NS19504: a novel BK channel activator with relaxing effect on bladder smooth muscle spontaneous phasic contractions

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Supplemental Materials and Methods

Selectivity testing

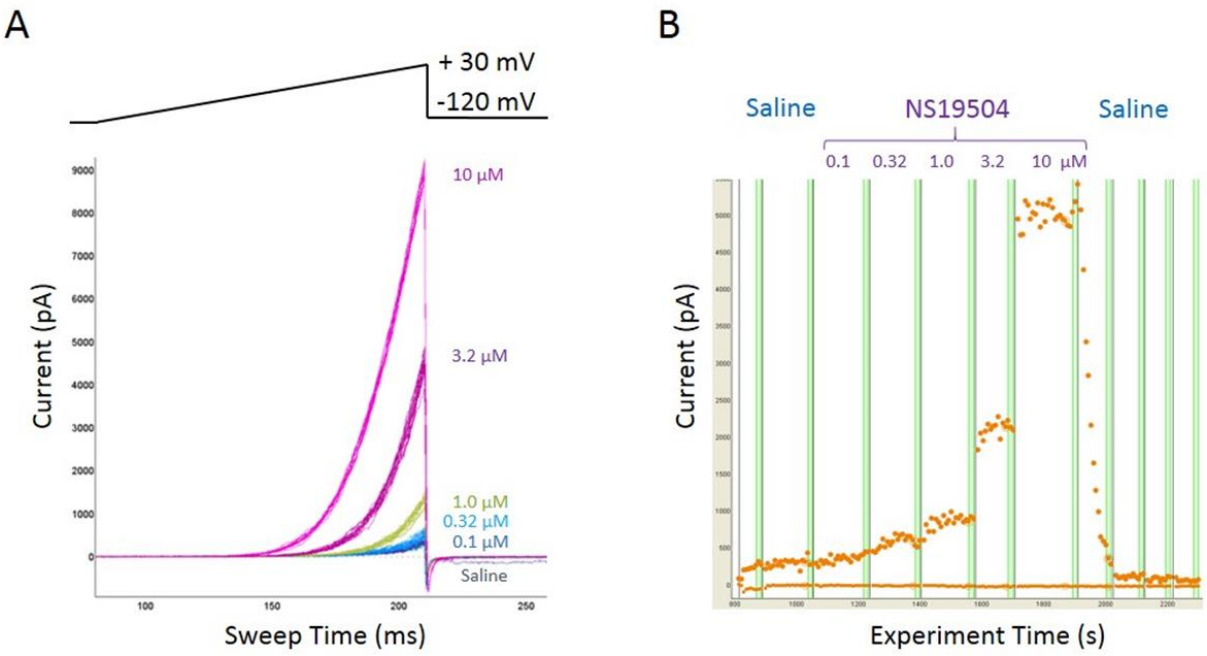
DRG neurons (Na_v and Ca_v channels):

DRG neurons were isolated from adult male Sprague Dawley rats by standard procedures and incubated for 1-2 days in Hank Buffered Salt Solution (HBSS) before recordings of voltage-dependent Na^+ and Ca^{2+}-currents were performed. Na_v currents were recorded using a bath saline containing in mM: 40 NaCl, 4 KCl, 110 TEA-Cl, 2 $CaCl_2$, 1 $MgCl_2$ and 10 HEPES (pH 7.4 with NaOH). Ca_v currents were recorded using a bath saline containing in mM: 144 TEA-Cl, 10 $BaCl_2$, 1 $MgCl_2$ and 10 HEPES (pH 7.4 with CsOH). The pipette saline contained in mM: 140 L-aspartic acid, 10 CsCl, 4 NaCl, 2.7 $MgCl_2$, 0.041 $CaCl_2$, 0.1 EGTA and 10 HEPES (pH 7.2 with CsOH). Both Na_v and Ca_v currents were recorded in the whole cell voltage clamp mode by 15 ms steps to 0 mV elicited every 5 s. The voltage-dependent Na_v and Ca_v currents were not further characterized i.e. no differentiation of TTX-sensitive and -insensitive currents and no pharmacological isolation in T-, P/Q-, L-, and N Ca_v currents.

HEK293 cells (hIK, hSK3, hSK2, $Na_v1.2$):

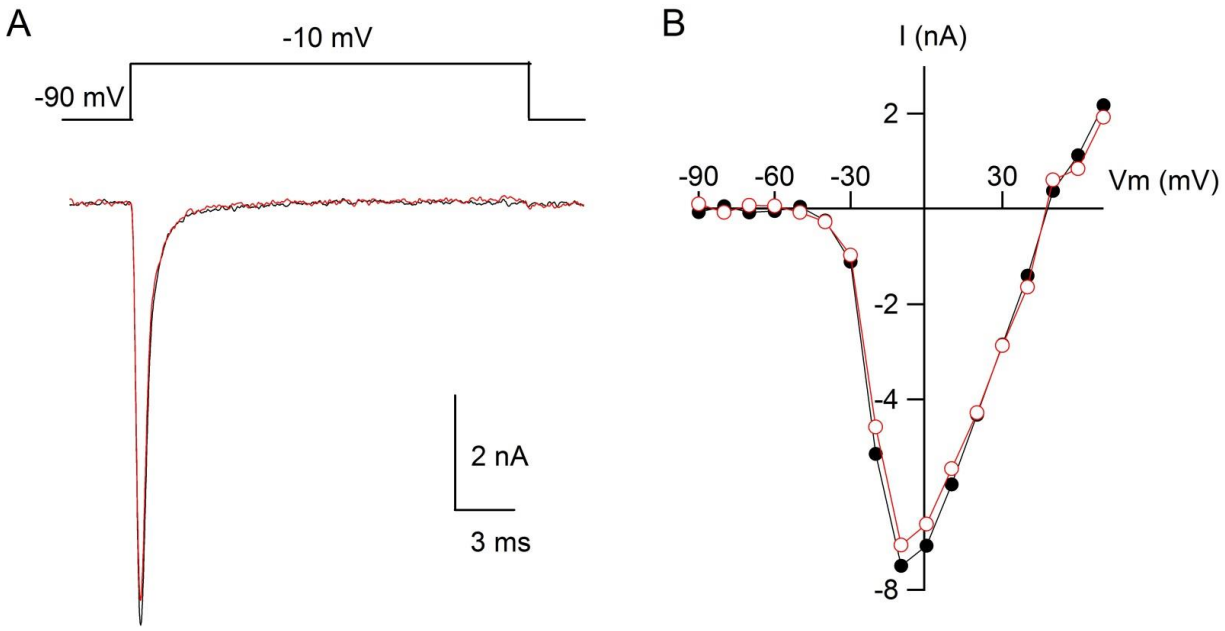
Cultured HEK293 cell lines stably expressing the ion channels in question were cultured and maintained as the stable hBK channel cell line. hIK, hSK3, hSK2 currents were recorded using the same experimental salines as for hBK and applying the same voltage ramp protocol. $Na_v1.2$ were recorded using the same extracellular saline as for hBK and an intracellular saline containing in mM: 146 KCl, 5.17 $CaCl_2$, 1.42 $MgCl_2$, 10 EGTA, 10 HEPES and 4 ATP (pH 7.2 with KOH).

Supplemental figure S1



Hit validation performed by automated electrophysiology using a 48-channel QPatchHTX screening station. The BK expressing HEK293 cell line were the same as used in the FLIPR HTS screen (Fig. 1, 2, and 3 in main text). The extracellular and intracellular buffers used on the QPatch were the same as used for the manual electrophysiology experiments, except that 24 mM KCl was substituted with 24 mM KF in the intracellular saline in order to improve giga-seal formation. A representative QPatchHTX concentration-response experiment with NS19504 is shown. BK currents were elicited by repeatedly applying voltage ramps from -120 mV to +30 mV in 150 ms every 10 s. After giga-seal formation and break-through to the whole cell configuration, extracellular buffer was added to record the current response during the saline period. This was followed by recording of the current responses to five cumulative additions of NS19504 spanning from 0.1 to 10 µM and finally a complete wash-out of the compound was obtained during addition of extracellular buffer. Fig 1A shows the overlay of the current traces during the saline period as well as during each of the five concentrations of NS19504. Fig. 1B shows the time course of the entire experiment measured at two different voltages (-100 mV and +28 mV) including 4 periods with addition of extracellular buffer at the end of the experiment, which completely reversed the current to control levels. The black vertical lines indicate the timing of liquid addition to the QPlate and the green vertical lines show the data points used in calculations of the compound effects.

Supplemental figure S2



Test for effects on rNav1.2 channels in whole-cell experiments

Whole-cell patch clamp experiments were performed using similar salines as for experiments with hBK but the pipette saline contained 4 mM ATP and a free concentrations Ca^{2+} of 0.1 µM. Voltage steps of 20 ms duration to potentials between -90 mV and +70 mV (10 mV increments) were applied every 2 seconds from a holding potential of -90 mV. A) Current traces recorded upon a step to -10 mV before (black) and in the presence (red) of 10 uM NS19504. B) IV curves measured from the peak currents before (filled black circles) and in the presence of 10 µM NS19504 (open red circles). Traces were leak subtracted off line using a P/5 protocol (step fraction = 0.1; leak holding = -100 mV)

Supplemental Table S1

In vitro receptor binding selectivity of NS19504. Significant results defined as ≥ 50% inhibition or stimulation. NS19504 was tested at a concentration of 10 μM.

Target	Species	Radioligand	% Inhibition at 10 μM
Adenosine A_1	Human	[^{3}H]DPCPX	3
Adenosine A_{2A}	Human	[^{3}H]CGS-21680	8
Adenosine A_3	Human	[^{125}I]AB-MECA	13
Adrenergic α_{1A}	Rat	[^{3}H]Prazosin	1
Adrenergic α_{1B}	Rat	[^{3}H]Prazosin	17
Adrenergic α_{1D}	Human	[^{3}H]Prazosin	11
Adrenergic α_{2A}	Human	[^{3}H]MK-912	8
Adrenergic β_1	Human	[^{125}I]Cyanopindolol	9
Adrenergic β_2	Human	[^{3}H]CGP-12177	6
Androgen (Testosterone) AR	Rat	[^{3}H]Mibolerone	7
Bradykinin B_1	Human	[^{3}H](Des-Arg^{10})-Kallidin	15
Bradykinin B_2	Human	[^{3}H]Bradykinin	-13
Ca^{2+} Chan. (L-type), Benzothiazepine	Rat	[^{3}H]Diltiazem	15
Ca^{2+} Chan. (L-type), Dihydropyridine	Rat	[^{3}H]Nitrendipine	12
Ca^{2+} Chan. (N-type)	Rat	[^{125}I]ω-Conotoxin GVIA	8
Dopamine D_1	Human	[^{3}H]SCH-23390	9
Dopamine D_{2S}	Human	[^{3}H]Spiperone	10
Dopamine D_3	Human	[^{3}H]Spiperone	11
Dopamine $D_{4,2}$	Human	[^{3}H]Spiperone	8
Endothelin ET_A	Human	[^{125}I]Endothelin-1	-1
Endothelin ET_B	Human	[^{125}I]Endothelin-1	0

Epidermal Growth Factor (EGF)	Human	[^{125}I]EGF (human)	3
Estrogen ERα	Human	[^{3}H]Estradiol	4
G Protein-Coupled Receptor GPR103	Human	[^{125}I]QRFP-43	0
$GABA_A$, Agonist Site	Rat	[^{3}H]Muscimol	11
$GABA_A$, Benzodiazepine, Flunitrazepam	Rat	[^{3}H]Flunitrazepam	3
$GABA_{B1A}$	Human	[^{3}H]CGP-54626	15
Glucocorticoid	Human	[^{3}H]Dexamethasone	3
Glutamate, Kainate	Rat	[^{3}H]Kainic Acid	4
Glutamate, NMDA, Agonism	Rat	[^{3}H]CGP-39653	20
Glutamate, NMDA, Glycine	Rat	[^{3}H]MDL-105519	-2
Glutamate, NMDA, Phencyclidine	Rat	[^{3}H]TCP	-12
Histamine H_1	Human	[^{3}H]Pyrilamine	16
Histamine H_2	Human	[^{125}I]Aminopotentidine	16
Histamine H_3	Human	[^{3}H]R(-)-α-Methylhistamine	-17
Imidazoline I_2, Central	Rat	[^{3}H] Idazoxan	-4
Interleukin IL-1	Mouse	[^{125}I]IL-1β	-2
Leukotriene, Cysteinyl $CysLT_1$	Human	[^{3}H]LTD_4	-5
Melatonin MT_1	Human	[^{125}I]2-Iodomelatonin	20
Muscarinic M_1	Human	[^{3}H]N-Methylscopolamine	6
Muscarinic M_2	Human	[^{3}H]N-Methylscopolamine	-4
Muscarinic M_3	Human	[^{3}H]N-Methylscopolamine	6
Neuropeptide Y Y_1	Human	[^{125}H]Peptide YY	-7
Neuropeptide Y Y_2	Human	[^{125}H]Peptide YY	-14
Nicotinic Acetylcholine	Human	[^{125}I]Epibatidine	0
Nicotinic Acetylcholine α_1, Bungarotoxin	Human	[^{125}I]α-Bungarotoxin	1
Opiate δ (OP1, DOP)	Human	[^{3}H]Naltrindole	9

Opiate κ (OP2, KOP)	Human	[^{3}H]Diprenorphine	16
Opiate μ (OP3, MOP)	Human	[^{3}H]Diprenorphine	-11
Phorbol Ester	Mouse	[^{3}H]PDBu	6
Platelet Activating Factor (PAF)	Human	[^{3}H]PAF	10
Potassium Channel [K_{ATP}]	Hamster	[^{3}H]Glyburide	1
Potassium Channel HERG	Human	[^{3}H]Astemizole	19
Prostanoid EP_4	Human	[^{3}H]Prostaglandin E_2 (PGE_2)	0
Purinergic P_{2X}	Rabbit	[^{3}H]α, β-Methylene-ATP	14
Purinergic P_{2Y}	Rat	[^{35}S]ATP-αS	4
Rolipram	Rat	[^{3}H]Rolipram	-16
Serotonin 5-HT_{1A}	Human	[^{3}H]8-OH-DPAT	5
Serotonin 5-HT_3	Human	[^{3}H]GR-65630	9
Sigma σ_1	Human	[^{3}H]Haloperidol	**59**
Sigma σ_2	Rat	[^{3}H]Ifenprodil	18
Sodium Channel, Site 2	Rat	[^{3}H]Batrachotoxin	32
Tachykinin NK_1	Human	[^{3}H]SR-140333	0
Thyroid Hormone	Rat	[^{125}I]Triiodothyronine	10
Transporter, Dopmanine (DAT)	Human	[^{125}I]RTI-55	**75**
Transporter, GABA	Rat	[^{3}H]GABA	-5
Transporter, Norepinephrine (NET)	Human	[^{125}I]RTI-55	**74**
Transporter, Serotonin (SERT)	Human	[^{3}H]Paroxetine	6