CLONING OF POTENTIAL CANDIDATES FOR GUINEA PIG OPIOID RECEPTORS

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The endogenous opioid system plays an important role in modulating endocrine, respiratory, cardiovascular and immune functions and others (1). Opioids exert their actions by binding to specific, G-protein coupled seven-transmembrane domaine receptors, which have been classified by their ligands as delta, mu, and kappa. Based on the recently cloned mouse delta opioid receptor DNA sequence (2,3), other opioid receptors were also successfully cloned (4,5). In light of the recent cloning and pharmacological characterization of the guinea pig (gp) kappa receptor in our lab (4), the aim of this work was to clone and characterize the gp brain delta- and mu receptors. We were able to clone a 675 bp guinea pig mu opioid receptor sequence, corresponding to the AA 153-383 of the rat mu opioid receoptor (5). Additionally we were able to clone a 571 bp fragment, homologous to the AA 138-329 of the mouse delta opioid receptor (1,2).

Methods: a) cDNA library construction and screening: we used a Lambda gt11 gp brain cDNA library (Clonetech) with 1.3 million independent clones, oligo(dT)-primed, cDNA's inserted into the EcoRI site of Lambda gt11 and ranging in size from 1.3kb to 4kb. For new libraries we used gp brain mRNAs to construct several cDNA libraries in the following vectors: pME18SNeo, pME18S, pcD-Neo-SRa-X. b) PCR: P1: 5'-CTCACCATGATGAGCG TGGA-3'; P2: 5'-AGCAGCGCTTGAAGTTCTCG-3', P3: 5'-TCGATCCACTGTATTAGCCG-3'; muPCR: P1+P3, 1x [94°C 1.5min], 30x [56°C 2min, 72°C 2.5min, 94°C 1min], 1x [56°C 2min, 72°C 8min]. deltaPCR: P1+P2, 1x [94°C 1.5min], 30x [60°C 1.5min, 72°C 2min, 94°C 1min], 1x [60°C 1.5min, 72°C 6min]. The resulting PCR fragments were isolated and finally cloned into pBSSKII+ (Stratagene). After transformation into E.coli and colony screening, the plasmids from positive clones were isolated, and both strands were sequenced using the PCR primer and the plasmid T7- and T3 RNA polymerase promoter primer. Results: The isolated DNAs from those gp cDNA libraries (including the Lambda gt11 gp brain cDNA library) have been hybridized with a rat delta opioid receptor (470bp, part of the ORF) and a rat mu opioid receptor probe (1.4kb, containing the complete ORF). All cDNA libraries gave positive signals with both probes and were subsequently used for the PCR reactions. Using the sequence analysis software GCG (University Wisconsin) we translated both sequences into open reading frames and aligned them to the rat and guinea pig kappa-, the rat mu- and the mouse delta opioid receptor sequences. The results are shown in Fig. 1.

Fig.1 Aminoacid-alignement of the cloned gp mu- and delta opioid receptor sequences

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   1                                                              60
Rk .......... ..MESPIQIF RGEPGPTCAP SACLLPNSSS WFPNWAESDS NGSVGSEDQQ
Gk .......... ..MGRRRQGP AQPASELPAR NACLLPNGSA WLPGWAEPDG NGSAGPQDEQ
Gm .......... .......... .......... .......... .......... ..........
Rm MDSSTGPGNT SDCSDPLAQA SCSPAPGSWL NLSHVDGNQS DPCGLNRTGL GGNDSLCPQT
Gd .......... .......... .......... .......... .......... ..........
Md .......... ..ME   LV P SARA ELQSS PLVNL   SD AFPSAFPSAG ANASGSPGAR
   61                                                            120
              ___________TM-I_____________              _______TM-II_
Rk LEPAHISPAI PVIITAVYSV VFVVGLVGNS LVMFVIIRYT KMKTATNIYI FNLALADALV
Gk LEPAHISPAI PVIITAVYSV VFVVGLVGNS LVMFVIIRYT KMKTATNIYI FNLALADALV
Gm .......... .......... .......... .......... .......... ..........
Rm GSP...SMVT AITIMALYSI VCVVGLFGNF LVMYVIVRYT KMKTATNIYI FNLALADALA
Gd .......... .......... .......... .......... .......... ..........
Md SAS...SLAL AIAITALYSA VCAVGLLGNV LVMFGIVRYT KLKTATNIYI FNLALADALA
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   121                                                               180
   _____                       __________TM-III_________
Rk TTTMPFQSAV YLMNSWPFGD VLCKIVISID YYNMFTSIFT LTMMSVDRYI AVCHPVKALD
Gk TTTMPFQSTV YLMNSWPFGD VLCKIVISID YYNMFTSIFT LTMMSVDRYI AVCHPVKALD
Gm .......... .......... .......... .......... LTMMSVDRYI AVCHPVKALD
Rm TSTLPFQSVN YLMGTWPFGT ILCKIVISID YYNMFTSIFT LCTMSVDRYI AVCHPVKALD
Gd .......... .......... .......... .......... LTMMSVDRYI AVCHPVKALD
Md TSTLPFQSAK YLMETWPFGE LLCKAVLSID YYNMFTSIFT LTMMSVDRYI AVCHPVKALD
   181                                                               240
          ___________TM-IV_______                                  ___
Rk FRTPLKAKII NICIWLLASS VGISAIVLGG TKVREDVDVI ECSLQFPDDE YSWWDLFMKI
Gk FRTPLKAKII NICIWLLSSS VGISAIILGG TKVREDVDII ECSLQFPDDD YSWWDLFMKI
Gm FRTPRNAKIV NVCNWILSSA IGLPVMFMAT TKYRQGS..I DCTLTFSHPT W.YWENLLKI
Rm FRTPRNAKIV NVCNWILSSA IGLPVMFMAT TKYRQGS..I DCTLTFSHPT W.YWENLLKI
Gd FRTPAKAKLI NICIWVLASG VGVPIMVMAV TQPRDGA..V VCTLQFPSPS W.YWDTVTKI
Md FRTPAKAKLI NICIWVLASG VGVPIMVMAV TQPRDGA..V VCMLQFPSPS W.YWDTVTKI
   241                                                               300
   ______TM-V______________                              __________TM-VI_
Rk CVFVFAFVIP VLIIIVCYTL MILRLKSVRL LSGSREKDRN LRRITKLVLV VVAVFIICWT
Gk CVFVFAFVIP VLIIIVCYTL MILRLKSVRL LSGSREKDRN LRRITRLVLV VVAVFIICWT
Gm CVFIFAFIMP VLIITVCYGL MILRLKSVRM LSGSKEKDRN LRRITRMVLV VVAVFIVCWT
Rm CVFIFAFIMP VLIITVCYGL MILRLKSVRM LSGSKEKDRN LRRITRMVLV VVAVFIVCWT
Gd CVFLFAFVVP TLIITVCYGL MLLRLRSVRL LSGSKEKDRS LRRITRMVLV VVGAFVVCWA
Md CVFLFAFVVP ILIITVCYGL MLLRLRSVRL LSGSKEKDRS LRRITRMVLV VVGAFVVCWA
   301                                                               360
   ________                    __________TM-VII_______
Rk PIHIFILVEA LGSTSHSTA. VLSSYYFCIA LGYTNSSLNP VLYAFLDENF KRCFRDFCFP
Gk PIHIFILVEA LGSTSHSTA. ALSSYYFCIA LGYTNSSLNP ILYAFLDENF KRCFRDFCFP
Gm PIAIYVIIKA LITI.PETTF QTVSWHFCIA LGYTNSCLNP VLYAFLDENF KRCFREFCIP
Rm PIHIYVIIKA LITI.PETTF QTVSWHFCIA LGYTNSCLNP VLYAFLDENF KRCFREFCIP
Gd PIHIFVIVWT LVDINRRDPL VVAALHLCIA LAYANSSLNP VLYAFLDENF KRC
Md PIHIFVIVWT LVDINRRDPL VVAALHLCIA LGYANSSLNP VLYAFLDENF KRCFRQLCRT
   361                                          409
Rk IKMRMERQST NRVRNTVQDP ASMRDVGGMN KPV....... .....
Gk IKMRMERQST SRVRNTVQDP AYMRNVDGVN KPV....... .....
Gm TSSTIEQQNS TRVRQNTREH PSTANTVDR. .......... .....
Rm TSSTIEQQNS TRVRQNTREH PSTANTVDRT NHQLENLEAE TAPLP
Md PCGRQEPGSL RRPRQATTRE RVTACTPSDG PGGGRAA... .....
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Legend: Rk: rat kappa opioid receptor (KOR), Gk: gp KOR, Rm: rat mu opioid receptor (MOR), Gm: gp MOR, Md: mouse delta opioid receptor (DOR), Gd: gp DOR; bold letters AA differences: Rk vs. Gk, Rm vs. Gm, and Md vs. Gd; TM: transmembrane domains.

The cloned receptor fragments show that both the gp delta- and mu opioid receptor AA-sequences are highly homologous to the rat and mouse opioid receptor sequences. Since we are particularly interested in the influence of opioids on the gastrointstinal motility, those clones may be used to investigate the distribution of opioid receptors across the gastrointestinal tract of guinea pig.

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