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REVIEW ARTICLE

The Cold Case of Metabotropic Glutamate Receptor 6: Unjust Detention in the Retina?

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Abstract: It is a common opinion that metabotropic glutamate receptor subtype 6 (mGluR6) is expressed exclusively in the retina, and in particular in the dendrites of ON-bipolar cells. Glutamate released in darkness from photoreceptors activates mGluR6, which is negatively associated with a membrane non-selective cation channel, the transient receptor potential melanoma-related 1, TRPM1, resulting in cell hyperpolarization. The evidence that mGluR6 is expressed not only in the retina but also in other tissues and cell populations has accumulated over time. The expression of mGluR6 has been identified in microglia, bone marrow stromal and prostate cancer cells, B lymphocytes, melanocytes and keratinocytes and non-neural tissues such as testis, kidney, cornea, conjunctiva, and eyelid. The receptor also appears to be expressed in brain areas, such as the hypothalamus, cortex, hippocampus, nucleus of tractus solitarius, superior colliculus, axons of the corpus callosum and accessory olfactory bulb. The pharmacological activation of mGluR6 in the hippocampus produced an anxiolytic-like effect and in the periaqueductal gray analgesic potential. This review aims to collect all the evidence on the expression and functioning of mGluR6 outside the retina that has been accumulated over the years for a broader view of the potential of the receptor whose retinal confinement appears understimated.

Keywords: mGluR6, homo-AMPA, anxiolytic-like effect, periaqueductal grey, rostroventromedial medulla.

1. INTRODUCTION

mGluR6 shows high sensitivity to glutamate and slow desensitization [1]. mGluR6 has a pharmacological profile similar to the other mGluRs of the III groups, characterized by high affinity for glutamate (EC50 = 10 μM), L-(+)-2-amino-4-phosphonobutyric acid, L-AP4 (EC50 = 0.6-0.9 μM), and L-serine-O-phosphate, L-SOP (EC50 = 0.39 ± 0.05 μM) [2], the latter being two broad-spectrum agonists of group III mGluRs [1, 3]. However, studies with human embryonic kidney (HEK) cells transfected with rat or human mGluR6 have detected an absence of specific binding with [^{3}H] L-AP4 [4] or low affinity towards L-AP4 and L-SOP [5]. It seems that the lower affinity towards L-AP4 and L-SOP is due to the replacement of lysine with glutamine in position 58 of mGluR6 protein, which leads to a hydrogen bond with the phosphonate group of the two agonists endowed with lower-energy [6].

Splice variants of the mGluR6 exist in humans, rats, mice, and zebrafish. It is noteworthy that the splicing pattern occurs differently in rats, humans, and mice producing different truncated proteins [7-9]. In rat, a stop codon in an additional exon produces a truncated protein of 508 amino acids [7, 8]. In man, there are two variants of splicing, one with 97 bp less (exon 6) and the other with 5 bp more (intron between exons 5 and 6). These splice variants encode truncated proteins of 405 and 425 amino [7]. In mice, the splicing in exon 8 produces a stop codon in exon 9 encoding a truncated protein of 545 amino acids [9]. In any case, the different splicing variants encode for truncated and soluble proteins characterized by the presence of the N-terminal domain containing the binding site but lacking the transmembrane and the intracellular domains [7-9]. Two paralogs coding for mGluR6, mglur6a, and mglur6b have been found in the genome of zebrafish. Interestingly, the expression pattern of the two paralogs overlaps that of the downstream effectors, gnaoa and gnaob, coding for the G protein Goα subunit [10].

A 3-isoxazolol bioisostere of 2-aminoadipic acid (homo-AMPA) which showed potent and selective agonist activity at mGluR6 (EC50 =58 ± 11 μM) was developed in 1996 [3, 11]. Despite homo-AMPA did not show activity at iGluRs and at other mGluRs proved to be a weak N-methyl-D-aspartic acid (NMDA) receptor antagonist (IC50 = 131 ± 18 μM) [3].

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2. THE DISTRIBUTION OF mGluR6

mGluR6 was first identified in the retina of rat [1] where it is expressed on the tips of ON bipolar cell dendrites [12]. ON-pathway is activated by an increase in light which results in a decrease in glutamate. This effect is due to mGluR6, whose activation leads to a Gαo protein-dependent [13-16] closure of the transient receptor potential melanoma-related 1 (TRPM1) and cell hyperpolarization [17-21]. A single report has, however, implicated Gβγ, rather than Gαo, in the negative regulation of TRPM1 [22]. Ultimately, the function of mGluR6 is just to reverse the sign of the signal from the photoreceptors.

Although the general opinion places mGluR6 receptor expression exclusively in the ON bipolar cells of the retina, mGluR6 expression was also detected on microglia in 1997 [23, 24]. The stimulation of group III mGluRs by L-AP-4 and RS-PPG caused a mild activation of resting microglia and an inhibition of that activated [24]. Since, L-AP4 is more potent at mGluR4, mGluR6, and mGluR8 receptors than at mGlu7 receptors [25, 26] while RS-PPG has a higher potency for mGluR8a than mGluR6 or mGluR4a and lowest potency at mGluR7b [27], the specificity or the involvement of mGluR6 in modulating microglia activity cannot be confirmed [24]. In the same year, the expression of mGluR6 was also demonstrated in the suprachiasmatic (SCN) and arcuate (ARC) nuclei of the hypothalamus [28]. The transcripts for mGluR6 have also been found to be expressed in nodose ganglia and the nucleus of the solitary tract [29, 30]. The transcripts for mGluR6 were also found in CA3 hippocampal slices, which were down-regulated after status epilepticus [31].

mGluR6 mRNA and protein were also detected in bone marrow stromal cells. Interestingly, mGluR6 in bone marrow stromal cells: i) showed an expression density comparable with (or even higher than) that of the retina, ii) inhibited Ca^{2+} influx hyperpolarizing cells (as it does on the ON bipolar cells), and iii) produced a decrease in Ca^{2+} influx and hyperpolarization which proved to be not related to cAMP levels [32]. The expression of mGluR6 was also detected in human prostate cancer cells and in particular in LNCaP androgen-sensitive cells suggesting its involvement in regulating cancer cell biology [33].

A broader overview of the mGluR6 receptor distribution was achieved through the construction of transgenic mouse lines with an enhanced green fluorescent protein (GFP) under the control of the mGluR6 promoter [9]. In the retina, GFP was expressed exclusively on ON bipolar cells. GFP expression was also found in non-neural tissues such as the corneal endothelium, testis, kidney (especially in the inner medulla collecting tubules and vasa recta but also in the parietal layer surrounding the glomerulus in the cortex), conjunctiva, eyelid and lymph nodes (B lymphocytes). The expression of mGluR6 on testis has been further confirmed later on [34]. Interestingly, the tissues which express mGluR6 in the transgenic mouse also transcribe mGluR6 in the wild-type mouse, which rules out a possible ectopic expression of mGluR6 in the transgenic mouse. Moreover, two splice variants of mGluR6 were found. GFP was also detected in the brain and in particular in certain cortical areas, superior colliculus, axons of the corpus callosum, accessory olfactory bulb, and cells of the subcommissural organ, a non-neuronal structure that bridges across the third ventricle [9]. In this study, GFP expression appears to be a good indicator of mGluR6 localization since i) GFP expression in the retina was uniquely localized on ON bipolar cells, ii) GFP expression coincides in two transgenic lines and iii) the expression of mRNAs for mGluR6 in wild type mice overlaps that of GFP in the transgenic mice. The question of whether the transcripts are indicative of the translated proteins is still under discussion. Protein translation has been positively correlated with mGluR transcription [35-37], however the transcription-translation binomial has been unequivocally confirmed only for housekeeping proteins [38-40]. Moreover, the presence of transcripts in tissues and cell populations that do not express the translated protein is indicative of their potential to be able to express the protein under specific conditions, such as after injury as a protective mechanism [23].

Recently, mGluR6 was also found to be expressed in human melanocytes where it promotes melanin production. mGluR6 signaling driving melanin production involves TRPM1 calcium channel activation, though not Gαo protein, which was proved to be absent in human melanocytes [41]. mGluR6 has also been found to be expressed in human keratinocyte cell lines where it controls keratinocyte phagocytosis by modulating calmodulin kinase II (CaM KII)/ERK/ myosin light chain (MLC) signaling pathway [42].

In a study aimed to investigate the expression of mGluR6 in zebrafish, the receptor expression has been found in retinal ON and OFF bipolar and ganglion cells. The expression of mGluR6 in zebrafish was also found outside the retina and in particular in certain brain areas such as habenula, medial and lateral tectum opticum, midbrain, mid-hindbrain boundary, and bilateral nucleus of the medulla oblongata. Interestingly, two paralogs encoding for mGluR6, mglur6a, and mglur6b were found. The expression of the two paralogs overlapped with the expression of two downstream effector molecules, gnaoa and gnaob, encoding for Gαo proteins [10]. Altogether these results suggest a more widespread expression of mGluR6, which includes other cell populations in addition to ON-bipolar cells, brain areas and non-neuronal peripheral tissues. A summary of mGluR6 distribution and function is shown in Table **1**.

3. PHARMACOLOGICAL ACTIVATION OF mGluR6

Only a few studies have investigated the effect of the pharmacological activation of mGluR6 *in vivo*. Homo-AMPA, a mGluR6 selective agonist [3, 11], given locally into the hippocampus produced an anxiolytic-like effect in the conflict drinking test in rats. This effect was reversed by (RS)-α-cyclopropyl-4-phosphonophenylglycine, CPPG, a broad spectrum group III mGluR antagonist [43]. The effect of homo-AMPA proved to be less potent than (1S,3R,4S)-1-aminocyclopentane-1,3,4-tricarboxylic acid, ACPT-I, a group III mGluR agonist [44], since the anxiolytic-like activity of homo-AMPA was produced at higher doses (250 nmol/rat) than ACPT-I (7.5 nmol/rat) [45]. This not only indicates pharmacologically the presence of the mGluR6 in the rat hippocampus but suggests its involvement in the anxiolytic-like action of group III mGluR agonists at this level

Table 1. A summary of the expression of mGluR6 outside the retina.

Location	Evidence	Tranduction	Function	Specie	Refs.
Cortical neurons/glial coltures Adult brain Microglia	mRNA mRNA and protein	- AC/cAMP - AC/cAMP	Neuroprotection + resting microglia and - activated microglia	Rat Rat	[23] [24]
Suprachiasmatic and arcuate nuclei of the hypothalamus Whole brain extract	mRNA			Rat	[28]
Brain: cortex, superior colliculus, corpus callosum, accessory olfactory bulb. Subcommissural organ cells *Cornea* (endothelium) *Kidney* (collecting tubules and vasa recta of inner medulla, parietal layer surrounding the glomerulus in the cortex) *B-lymphocytes* (spleen, germinal center of Peyer's patches and ribcage bone marrow) Testis, conjunctiva, spinal cord, and eyelid	GFP under mGluR6 promoter mRNA	+ Ca^{2+} (L-AP4)	Secretion ?	Transgenic mouse	[9] [34]
Prostate (LNCaP androgen-dependent cells).	mRNA		Prostate cancer cell biology	Human	[33]
Bone marrow stromal cells	mRNA and protein	- Ca^{2+} influx and NO production	Regulation of bone matrix	Rat	[32]
Epidermal keratinocytes		CaM KII/ERK/MLC	Cytoskeletal reorganization Regulation of phagocytosis	Human	[42]
Melanocytes	mRNA and protein	Gαo (absent in melano-cytes) restored TRPM1 inhibition	Melanin production	Human	[41]
Nodose ganglia and nucleus of the solitary tract	mRNA	- voltage-gated Ca^{2+} channels		Rats	[29, 30]
CA3 hippocampal slices	mRNA		Synaptic depression Down-regulated after status epilepticus		[31]

The expression of mRNA or protein, associated transduction mechanism, the function and specie are also indicated. + and - indicate stimulation or inhibition, respectively.

[46]. The lower anxiolytic potency of homo-AMPA compared to ACPT-I could be due to i) the recruitment of mGluR6 alone rather than several mGluRs belonging to group III, whose anxiolytic activity in the hippocampus has already been reported [46-48] and ii) the lowest expression density of mGluR6 compared to mGluR4 and mGluR8 in the rat hippocampus [31].

Homo-AMPA was also microinjected into the ventrolateral periaqueductal grey (vl-PAG), (Palazzo E., Marabese I., Boccella S, and Maione S., unpublished results). It turned out that homo-AMPA modulated the activity of pain-responding neurons in the rostral ventromedial medulla (RVM), the ON and OFF cells. PAG projections to the spinal dorsal horn pass through the RVM and constitute the pain descending modulatory system, whose activation produces analgesia [20, 49]. In the RVM, ON and OFF cells are characterized by their response to a noxious stimulus, normally an intense light beam focused on the rodent's tail which evokes an abrupt flick (tail flick). The electrical activity of the ON cell, the ongoing frequency, burst of activity and onset of burst, is enhanced by the noxious stimulation of the tail whereas it is inhibited by centrally acting analgesics such as morphine. For these characteristics ON cells are defined as "pronociceptive". On the contrary, the electrical activity of the OFF cell (spontaneous frequency, duration of the pause, a complete cessation of activity evoked by the noxious stimulation, and onset of the pause) is reduced by the noxious stimulation of the tail while it is enhanced by morphine: therefore they are defined as "antinociceptive" [50-56]. The electrical activity of the ON and OFF cells of the RVM thus represents a tool to evaluate or predict the analgesic (or pain facilitating) potential of a drug [52, 57-59].

Homo-AMPA delivered locally into the vl-PAG decreased the activity of the ON cells; in particular, it inhibited the frequency and the burst of the activity and delayed the onset of the burst. Homo-AMPA instead increased the frequency of activity and the onset of the pause while it decreased the duration of the pause of the OFF cell. These electrophysiological changes in ON and OFF cell activity within RVM are consistent with a pain-inhibitory role of mGluR6 within the PAG. These preliminary data represent pharmacological evidence of the presence of mGluR6 in the vl-PAG and its involvement in the modulation of pain at the PAG level. Although preliminary, these data are in agreement with those

that indicate the involvement of the other mGluRs of group III in the modulation of pain at the level of the descending system of pain [60-66]. Obviously, further studies are needed to confirm these results and in particular it is believed that it is still necessary to further evaluate: i) the expression of mGluR6 in PAG, ii) the behavioral responses to pain after the administration of homo-AMPA in vl-PAG and finally iii) if the effect of homo-AMPA in the PAG is closely related to the stimulation of mGluR6 and not with the action of homo-AMPA as a weak antagonist on the NMDA receptor. Regarding this possible action of homo-AMPA as a weak antagonist on the NMDA receptor, it is considered unlikely since NMDA receptor blockade at PAG level would lead to the inhibition rather than the stimulation of the pain descending modulatory system. Indeed the intra-PAG microinjection of selective NMDA antagonists enhances the activity of the ON cells while inhibiting that of the OFF cells in the RVM [67] and facilitates pain accordingly [68, 69].

CONCLUSION

The history of mGluR6 began with the discovery of its expression in the retina, where its role in the modulation of the responses with the light of the ON bipolar cells is well characterized. At the same time, over the years, evidence has been collected regarding the presence of the receptor outside the retina, in several cell populations, non-neuronal tissues, and brain. The receptor would be involved in melanin release in melanocytes, phagocytosis in keratinocytes, inhibition of activated microglia, regulation of bone matrix in bone marrow stromal cells, and tumor cell biology in prostate cancer cells. Thus, the collected evidence on the expression and the function of the mGluR6 at the peripheral and central levels begins to outline a new aspect for this receptor which was unjustly understimated.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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