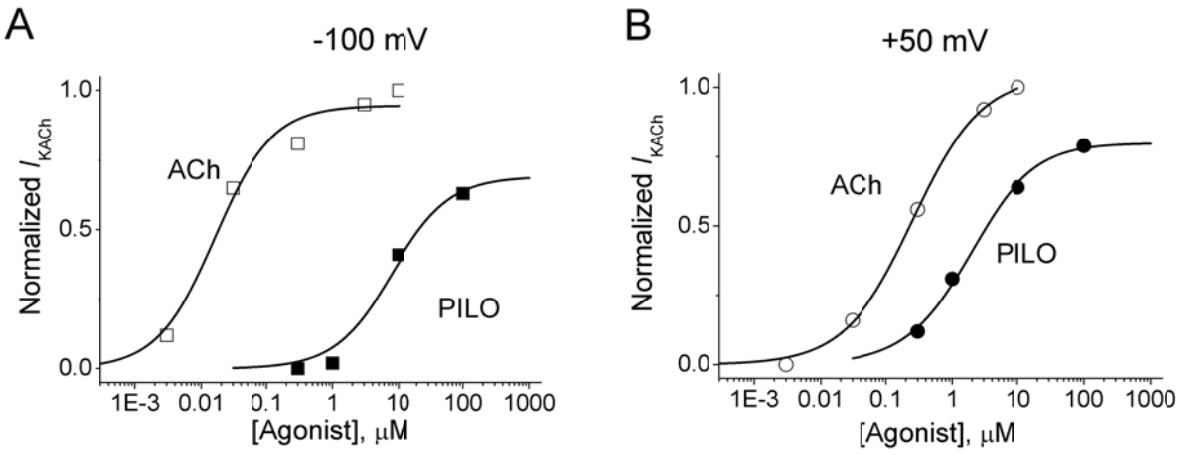
Suppl. Figure 2. Opposite voltage dependent effects of ACh and PILO on heterologously expressed M2R-Kir3.1/3.4 in HEK-293 cells. *A*, Effects of ACh and PILO on reconstituted I_{KACh} recorded at HP +50 mV (top traces) and -100 mV (bottom traces). *B*, Concentration-response curves for ACh (open symbols) and PILO (closed symbols) activation of I_{KACh} at HP +50 mV (circles) and -100 mV (squares). The lines represent data fits to a Hill equation. For ACh, the EC_{50} and Hill coefficient were 14 ± 3 nM and 0.8 ± 0.1 (HP -100 mV) and 62 ± 6 nM and 0.9 ± 0.1 (HP +50 mV). The EC_{50} and Hill coefficient for PILO were 1.7 ± 0.4 μM and 0.8 ± 0.1 (HP -100 mV) and 0.6 ± 0.2 μM and 0.8 ± 0.1 (HP +50 mV), n = 7-9 cells.

Suppl. Figure 3. Estimation of affinity and efficacy values of PILO by the comparative and operational methods. Example of typical experimental data for I_{KACh} activation through M2R in feline atrial myocytes. Continuous fitting lines were obtained by equations (2) and (3) as defined in Methods. C-R curves for PILO (closed symbols) and the reference full agonist, ACh (open

symbols), assayed at membrane voltages of -100 mV (*A*) and +50 mV (*B*). The data for both panels were obtained in a single atrial myocyte using the sequential protocol. The resulting affinity (K_A) and efficacy (τ) values of PILO from this individual analysis are K_A = 30 μM and τ = 2.7 at -100 mV *versus* K_A = 13 μM and τ = 4.3 at +50 mV. Average estimates of K_A for PILO were 28.6 ± 5.4 μM and 15.2 ± 4.6 μM at -100 and +50 mV, respectively and τ 2.0 ± 0.3 and 3.9 ± 0.8 at -100 and +50 mV, respectively. n = 10 cells.


A
PILO-1
(1 mM)
PILO-2
(1 mM)
ACh-1
(10 μM)
ACh-2
(10 μM)
HP +50 mV
50 pA
10 s
HP -100 mV
200 pA
10 s
B
PILO-2 / PILO-1
1.0
0.5
0.0
HP +50 mV
HP -100 mV
ACh-2 / ACh-1
1.0
0.5
0.0
HP +50 mV
HP -100 mV


A
ACh
0.010
0.030
10 (μM)
PILO
1
10
ACh
10 (μM)
25 pA
10 s
HP= +50 mV
HP= -100 mV
500 pA
20 s
B
ACh
PILO
-100 mV
+50 mV
+50 mV
-100 mV
IK_ACh normalized
1.0
0.5
0.0
1E-4
1E-3
0.01
0.1
1
10
100
[Agonist], μM


A
-100 mV
Normalized I_{KACh}
1.0
0.5
0.0
ACh
PILO
1E-3
0.01
0.1
1
10
100
1000
[Agonist], μM
B
+50 mV
Normalized I_{KACh}
1.0
0.5
0.0
ACh
PILO
1E-3
0.01
0.1
1
10
100
1000
[Agonist], μM