



Legend:

Real-Time quantitative RT-PCR analysis. Relative expression of TREK-1, TREK-2, TRAAK, TRPV1, TRPV2, TRPV3 and TRPV4 channels in DRG from TREK-1^{+/+} and TREK-1^{-/-} mice. Data were normalized to Cyclophilin D expression level which was arbitrary fixed to 1. Bars indicate the standard deviation (n=4; P = 5 10⁻⁷, P= 6 10⁻³, all others P are > 0.1, t-test).

Material and methods:

Total RNAs from DRG of TREK-1^{+/+} and TREK-1^{-/-} mice were isolated with the NucleoSpin RNA II kit (Macherey Nagel). 2 µg of total RNAs were used for reverse transcription reaction carried out with the Superscript II reverse transcriptase (Invitrogen) according to the protocol of the supplier. Real-time PCR analysis (SYBR green mastermix plus, Eurogentec) was performed to estimate the level of expression of TREK-1, TREK-2, TRAAK, TRPV1, TRPV2, TRPV3 and TRPV4, the endogenous reference was the cyclophilin D (CycloD). Real-time PCR assays (triplicate for each target gene tested) were performed in 96-well plates on an ABI GenAmp 5700 apparatus. Data were analyzed using the comparative Ct method where the amount of target was normalized to the endogenous reference (User bulletin N°2 Applied Biosystems). Primers used for the different amplicons were as follows:

TREK-1 forward TTTTCCTGGTGGTCGTCCTC;

TREK-1 reverse GCTGCTCCAATGCCTTGAAC;

TREK-2 forward CCGGAATTACTCTCTGGATGAAGA;

TREK-2 reverse CATGGCTGTGCTGGAGTTGT;

TRAAK-Forward GGGCGCAAACCCAGAAA;

TRAAK- Reverse CCCAGGTTCCAAGCTGATGA

TRPV1-Forward CGTGCACTCCTCCCTTTATGA;

TRPV1-Reverse CGATCACCTCCAGCACTGAA;

TRPV2-Forward CGCTTCCTGCTGGTCTACCT;

TRPV2-Reverse CCTCCCGGCTCAAGCTTACT;

TRPV3-Forward GGCCCTGACACACAAAATGAG;

TRPV3-Reverse ATGCATGTGGCCAGATGA;

TRPV4-Forward CAGCCGCACATCGTCAACTA;

TRPV4-Reverse TCGCCTCATGTCAGCTTTCTT;

CycloD forward GGCTCTTGAAATGGACCCTTC;

CycloD reverse CAGCCAATGCTTGATCATATTCTT.