Charest-Morin et al.: Supporting Information

Supplementary Materials and Methods

Novel bradykinin (BK) B_2 receptor (B_2R) vectors

The human B_2R vector was constructed following the amplification of the entire coding region of the exon 3 part of the human bradykinin B_2R gene (Leeb-Lundberg et al., 2005; 5'-CGTTTAAACGGGCCCTATGCTCAATGTCACCTTGC-3' was the sense PCR oligonucleotide primer and 5'- TTGGTACCGAGCTCGTCACTGTCTGCTCCCTGC-3' the anti-sense) from the genomic DNA of human umbilical vein endothelial cells extracted as directed using the Trizol reagent (Life Technologies). These primers were derived from Eggerickx et al., (1992). The pcDNA3.1 vector was digested with XbaI and BamHI (Life Technologies) for 2 h at 37°C. Both the PCR product and the plasmid were purified by migration on agarose gels, extracted from the gel using QIAEX II Extraction Kit (Qiagen) and assembled (Gibson Assembly Cloning Kit, New England Biolabs.). The sequence of the properly selected and amplified clone was verified (*Plateforme de Séquençage et de Génotypage, Centre de Recherche du CHU de Québec*). The result of sequencing is shown in Supplementary Fig. 1A and the predicted amino acid sequence, in Supplementary Fig. 1B. The human myc-B_2R vector was constructed by extracting the hu B_2R sequence from the hu B_2R vector using the following primers: 5'-CGTTTAAACGGGCCCTATGGAGCAGAAACTCATCTCAGAAGAGGATCTCAATGTCACCTTGCAAGG-3' (sense) and

5'-TTGGTACCGAGCTCGTCACTGTCTGCTCCCTGC-3' (antisense). The pcDNA3.1 was digested with XbaI and BamHI, extracted from agarose gel, assembled and analyzed precisely as described above.

Rat DNA was isolated from a primary culture of Leydig cells (kind gift from Dr. Jacques Tremblay, CHU de Québec) using the QIAamp DNA Blood kit (Qiagen). The rat B_2R vector was constructed following the amplification of the entire coding region of the exon 3 part of the bradykinin B_2R gene (McEachern et al., 1991; 5'-CGTTTAAACGGGCCCTATGTTCAACATCACCACGCAAG -3' was the sense PCR oligonucleotide primer and 5'- TTGGTACCGAGCTCGTCACTGCTTGTTCCCCGC -3' the anti-sense) from the rat genomic DNA. For the rat myc-B_2R construction, the following primers were used: 5'-CGTTTAAACGGGCCCTATGGAGCAGAAACTCATCTCAGAAGAGGATCTGATGTTCAACATCACCACGCAAG - 3' (sense) and 5' - TTGGTACCGAGCTCGTCACTGCTTGTTCCCCGC - 3'. The pcDNA3.1 vector was digested with XbaI and BamHI (Life Technologies) for 2 h at 37°C. Both PCR product and the plasmid were purified by migration on agarose gels, extracted and assembled precisely as outlined above. Both rat B_2R and rat myc-B_2R vectors have been sequenced (Supplementary Fig. 2, A-B).

The B_2R coding sequences from conserved exon 3 are fully functional in various mammalian species; all possess a functional start codon initiating the synthesis of a GPCR with an N-terminal extracellular domain (McEachern et al., 1991; Eggerickx et al., 1992; Bachvarov et al., 1995), but they lack a codon start present in exon 2 that determines a further N-terminal extension of 27

amino acids in the human sequence (AbdAlla et al., 1996). Thus, the present myc-B_2R constructs lack this extension.

Supplementary references

AbdAlla S, Godovac-Zimmermann J, Braun A, Roscher AA, Müller-Esterl W, Quitterer U (1996). Structure of the bradykinin B_2 receptors' amino terminus. Biochemistry 35: 7514-759.

Bachvarov DR, Saint-Jacques E, Larrivée JF, Levesque L, Rioux F, Drapeau G, Marceau F (1995). Cloning and pharmacological characterization of the rabbit bradykinin B_2 receptor. J Pharmacol Exp Ther 275: 1623-1630.

Eggerickx D, Raspe E, Bertrand D, Vassart G, Parmentier M (1992). Molecular cloning, functional expression and pharmacological characterization of a human bradykinin B_2 receptor gene. Biochem Biophys Res Commun 187: 1306-1313.

McEachern AE, Shelton ER, Bhakta S, Obernolte R, Bach C, Zuppan P, Fujisaki J, Aldrich RW, Jarnagin K (1991). Expression cloning of a rat B_2 bradykinin receptor. Proc Natl Acad Sci USA 88: 7724-7728.

Supplementary figures

Suppl. Fig. 1

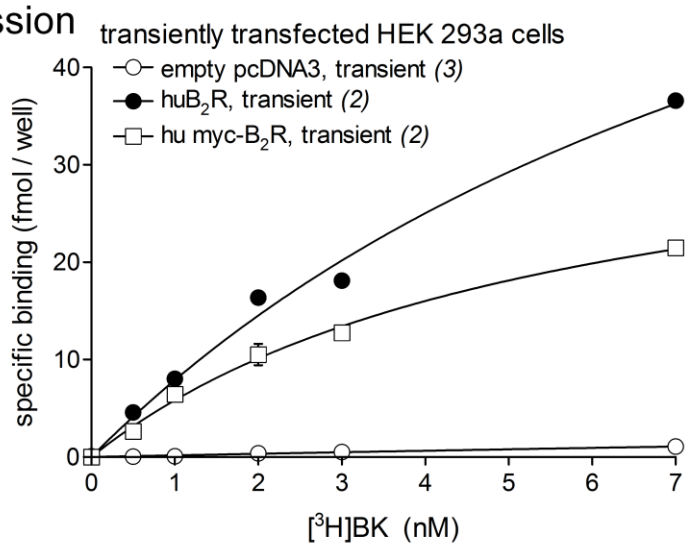
A. Coding sequence inserted into the hu myc-B_2R vector

ATGCTCAATGTCACCTTGCAAGGGCCCACTCTTAACGGGACCTTTGCCCAGAGCAAATGCCCCCAAGTG
GAGTGGCTGGGCTGGCTCAACACCATCCAGCCCCCCTTCCTCTGGGTGCTGTTCGTGCTGGCCACCCT
AGAGAACATCTTTGTCCTCAGCGTCTTCTGCCTGCACAAGAGCAGCTGCACGGTGGCAGAGATCTACCT
GGGGAACCTGGCCGCAGCAGACCTGATCCTGGCCTGCGGGCTGCCCTTCTGGGCCATCACCATCTCCA
ACAACTTCGACTGGCTCTTTGGGGAGACGCTCTGCCGCGTGGTGAATGCCATTATCTCCATGAACCTGT
ACAGCAGCATCTGTTTCCTGATGCTGGTGAGCATCGACCGCTACCTGGCCCTGGTGAAAACCATGTCCA
TGGGCCGGATGCGCGGCGTGCGCTGGGCCAAGCTCTACAGCTTGGTGATCTGGGGGTGTACGCTGCT
CCTGAGCTCACCCATGCTGGTGTTCCGGACCATGAAGGAGTACAGCGATGAGGGCCACAACGTCACCG
CTTGTGTCATCAGCTACCCATCCCTCATCTGGGAAGTGTTCACCAACATGCTCCTGAATGTCGTGGGCTT
CCTGCTGCCCCTGAGTGTCATCACCTTCTGCACGATGCAGATCATGCAGGTGCTGCGGAACAACGAGAT
GCAGAAGTTCAAGGAGATCCAGACGGAGAGGAGGGCCACGGTGCTAGTCCTGGTTGTGCTGCTGCTAT
TCATCATCTGCTGGCTGCCCTTCCAGATCAGCACCTTCCTGGATACGCTGCATCGCCTCGGCATCCTCT
CCAGCTGCCAGGACGAGCGCATCATCGATGTAATCACACAGATCGCCTCCTTCATGGCCTACAGCAACA
GCTGCCTCAACCCACTGGTGTACGTGATCGTGGGCAAGCGCTTCCGAAAGAAGTCTTGGGAGGTGTAC
CAGGGAGTGTGCCAGAAAGGGGGCTGCAGGTCAGAACCCATTCAGATGGAGAACTCCATGGGCACACT
GCGGACCTCCATCTCCGTGGAACGCCAGATTCACAAACTGCAGGACTGGGCAGGGAGCAGACAGTGA

B. Translation, hu myc-B_2R

M E Q K L I S E E D L N V T L Q G P T L N G T F A Q S K C P Q V E W L G W L N T I Q P P F L W V L F V
L A T L E N I F V L S V F C L H K S S C T V A E I Y L G N L A A A D L I L A C G L P F W A I T I S N N F D
W L F G E T L C R V V N A I I S M N L Y S S I C F L M L V S I D R Y L A L V K T M S M G R M R G V R W A
K L Y S L V I W G C T L L L S S P M L V F R T M K E Y S D E G H N V T A C V I S Y P S L I W E V F T N M
L L N V V G F L L P L S V I T F C T M Q I M Q V L R N N E M Q K F K E I Q T E R R A T V L V L V V L L L F
I I C W L P F Q I S T F L D T L H R L G I L S S C Q D E R I I D V I T Q I A S F M A Y S N S C L N P L V Y V I
V G K R F R K K S W E V Y Q G V C Q K G G C R S E P I Q M E N S M G T L R T S I S V E R Q I H K L Q D
W A G S R Q Stop

C. Expression



Supplementary figure 1. Validation of novel human B_2R vectors. A. Coding DNA sequence inserted into the hu B2R vector based on the pcDNA3.1 vector, as determined by post-hoc sequencing. The covered sequence is identical to the NCBI Reference Sequence: NP_000614.1. B. Translation of the DNA sequence into an amino acid sequence. The myc-B_2R construct is made by inserting 9 residues in the N-terminal region to reconstitute the 10-residue myc tag (underlined). C. Expression of hu B_2R and of its myc-tagged variant in HEK 293a cells as measured by saturation curves of [^{3}H]BK in cells (transfected with the indicated vector). Values are the mean of 2-3 values, each composed of duplicate determinations.

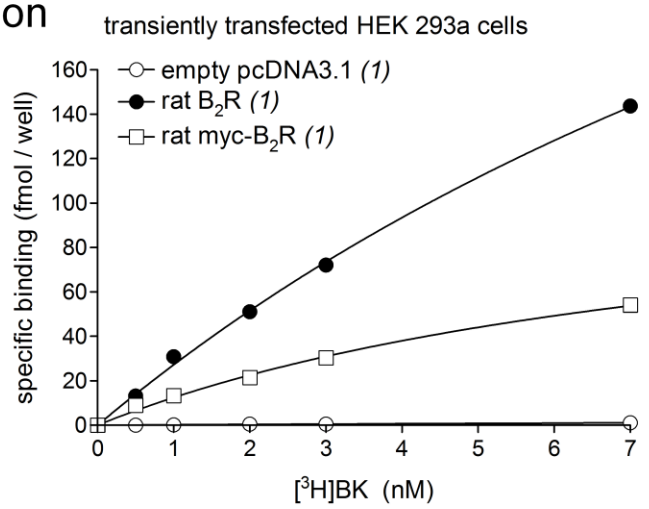
A. Coding sequence inserted into the *rat myc-B_2R vector*

ATG*GAGCAGAAACTCATCTCAGAAGAGGATCTG*ATGTTCAACATCACCACGCAAGCCCTGGGGTCTGCT
CATAACGGGACCTTTTCAGAGGTCAACTGCCCAGACACTGAGTGGTGGAGCTGGCTCAATGCCATCCAG
GCCCCCTTCCTCTGGGTCCTCTTCCTGCTGGCCGCACTGGAGAACATCTTTGTCCTCAGCGTGTTCTGT
CTGCACAAAACCAACTGCACTGTGGCTGAGATCTACCTGGGCAACCTGGCAGCCGCGGACCTCATCCT
GGCCTGCGGATTACCCTTCTGGGCCATCACCATCGCCAATAACTTCGACTGGCTGTTCGGAGAGGTGCT
GTGCCGCGTGGTGAATACCATGATCTACATGAACCTCTACAGCAGCATCTGCTTCCTGATGCTTGTGAGT
ATCGACCGATACCTGGCGCTGGTGAAGACCATGTCCATGGGCCGGATGCGCGGGGTACGCTGGGCCA
AACTGTACAGCCTGGTGATCTGGAGCTGTACGCTGCTTCTGAGTTCACCCATGTTGGTGTTCAGGACCA
TGAAGGACTACAGGGAAGAGGGCCACAACGTCACGGCCTGCGTCATTGTCTACCCGTCCCGCTCCTGG
GAGGTGTTCACCAACATGCTGCTGAACCTGGTGGGTTTCCTCCTACCCCTGAGTATCATCACCTTCTGCA
CGGTGCGAATCATGCAGGTGCTGAGGAACAACGAGATGAAGAAGTTCAAGGAGGTCCAGACGGAGAAG
AAGGCCACTGTGCTGGTGCTGGCTGTCCTGGGGCTCTTTGTGCTGTGTTGGTTTCCTTTCCAGATCAGC
ACCTTCCTGGACACGCTGCTGCGGCTTGGCGTGCTGTCGGGATGCTGGAACGAGCGTGCCGTGGATAT
TGTCACCCAGATCAGTTCCTACGTGGCCTATAGCAACAGCTGCCTCAACCCGCTGGTGTACGTGATTGT
GGGCAAGCGCTTCCGAAAGAAGTCCCGAGAGGTGTACCAGGCAATATGCCGGAAGGGAGGCTGCATG
GGAGAGTCCGTCCAGATGGAGAACTCCATGGGGACTCTGAGGACCTCTATCTCGGTGGACCGGCAGAT
CCACAAGCTGCAGGATTGGGCGGGGAACAAGCAGTGA

B. Translation

M *E Q K L I S E E D L*
MFNITTQALGSAHNGTFSEVNCPDTEWWSWLNAIQ**APFLWVLFLLAALENIFVLSVFCL**HKTNCTVAE**IYLG**
NLAAADLILACGLPFWAITIANNFDWLFGEVLCR**VVNTMIYMNLYSSICFLMLVSI**DRYLALVKTMSMGRMRG
VRWAK**LYSLVIWSCTLLLSSPMLVFRTM**KDYREEGHNVTACVIVYPSRSWE**VFTNMLLNLVGFLLPLSIITFC**
TVRIMQVLRNNEMKKFKEVQTEKK**ATVLVLAVLGLFVLCWFPFQISTFLD**TLLRLGVLSGCWNERAVD**IVTQI**
SSYVAYSNSCLNPLVYVIVGKRFRKKSREVYQAICRKGGCMGESVQMENSMGTLRTSISVDRQIHKLQDWA
GNKQ

C. Expression



Supplementary figure 2. Validation of novel rat B_2R vectors. A. Coding DNA sequence inserted into the rat B_2R vector based on the pcDNA3.1 vector, as determined by post-hoc sequencing. The covered sequence is identical to the NCBI Reference Sequence: NP_001257642. B. Translation of the DNA sequence into an amino acid sequence. Predicted transmembrane domains in boldface. The myc-B_2R construct was made by inserting the 10-residue myc tag (underlined). C. Expression of rat B_2R and of its myc-tagged variant in HEK 293a cells as measured by saturation curves of [^{3}H]BK in cells (transfected with the indicated vector). Presentation as in Supplementary figure 1.