

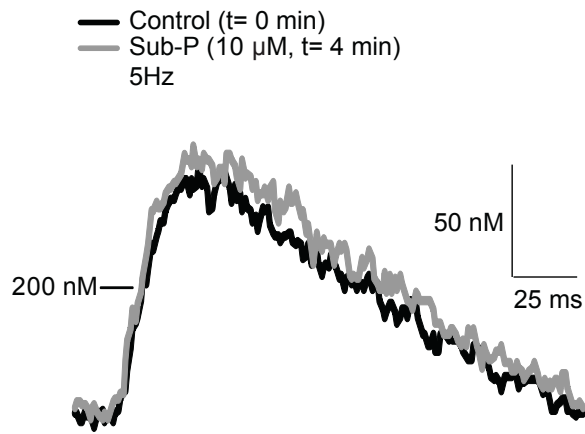
**Neurokinin-3 receptor activation prolongs atrial refractoriness by
inhibition of a background K⁺ channel**

Veldkamp et al.

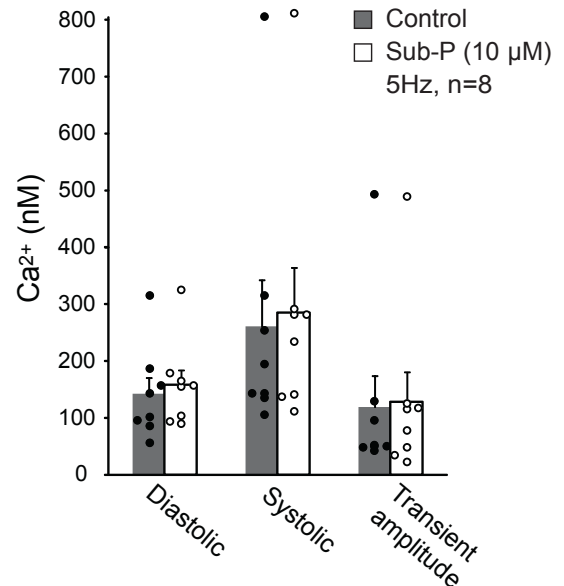
Supplementary FIGURES

Supplementary Figure 1

a

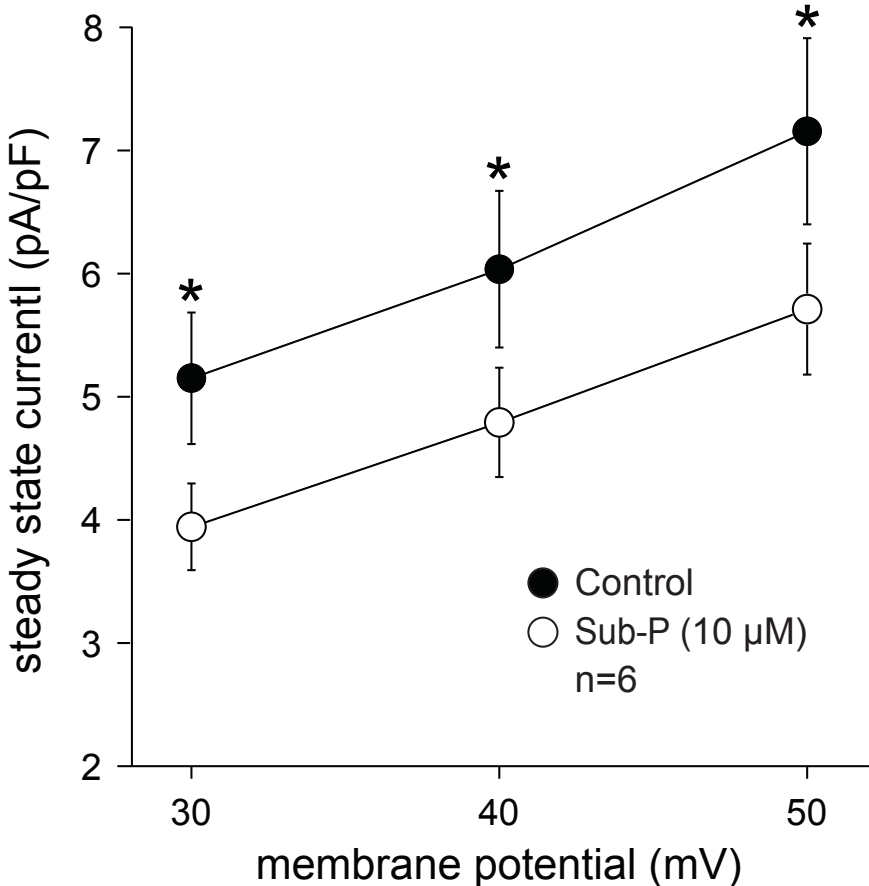


b



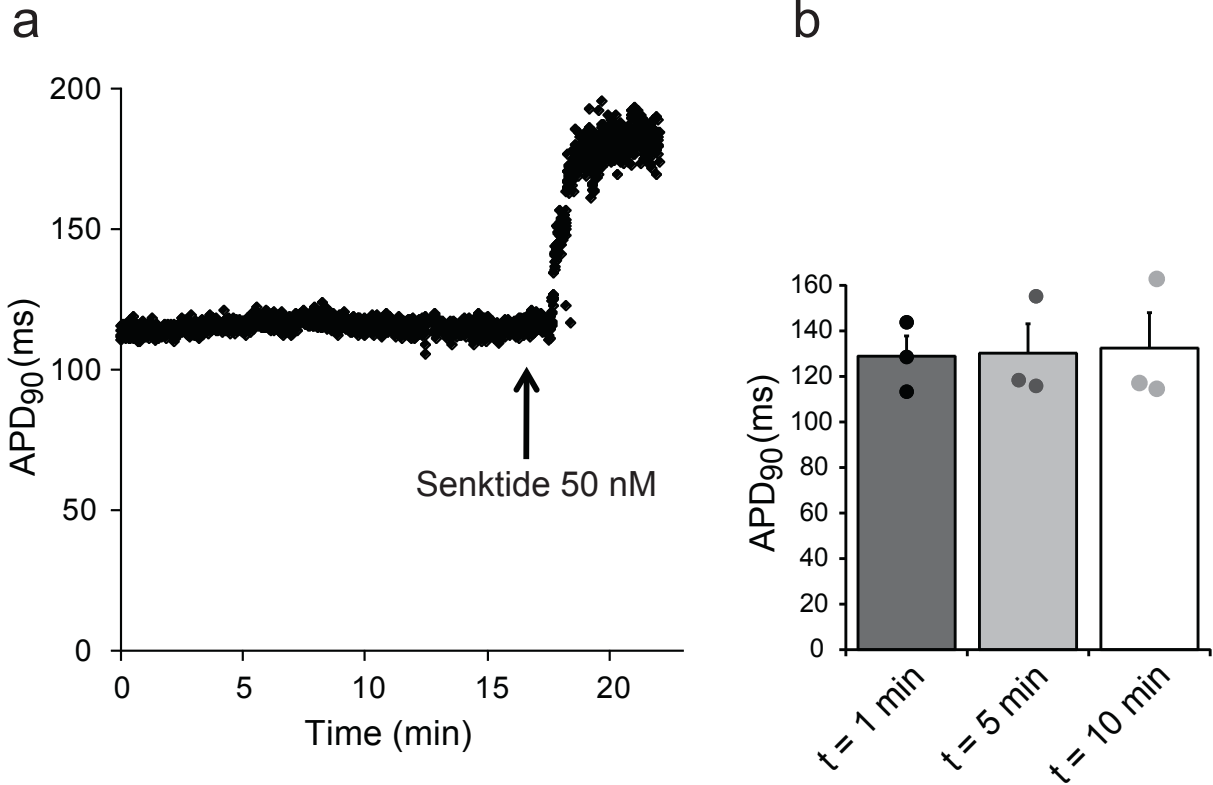
SUPPLEMENTARY FIGURE 1. Effect of Substance-P on intracellular calcium concentration. (a) Intracellular calcium (Ca^{2+}_i) transients in Control conditions and in the presence of 10 $\mu\text{mol/L}$ Sub-P. (b) Bar histograms showing average diastolic, systolic and peak Ca^{2+}_i before (Control) and after application of 10 μM Sub-P (n=8). All values shown are mean \pm SEM. Paired *t*-test. * $P < 0.05$

Supplementary Figure 2



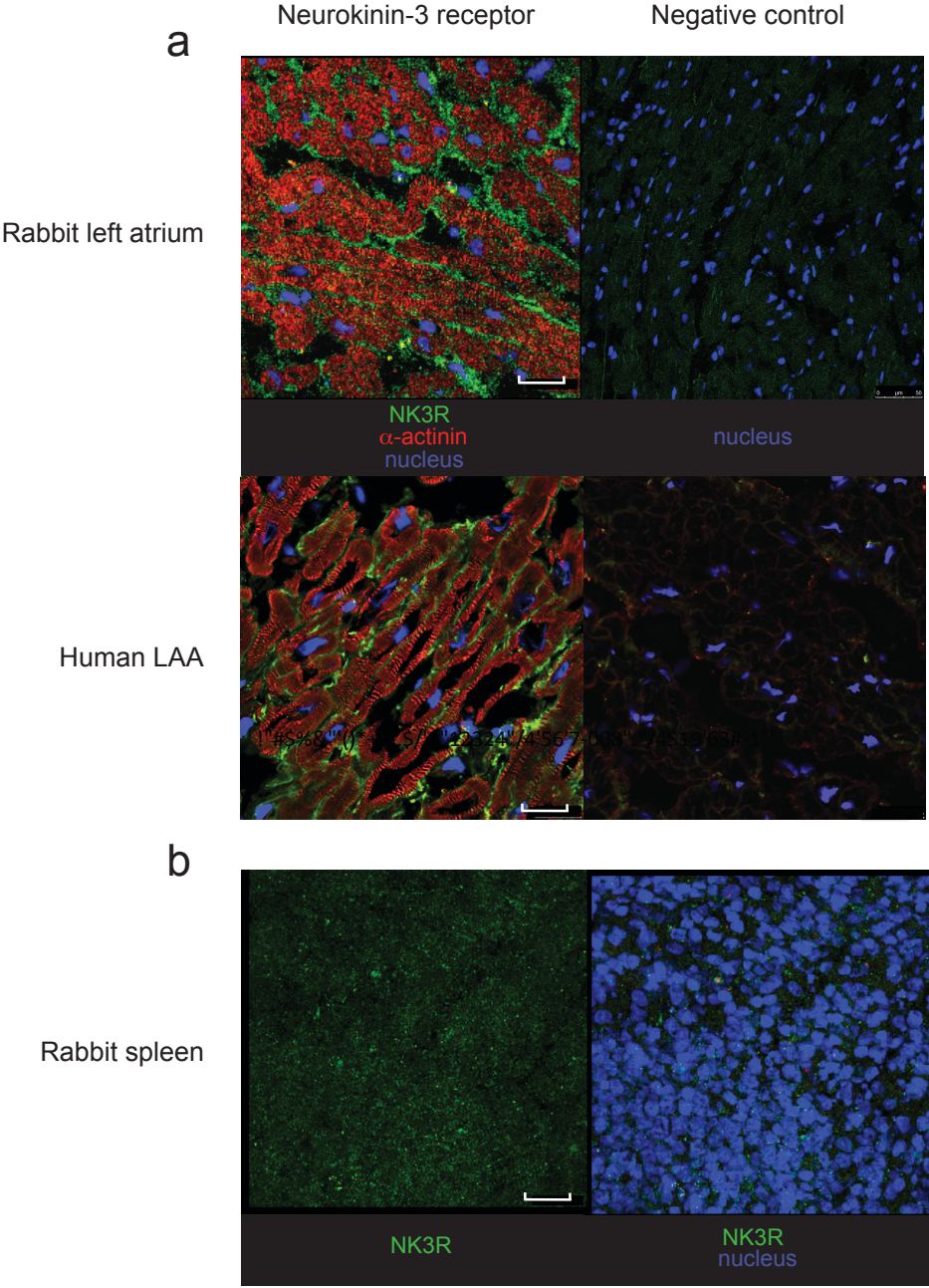
SUPPLEMENTARY FIGURE 2. Effect of neurokinin-3 receptor (NK-3R) stimulation on steady-state currents in the presence of blockers for Na⁺, transient outward and ultra-rapid delayed rectifier K⁺ currents. Average current-voltage relationships of steady-state current at t=300 ms before (Control) and after application of 10 μM Sub-P (n=6). All values shown are mean±SEM. Two-way RM ANOVA. * P<0.05.

Supplementary Figure 3.



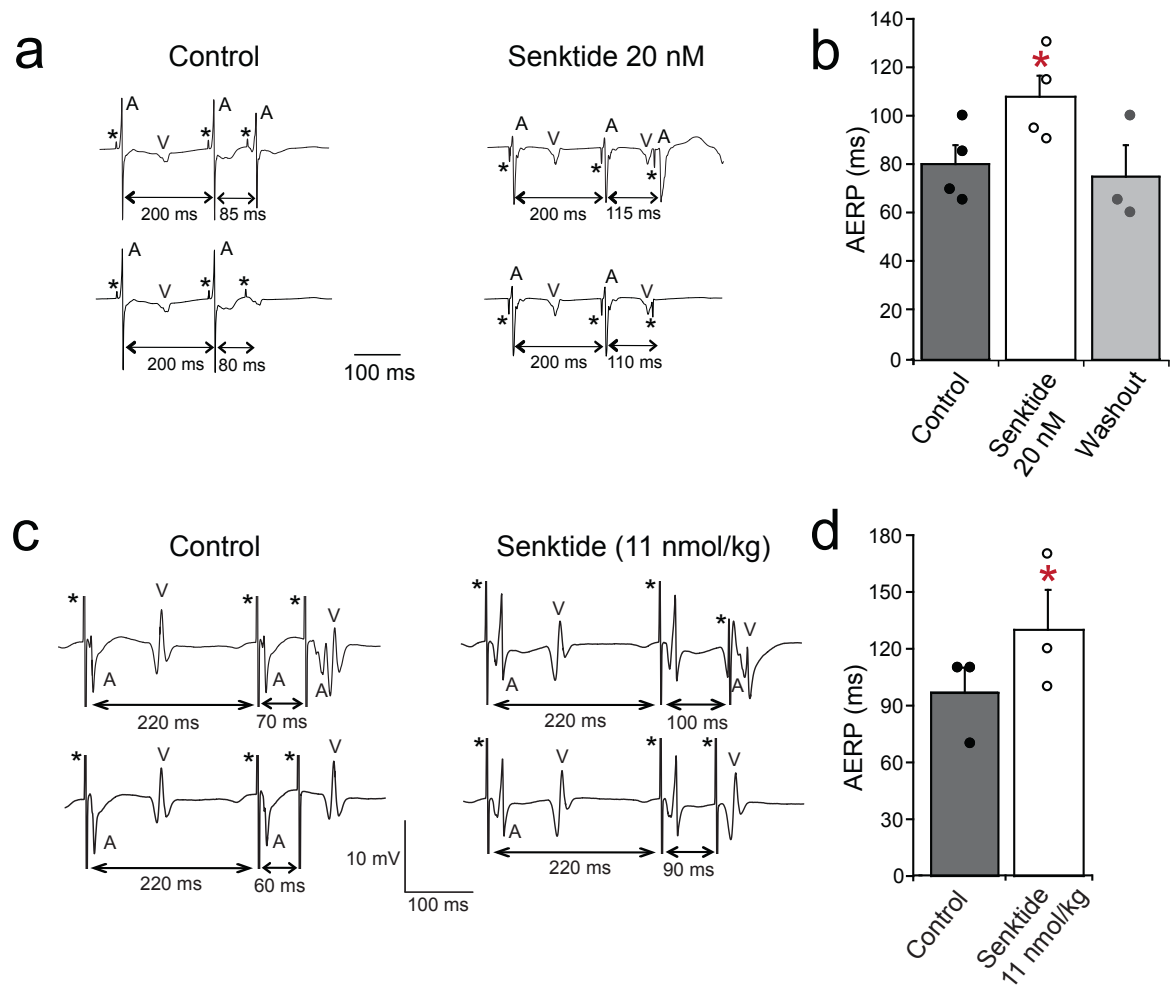
SUPPLEMENTARY FIGURE 3. Action potential duration is stable in time. (a) Action potential duration at 90% of repolarization (APD₉₀) as a function of time. At t=16 min. 50 nM Senktide was applied. (b) APD₉₀ at 1, 5, and 10 min after the start of the experiment (n=3). All values shown are mean±SEM. Paired *t*-test. * P<0.05.

Supplementary Figure 4



SUPPLEMENTARY FIGURE 4. Negative controls for NK-3R staining in rabbit and human atrium. (a) NK-3R positive staining (green) in tissue sections of rabbit left atrium (top, left) and human LAA (bottom, left). In rabbit sections, the fluorophore was conjugated to the primary antibody directly. In human sections, the fluorophore was conjugated to the secondary antibody. (Top, right) No primary antibody controls for rabbit and human (bottom, right). Tissue sections were incubated with secondary antibody only. (b) A section from rabbit spleen incubated with fluorophore-conjugated primary NK-3R antibody. Spleen has been reported not to express the target antigen NK-3R¹.

Supplementary Figure 5



SUPPLEMENTARY FIGURE 5. . Effect of neurokinin-3 receptor (NK-3R) stimulation on effective refractory period (AERP) in rabbit intact left atrium *ex vivo* and *in situ*. (a) Unipolar electrograms recorded from the left atrium of a rabbit Langendorff perfused heart before (Control) and after application of 20 nM of the NK-3R agonist Senktide. (b) Bar graph showing average values for left AERP in before (Control) and after application of 20 nM Senktide (n=4). (c) Unipolar electrograms recorded from the left atrium of the *in situ* rabbit heart in before (Control) and after infusion of 11 nmol/kg of Senktide. (d) Bar graph showing average values for AERP before (Control) and after infusion of 11 nmol/kg Senktide (n=3). All values shown are mean±SEM. Two-way RM ANOVA. * P<0.05

Electograms: A, atrial activation; V, ventricular activation; *, stimulus.

Supplementary Table 1. Effects of selective TASK-1, TASK-3, TREK-1 channel blockers, and Senktide on action potential parameters in atrial myocytes

	RMP (mV)	APA (mV)	V_{max} (V/s)	APD₂₀ (ms)	APD₅₀ (ms)	APD₉₀ (ms)
Atrial myocyte						
Control	-83.0±0.9	110.5±2.1	357.2 ±29.1	4.9 ±0.6	23.3±5.0	95.6 ±10.5
ML-365 (100 nM, n=8)	-83.5±1.0	99.2±12.7	382.8±30.8	5.8±1.0	27.2±5.3*	100.4±10.9*
ML-365 + Senktide (100 nM, 50 nM, n=8)	-83.6±0.7	110.3±2.5	357.9±31.4	7.9±2.0	48.9±6.9*#	139.0±15.9*#
Control	-81.2±0.9	104.2±1.9	341.6 ±30.9	4.0 ±0.6	14.0 ±4.7	73.5 ±3.2
PK-THPP (100 nM, n=7)	-80.5±0.4	103.1±2.1	322.2±28.2	4.5±0.7*	17.9±5.4*	79.0±3.1*
PK-THPP + Senktide (100 nM, 50 nM, n=7)	-78.2±2.3	95.8±4.7	250.6±47.8	5.2±0.4*	40.8±8.6*#	119.1±5.1*#
Control	-82.4±1.5	107.2±3.7	168.6 ±36.7	7.2 ±1.0	26.1±4.6	80.1 ±6.7
Spadin (100 nM, n=7)	-82.8±1.6	108.3±4.1	193.7±41.5	6.2±1.0	27.3±4.7	82.1±6.8
Spadin + Senktide (100 nM, 50 nM, n=7)	-81.2±2.6	105.4±6.0	162.4±46.0	8.1±1.6	48.2±8.4*#	114.6±13.0*#

All values are mean±SEM. RMP, resting membrane potential; APA, action potential amplitude; V_{max}, upstroke velocity; APD₂₀, action potential duration at 20% repolarization; APD₅₀, action potential duration at 50% repolarization; APD₉₀, action potential duration at 90% repolarization. One-way RM ANOVA followed by post hoc test. *p<0.05 vs. Control; #p<0.05 vs. Blocker ML-365, PK-THPP or Spadin, respectively.

Supplementary Table 2. Effects of Senktide on incidence and duration of atrial fibrillation (AF) in a rabbit isolated heart model of AF based on atrial dilatation

	Control		Senktide (20 nM)		Wash out	
	0 cm H ₂ O	20 cm H ₂ O	0 cm H ₂ O	20 cm H ₂ O	0 cm H ₂ O	20 cm H ₂ O
Incidence						
No Arrhythmia	0.23±0.06	0.11±0.04	0.46±0.06 [#]	0.39±0.14 ^{\$}	0.24±0.08	0.02±0.02
Arrhythmia 0 < Δt > 1 s	0.69±0.05	0.30±0.07	0.54±0.06	0.44±0.10	0.66±0.07	0.34±0.05
AF Episode Δt = >1 s	0.08±0.04	0.59±0.09*	0.0±0.0 [#]	0.17±0.08* ^{\$}	0.10±0.08	0.64±0.07*
Duration						
AF Total duration	4.0±0.6	20.6±5.3*	1.5±0.2	6.6±2.5* ^{\$}	3.7±1.0	30.0±8.7*

All values are mean ± SEM. Two-way RM ANOVA. *p<0.05 vs. 0 cm H₂O within each group (Control, Senktide, Wash out). \$p<0.05 vs. 20 cm H₂O in Control and Wash out. #p<0.05 vs. 0 cm H₂O in Control and Wash out.

Supplementary References:

1. Pinto F.M., Almeida T.A., Hernandez M., Devillier P, Advenier C., Candenas M.L. mRNA expression of tachykinins and tachykinin receptors in different human tissues. *Eur J Pharmacol.* 494, 233–239 (2004). doi:10.1016/j.ejphar.2004.05.016