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Cloning and expression of human TRAAK, a polyunsaturated fatty acids-activated and mechano-sensitive K^+ channel

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Abstract The two P domain hTRAAK K^+ channel has been cloned from human brain. hTRAAK cDNA encodes a 393 amino acid polypeptide with 88% of homology with its mouse counterpart. The hTRAAK gene has been mapped to chromosome 11q13 and the study of its organization indicates that the hTRAAK open reading frame is contained in six exons. hTRAAK is expressed abundantly in brain and placenta. In COS cells, hTRAAK currents are K^+-selective, instantaneous and non-inactivating. These currents are insensitive to the classical K^+ channels blockers 4-aminopyridine, tetraethylammonium, barium and quinidine, but are strongly stimulated by application of arachidonic acid as well as other polyunsaturated fatty acids. hTRAAK can also be activated by a stretch of the membrane.

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Key words: Baseline K^+ channel; Two P domain K^+ channel; Mechano-sensitivity

1. Introduction

Mammalian K^+ channels with four transmembrane segments and two pore domains in tandem form a novel class of K^+ channels. To date, seven members of this family have been cloned [1–8]. Despite an overall similar structure, the sequence identity between these channels is low (less than 30%). TWIK-1 and TWIK-2 are weak inward rectifying K^+ channels. TASK-1 and TASK-2 are outward rectifying K^+ channels sensitive to variations of extracellular pH in a narrow physiological range. TREK-1, another outward rectifying channel, is activated by membrane stretch, polyunsaturated fatty acids, intracellular acidosis and inhalational anesthetics [9–11]. All these two P domain K^+ channels have a widespread tissue distribution. TRAAK, the second cloned mechano-gated and polyunsaturated fatty acids-activated K^+ channel, is the sole to be expressed exclusively in the mouse central nervous system [5,12,13]. Here we describe the cloning and functional expression of human TRAAK. hTRAAK shares all the functional properties of its mouse counterpart and is also mainly expressed in neuronal tissues [5,12].

2. Materials and methods

2.1. cDNA cloning

Sequences of two P domain K^+ channels were used to search homologs in public DNA databases by using the Blast program [14]. This led to the identification of a genomic sequence (GenBank accession number AC005848) which showed significant similarities with mouse TRAAK. Two oligonucleotides were designed from this genomic sequence corresponding to the equivalent sequences flanking the first initiation codon and the stop codon of mTRAAK: sense strand: 5′-AGAATTCGCGCCATGCGCAGCACCACG-3′ and antisense strand: 5′-TTTCTCGAGGCCCGGCCAGGGATCCTG-3′ introducing *Eco*RI and *Xho*I restriction sites, respectively. The entire coding sequence was amplified from human brain cDNA by PCR using these primers and a low-error rate DNA polymerase, then subcloned into the pIRES-CD8 vector to give pIRES-CD8-hTRAAK. Inserts from different independent PCR-ligation experiments were sequenced on both strands and found to be identical.

2.2. Chromosomal mapping

The Genebridge 4 RH DNA panel (Research Genetics) was screened by PCR using primers deduced from intron 5 (sense primer: 5′-ACCCAGTGGAGGAGCCCTTC-3′) and exon 6 (antisense primer: 5′-GAGGCCCGGCCAGGGATCCTG-3′). PCR conditions were 39 cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C. PCR products were separated by electrophoresis on agarose then transferred onto charged nylon membranes. Blots were probed with a ^{32}P-labeled oligonucleotide 5′-CCAGGCTGCCAGCTGGACRG-3′. The results were analyzed by using the RH-MAPPER program at Whitehead Institute (http://www.genome.wi.mit.edu).

2.3. RT-PCR experiment

Multiple Tissue cDNA panels (Clontech) were used as template according to the manufacturer's protocol. Sequences of primers were sense primer: 5′-CTCAGTGCTCACCACCATCG-3′ (exon 5) and antisense primer: 5′-GAGGCCCGGCCAGGGATCCTG-3′ (exon 6). The PCR conditions were 34 cycles of 30 s at 94°C, 30s at 55°C, and 1 min at 72°C. PCR products were separated, transferred, and probed as described for chromosomal mapping.

2.4. Cell culture and transfection

COS-7 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. The pIRES-cD8-hTRAAK plasmid was transfected using the classical DEAE dextran procedure. The positive cells were visualized 48 h after transfection using the anti-CD8 antibody-coated bead method.

2.5. Electrophysiology

For whole cell and outside-out experiments, the pipette solution (INT) contained 150 mM KCl, 3 mM $MgCl_2$, 5 mM EGTA and 10 mM HEPES, pH 7.2 adjusted with KOH. The bath solution (EXT) contained 150 mM NaCl, 5 mM KCl, 3 mM $MgCl_2$, 1 mM $CaCl_2$ and 10 mM HEPES, pH 7.4 adjusted with NaOH. For inside-out experiments the solution in the pipette was EXT and the bath solution was INT. The EXT K^+-rich solution contained 150 mM KCl instead of 150 mM NaCl. To study the ion selectivity, current–voltage relationships were obtained at different [K^+]ext. For each concentrations, NaCl was substituted in the EXT solution with equimolar KCl.

All products were obtained from Sigma. Fatty acids were dissolved in the ethanol at the concentration of 100 mM, flushed under argon and kept at −20°C for a week. Mechanical stimulation was applied through an open loop pressure generating system and monitored at the level of the patch pipette throughout the experiment by a calibrated pressure sensor.

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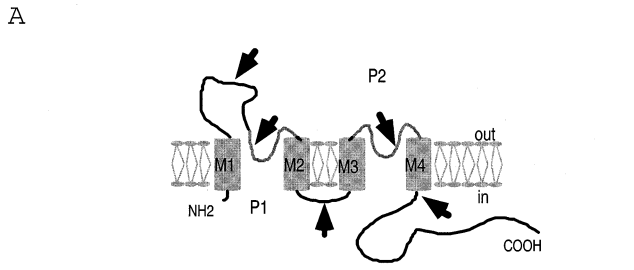
3. Results and discussion

3.1. Primary structure and gene organization and mapping

Searches of DNA databases using the Blast sequence alignment program [14] led to the identification of human sequences restrained to a single genomic contig. The analysis of these sequences suggested the presence of introns and exons forming a gene encoding a two P domain K^+ channel. Oligonucleotides were deduced from the potential exon sequences and used to PCR-amplify a DNA fragment containing the corresponding open reading frame from brain cDNAs. This ORF is 1182 nucleotides long and encodes a 393 amino acid polypeptide (Fig. 1). This protein is closely related to the mouse TRAAK channel with 82% of identity and 88% of homology. This homology level, together with the conserved tissue distribution and functional properties that are shown hereafter, indicates that the novel channel is the human counterpart of the mouse TRAAK. Fig. 1B shows the TRAAK cDNA sequence and genomic organization in human. The ORF is composed of six exons. The transmembrane segment M1 is encoded by exon 1, M2 by exon 3, M3 by exon 4, and M4 by exon 5. The second exon encodes the carboxy-terminal part of the M1P1 interdomain and the sixth one encodes the large carboxy-terminal part of the channel (Fig. 1A,B). Introns are short except the first one that is more than 3.8 kb long (Fig. 1B). A gene encoding another mammalian two P domain K channel, *TWIK-1*, has already been characterized [15]. The genomic organizations of *TRAAK* and *TWIK-1* are quite different because *TWIK-1* contains only three exons separated by two large introns. However, a common feature between the two genes is the presence of an intron in the first pore-domain P1. The intron site is between the first and the second nucleotide of the codon for the first glycine residue of the pore signature sequence GYG [15]. An intron in the same position is found in 20 genes among the 36 examined that encode potential two P domain K^+ channels in the nematode *Caenorhabditis elegans* [16]. The significance of this conserved intron position is not known, however it is worth to note that this intron has been conserved in mammals where it might eventually have the same role as in the nematode.



B

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    ATGCGCAGCACCACGCTCCTGGCCCTGCTGGCGCTGGTCTTGCTTTACTTGGTGTCTGGTGCCCTGGTGTTCCGG
1     M  R  S  T  T  L  L  A  L  L  A  L  V  L  L  Y  L  V  S  G  A  L  V  F  R

    GCCCTGGAGCAGCCCCACGAGCAGCAGGCCCAGAGGGAGCTGGGGGAGGTCCGAGAGAAGTTCCTGAGGGCCCAT
26    A  L  E  Q  P  H  E  Q  Q  A  Q  R  E  L  G  E  V  R  E  K  F  L  R  A  H

    CCGTGTGTGAGCGACCAGGAGCTGGGCCTCCTCATCAAGgtgcgt/intron~3.8kb/cctccttctgcacctt
51    P  C  V  S  D  Q  E  L  G  L  L  I  K

    gtcctgcagGAGGTGGCTGATGCCCTGGGAGGGGGTGCGGACCCAGAAACCAACTCGACCAGCAACAGCAGCCAC
64              E  V  A  D  A  L  G  G  G  A  D  P  E  T  N  S  T  S  N  S  S  H

    TCAGCCTGGGACCTGGGCAGCGCCTTCTTTTTCTCAGGGACCATCATCACCACCATCGgtgggg/intron~0.1
86    S  A  W  D  L  G  S  A  F  F  F  S  G  T  I  I  T  T  I

    2kb/cttcctgcatcgccccctctgcccagGCTATGGCAATGTGGCCCTGCGCACAGATGCCGGGCGCCTCTTCTG
105                                 G  Y  G  N  V  A  L  R  T  D  A  G  R  L  F  C

    CATCTTTTATGCGCTGGTGGGGATTCCGCTGTTTGGGATCCTACTGGCAGGGGTCGGGGACCGGCTGGGCTCCTC
121   I  F  Y  A  L  V  G  I  P  L  F  G  I  L  L  A  G  V  G  D  R  L  G  S  S

    CCTGCGCCATGGCATCGGTCACATTGAAGCCATCTTCTTGgtgagc/intron~0.19kb/tcgtgcctactgcc
146   L  R  H  G  I  G  H  I  E  A  I  F  L

    ccatcccgcagAAGTGGCACGTGCCACCGGAGCTAGTAAGAGTGCTGTCGGCGATGCTTTTCCTGCTGATCGGCT
159             K  W  H  V  P  P  E  L  V  R  V  L  S  A  M  L  F  L  L  I  G

    GCCTGCTCTTTGTCCTCACGCCCACGTTCGTGTTCTGCTATATGGAGGACTGGAGCAAGCTGGAGGCCATCTACT
180   C  L  L  F  V  L  T  P  T  F  V  F  C  Y  M  E  D  W  S  K  L  E  A  I  Y

    TTGTCATAGTGACGCTTACCACCGTGGGCTTTGGCGACTATGTGGCCGgtgagg/intron~0.46kb/tctacc
205   F  V  I  V  T  L  T  T  V  G  F  G  D  Y  V  A

    ttccctggtgggtacccagGCGCGGACCCCAGGCAGGACTCCCCGGCCTATCAGCCGCTGGTGTGGTTCTGGATC
221                       G  A  D  P  R  Q  D  S  P  A  Y  Q  P  L  V  W  F  W  I

    CTGCTCGGCCTGGCTTACTTCGCCTCAGTGCTCACCACCATCGGGAACTGGCTGCGAGTAGTGTCCCGCCGCACT
240   L  L  G  L  A  Y  F  A  S  V  L  T  T  I  G  N  W  L  R  V  V  S  R  R  T

    CGGGCAGAGgtaggc/intron~1.1kb/agggctgctttccctctccgtgcagATGGGCGGCCTCACGGCTCAG
265   R  A  E                                                 M  G  G  L  T  A  Q

    GCTGCCAGCTGGACTGGCACAGTGACAGCGCGCGTGACCCAGCGAGCCGGGCCCGCCGCCCGCCGCGGAGAAG
275   A  A  S  W  T  G  T  V  T  A  R  V  T  Q  R  A  G  P  A  A  P  P  P  E  K
    GAGCAGCCACTGCTGCCTCCACCGCCCTGTCCAGCGCAGCCGCTGGGCAGGCCCCGATCCCCTTCGCCCCCGAG
300   E  Q  P  L  L  P  P  P  C  P  A  Q  P  L  G  R  P  R  S  P  S  P  P  E
    AAGGCTCAGCCGCCTTCCCGCCCACGGCCTGGCCCCTGGATTATCCCAGCGAGAACCTGGCCTTCATCGACGAG
325   K  A  Q  P  P  S  P  P  T  A  S  A  L  D  Y  P  S  E  N  L  A  F  I  D  E
    TCCTCGGATACGCAGAGCGAGCGCGGCTGCCCGCTGCCCCCGCGCCGAGAGGTCGCCGCCGCCCAAATCCCCCC
350   S  S  D  T  Q  S  E  R  G  C  P  L  P  R  A  P  R  G  R  R  R  P  N  P  P
    AGGAAGCCCGTGCCGGCCCCGCGGCCCCGGGCGTCCCCGAGACAAAGGCGTGCCGGTGTAG
375   R  K  P  V  R  P  R  G  P  G  R  P  R  D  K  G  V  P  V  B
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Fig. 1. A: Topology of human TRAAK. The relative positions of introns are indicated by arrows. B: TRAAK cDNA and genomic sequences. Coding sequence is in upper cases and genomic sequence corresponding to splice sites in lowercase. M1 to M4 transmembrane segments are in light gray, and P1 and P2 pore-domains in dark gray. The size of introns is indicated in bold.

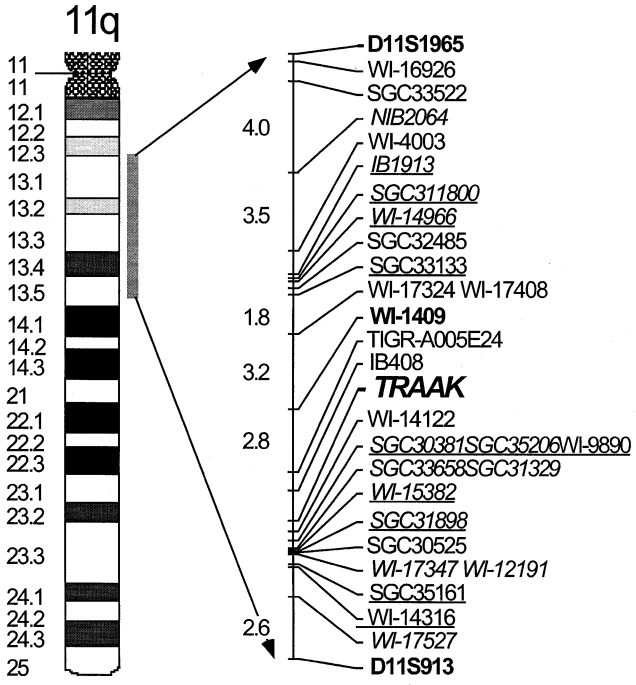

Fig. 2. Idiogram of human G-banded chromosome 11q and location of the *TRAAK* gene relative to markers mapped in the Genebridge 4 RH panel.

The chromosomal assignment of human TRAAK was carried out by radiation hybrid panel analysis. As shown in Fig. 2, the gene encoding TRAAk lies on chromosome 11q and is 5.34 cRays telomeric to the framework marker WI-1409 (Logarithm of odds score >21). Although radiation hybrid maps are not anchored to the cytogenic maps, the most likely localization of the TRAAK gene is 11q13. *KCNK7* that encodes a related two P domain K^+ channel has been mapped on chromosome 11q13 at 6.4 cRays telomeric to WI-1409. This suggests that *TRAAK* and *KCNK7* are very close each other [8].

3.2. Tissue distribution

The expression of TRAAK in various adult human tissues was examined by RT-PCR analysis. As shown in Fig. 3, TRAAK is expressed at the highest levels in brain and placenta. Only very faint signals were obtained in testis, small

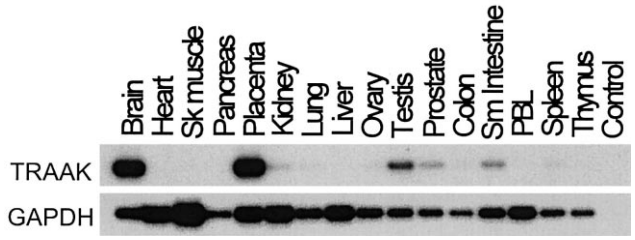

Fig. 3. Tissue distribution of TRAAK in human adult tissues as determined by RT-PCR analysis. The amplified products were analyzed by Southern blot. GAPDH was amplified to check the integrity of cDNA.

intestine, prostate and kidney. TRAAK was not detected in the mouse placenta [5]. The reason of this discrepancy is not known. In the mouse, TRAAK is highly expressed only in neuronal tissues, as in humans. In situ hybridization [5] and immunohistology [13] have demonstrated that mouse TRAAK is specifically expressed in neuronal cells. The tissue distribution shown in Fig. 3 suggests that TRAAK could have the same restricted pattern of expression in humans.

3.3. Biophysical properties

The electrophysiological experiments were performed in transiently transfected COS cells. hTRAAK current has no apparent voltage activation threshold, is time-independent and non-inactivating (Fig. 4A). The *I–V* curve is outward rectifying and noisy at positive potentials (Fig. 4A,B). In a physiological condition (5 mM K^+ ext), hTRAAK current reverses at the predicted value for the K^+ equilibrium (-87.1 ± 1.2 mV, $n = 6$). When the external Na^+ is substituted with K^+ the reversal potential closely follows the value for the K^+ equilibrium (Fig. 4C). The slope of the regression line is 58.6 ± 0.6 mV per 10-fold change in $[K^+]$ext ($n = 6$) which is in agreement with the Nernst equation for a K^+ selective channel. In a symmetrical condition (155 mM K^+ ext), the *I–V* curve is less outward rectifying (Fig. 4A,B) and reverses at 0.8 ± 1.1 mV ($n = 6$). The pharmacological properties of hTRAAK were investigated in the whole cell configuration. hTRAAK is insensitive to the classical K^+ channel blockers quinidine (100 μM), 4AP (3 mM), TEA (10 mM), barium (1 mM) and glibenclamide (10 μM). Some members of the two P domains K^+ channels family (TREK-1 and TASK-1) have been shown to be opened by volatile general anesthetics [11]. We then investigated the effect of chloroform on hTRAAK. Application of chloroform (0.8 mM, $n = 8$) has no effect on the channel activity. The single channel properties of hTRAAK are illustrated in Fig. 1D. At the microscopic level, in physiological K^+ condition, the hTRAAK current is outwardly rectifying and is characterized by a flickering behavior.

The mouse TRAAK is stimulated by the poly-unsaturated fatty acids [5,12]. Fig. 5A,B shows that human TRAAK activity is also potentiated by 10 μM of arachidonic acid (AA) in the whole cell configuration ($630 \pm 101\%$ at 0 mV, $n = 19$). This activation is completely reversible upon washout (Fig. 5A, inset). In physiological condition, the AA-induced current is outward rectifying and reverses at -80.6 ± 0.9 mV, $n = 7$ (Fig. 5A). When the external Na^+ is substituted with K^+, the current becomes linear and the reversal potential shifts to -0.6 ± 0.8 mV, $n = 7$ (Fig. 5B). hTRAAK is also activated by the poly-unsaturated fatty acid docosahexaenoate (10 μM, $n = 3$) but insensitive to the saturated fatty acids myristate, palmitate, stearate, arachidate (10 μM, $n = 6$–8). Moreover, AA derivatives with an alcohol or a methyl ester substituted in the carboxylic function are inactive ($n = 5$). The AA-stimulation of hTRAAK remains when the patch is excised (Fig. 5C). The AA-induced current observed in the outside-out configuration is outward rectifying and reverses at the K^+ reversal potential (Fig. 5C, inset). We have demonstrated that the two P domains K^+ channels activated by the polyunsaturated fatty acids (mTREK-1 and mTRAAK) are mechano-gated K^+ channels. Channel opening is mediated by membrane deformation [10,12]. Fig. 5D illustrates the mechano-sensitivity of hTRAAK. In the inside-out patch configuration, channel activity is almost absent at atmospheric pressure. Application of negative pressure opens the channels in a dose-dependent manner. Taken together, these results demonstrate that human TRAAK shares the same biophysical and pharmacolog-

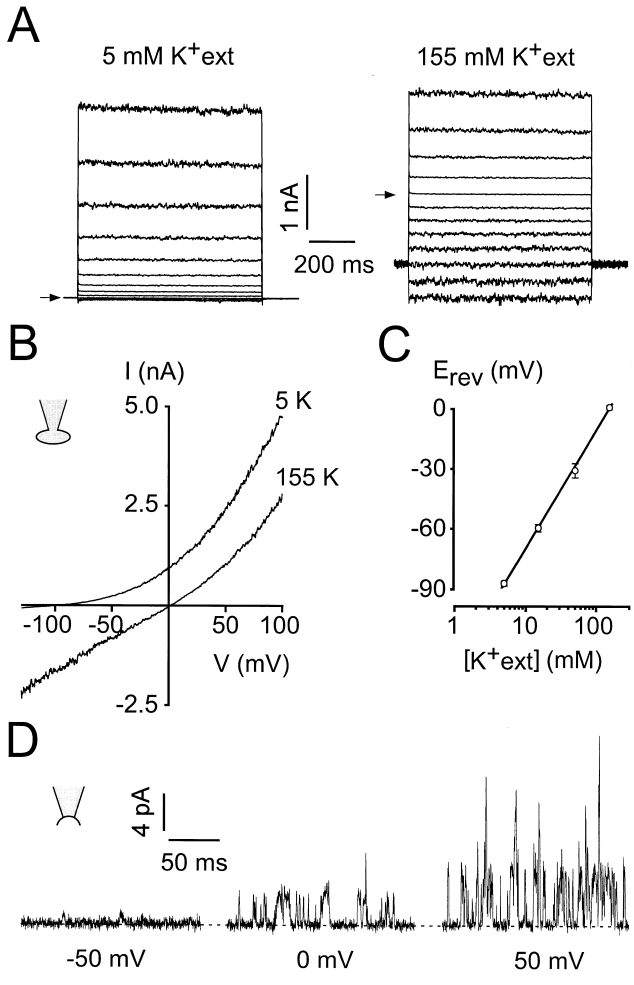

Fig. 4. Biophysical properties of the human TRAAK. A: Currents elicited by voltage pulses in 20 mV increments from −120 mV to 100 mV in a physiological condition (5 mM K^+ ext), left panel or in a symmetrical condition (155 mM K^+ ext), right panel. The holding potential is −80 mV and the zero current is indicated by horizontal arrows. B: current–voltage (*I–V*) curves of the hTRAAK in physiological and symmetrical K^+ conditions. The holding potential is −80 mV and voltage ramps of 800 ms in duration are applied every 10 s from −130 to 100 mV. C: Reversal potential of hTRAAK current (E_{rev}) as a function of $[K^+]$ext ($n = 6$). D: Single channel hTRAAK currents recorded in physiological K^+ condition, in the inside-out configuration at −50, 0 and 50 mV.

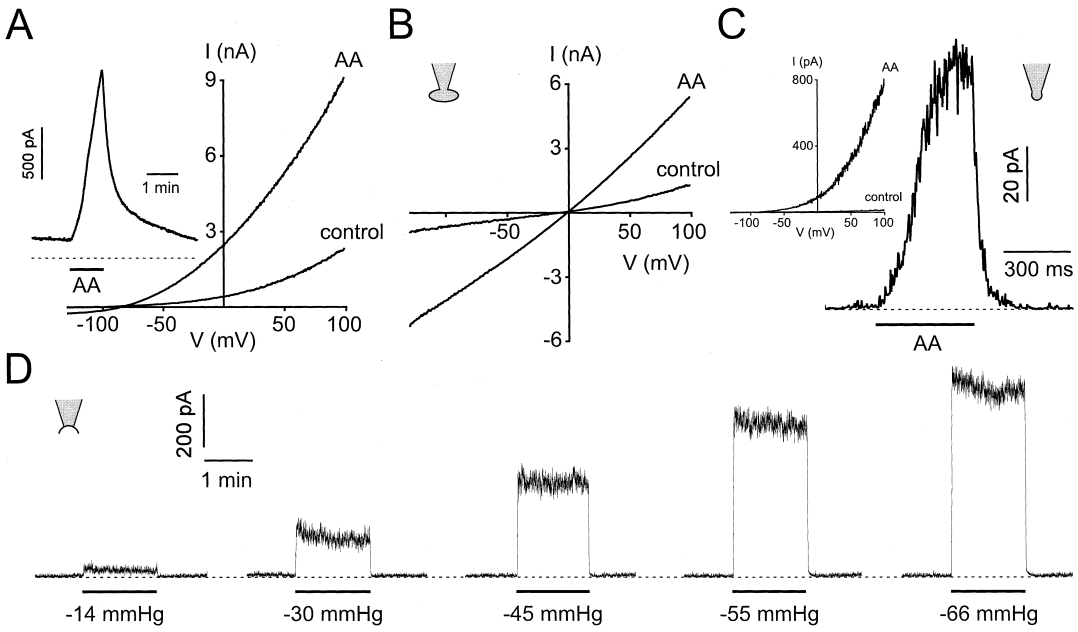

Fig. 5. hTRAAK is an AA-activated and a mechano-gated K^+ channel. A: Whole cell recording of a hTRAAK-transfected COS cell illustrating the effect of 10 μM AA. *I–V* curves were constructed using the same protocol as in Fig. 4B. The bath solution is EXT (5 mM K^+). The inset shows a typical current recorded at 0 mV during the application of 10 μM AA. The zero current is indicated by a horizontal dashed line (a different cell from A). B: *I–V* curve performed with the EXT K^+-rich solution. (same cell as in A). C: Outside-out patch clamp experiment showing the effect of 10 μM AA on a hTRAAK-transfected COS cell. The patch is maintained at 0 mV. The zero current is indicated by a horizontal dashed line. The inset shows the current–voltage relationship of such an experiment (a different cell from C). D: Graded reversible negative pressure activation of hTRAAK in physiological K^+ condition, in the inside-out configuration. The patch is held at 0 mV and the zero current is indicated by a dashed line.

ical properties as its mouse counterpart and has similar properties to K^+ currents previously identified in the central nervous system [17]. Together with hTREK-1, hTRAAK constitutes a novel class of AA- and stretch-activated K^+ channels in human.

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