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$HmGlu_{1d}$, a novel splice variant of the human type I metabotropic glutamate receptor

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Abstract

A novel splice variant, $hmGlu_{1d}$, of the human $mGlu_1$ metabotropic glutamate receptor has been isolated from a human brain library. This clone is identical to human $mGlu_{1a}$ except that it lacks 35 nucleotides in the 3′ coding sequence, which predicts a truncated protein and a novel carboxy terminus. After injection of the encoding sequence into mouse A9 fibroblasts, quisqualate and (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid ((1*S*,3*R*)-ACPD) elicited concentration-dependent increases in intracellular Ca^{2+} (pEC50 values of 6.09 and 4.33, respectively).

Keywords: Human $mGlu_{1d}$; Metabotropic glutamate receptor; Ca^{2+} fluorescence

The metabotropic glutamate receptor family is of increasing interest in studies of synaptic plasticity, epilepsy and excitotoxicity. To date eight members have been identified and assigned to three classes: Class I receptors ($mGlu_1$ and $mGlu_5$) couple positively to phospholipase C and are preferentially activated by quisqualate, whereas receptors of Class II ($mGlu_2$ and $mGlu_3$) and Class III ($mGlu_4$, $mGlu_6$, $mGlu_7$, $mGlu_8$) couple negatively to adenylate cyclase, and display agonist preferences for (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid ((1*S*,3*R*)-ACPD) and L(+)-2-amino-4-phosphonobutyric acid (L-AP4), respectively (reviewed by Pin and Duvoisin, 1995). The variety of receptor types is increased by alternative splicing of $mGlu_1$ (a(or α), b (or β) and c), $mGlu_4$ (a and b) and $mGlu_5$ (a and b) (Pin and Duvoisin, 1995) which may direct the coupling of these receptors to different effectors or engender them differentially susceptible to intracellular regulation. The sequences of the human $mGlu_{1a}$ and $mGlu_{1b}$, $mGlu_2$, $mGlu_{4a}$, and $mGlu_{5a}$ and $mGlu_{5b}$ receptors have recently been published (Minakami et al., 1994; Desai et al., 1995; Flor et al., 199a, b). We describe here a novel splice form of the human $mGlu_1$ receptor which can induce mobilisation of intracellular Ca^{2+} in response to metabotropic receptor agonists.

A human brain cDNA library in bacteriophage λgt10 (Clontech) was screened using [^{32}P]dCTP-radiolabelled fragments of the rat $mGlu_{1a}$ receptor cDNA (kindly supplied by Prof. Nakanishi). Two clones over 3 kb in length and with high sequence homology to rat $mGlu_{1a}$ were isolated. These were identical to human $mGlu_{1a}$ (Desai et al., 1995) up to the splice point at which, in $hmGlu_{1b}$, an 85 base pair exon in inserted. Thereafter, one clone (mGHB2) contained a presumed intron sequence, while the other (mGHB1) was identical to the $hmGlu_{1a}$ sequence except that 35 base pairs were lacking immediately after the splice point (Fig. 1a[i]). This introduces a shift of the reading frame and predicts a truncated protein of 908 residues with a novel carboxy terminus (Fig. 1a[ii]). This splice form was termed $hmGlu_{1d}$.

The co-existence of the $hmGlu_{1a}$, $hmGlu_{1b}$ and $hmGlu_{1d}$ splice variants was examined by PCR on human cerebellar cDNA using primers specific for the $hmGlu_1$ sequence (5′-CTGGGATCCATGTTCACTC-CCAAGATCTAC-3′, 5′-ATTAAGCT-TCACGTGCACAGAGAGGCGGTG-3′). Bases mutated to introduce BamHI and HindIII restriction sites are underlined). The PCR reactions used 'Expand-High Fidelity' polymerase (Boehringer Mannheim) in 50 cycles (94°C 1 min, 58°C 1 min, 72°C 1 min). No product was amplified in reactions lacking the cerebellar cDNA.

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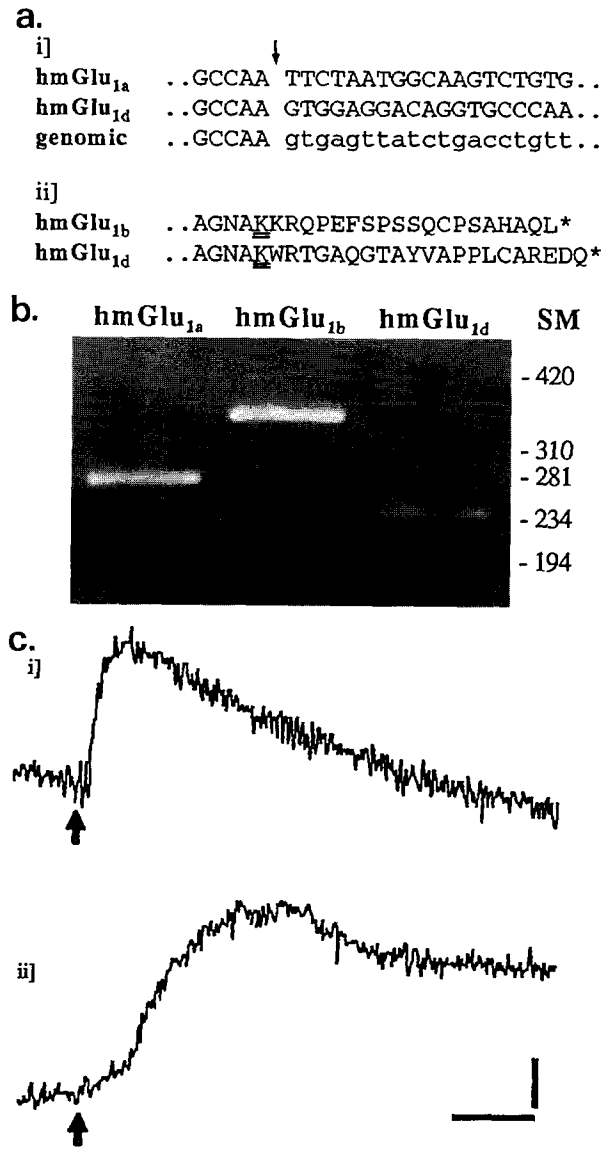

Fig. 1. (a) [i] Comparison of the human $mGlu_{1a}$, $mGlu_{1d}$ and genomic sequences around the splicing site which is indicated by an arrow. [ii]. Deduced amino-acid sequences of the carboxy termini of $hmGlu_{1b}$ and $hmGlu_{1d}$ (standard single-letter code). Sequences begin with amino acid number 883. The amino acid at the splice site is doubly underlined. (b) Fragments liberated by BamHI/HindIII digest of subcloned and sequenced PCR products. Digests were run on a 4% agarose gel (NuSeive) and visualised by ethidium bromide fluorescence. Predicted band sizes: $mGlu_{1a}$, 275 bp; $mGlu_{1b}$, 360 bp; $mGlu_{1d}$, 240 bp. Size markers (SM) from λgt10 (StyI digest) and PhiX174 (HaeIII digest). (c) Elevation of $[Ca^{2+}]_i$ in $hmGlu_{1d}$-injected A9 fibroblasts. Calcium/Fluo-3 fluorescence responses to [i] (1*S*,3*R*)-ACPD (3×10^{-4} M) and [ii] quisqualate (10^{-5} M). Agonists were applied at arrows and continued throughout traces. Horizontal bar: 10 s, vertical bar: 0.15 dF/F_{max} (dF, fluorescence change; F_{max}, maximum fluorescence on application of ionomycin, 10^{-6} M).

The PCR products were digested with BamHI and HindIII, subcloned into pBluescript and identified by sequencing as the $hmGlu_{1a}$, $hmGlu_{1b}$ and $hmGlu_{1d}$ splice variants. The subcloned PCR products from the three splice variants were predicted to be 275, 360 and 240 base pairs, respectively (Fig. 1b).

In order to examine functionality, the sequence encoding the $hmGlu_{1d}$ receptor was subcloned into the EcoRI site of a mammalian expression vector downstream from a cytomegalovirus promotor. This expression plasmid was injected (50 μg/ml, 0.2 s) into mouse A9 fibroblasts and on the next day Ca^{2+} responses were examined using the calcium indicator Fluo-3 and a HR Deltaron 1700 MOS-sensor camera (Fuji) mounted on an inverted microscope. The metabotropic receptor agonists quisqualate and (1*S*,3*R*)-ACPD elicited concentration-dependent rises in intracellular calcium ($[Ca^{2+}]_i$) (Fig. 1c) with pEC_{50} values of 6.09 ± 0.08 ($n = 7$) and 4.33 ± 0.06 ($n = 6$), respectively. These responses were independent of extracellular Ca^{2+} levels. Non-injected A9 fibroblasts did not respond to the agonists.

We have isolated $hmGlu_{1d}$, a novel splice variant of the human $mGlu_1$ metabotropic glutamate receptor, which is produced by omission of a 35 base pair exon. This contrasts both with the $mGlu_{1b}$ splice variant, in which an additional exon is introduced at the same splice point (Tanabe et al., 1992; Desai et al., 1995), and with the rat $mGlu_{1c}$ where a completely novel 3′ sequence is present (Pin et al., 1992). However, the predicted length of the $hmGlu_{1d}$ protein (908 residues) is similar to those of the $mGlu_{1b}$ and $mGlu_{1c}$ splice variants (906 and 897 residues, respectively) (Fig 1a[ii]). Activation of the $hmGlu_{1d}$ receptor by quisqualate and (1*S*,3*R*)-ACPD in injected A9 fibroblasts raises $[Ca^{2+}]_i$ and the relative potencies of the two agonists are typical for a Class I metabotropic glutamate receptor (Pin and Duvoisin, 1995). This elevation of $[Ca^{2+}]_i$ is presumably mediated through activation of phospholipase C, since this enzyme is also activated by the other splice variants of $mGlu_1$. Coupling of $hmGlu_{1d}$ to phospholipase C and regulation of the coupling to this effector system are currently under investigation.

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