Biochemical Pharmacology xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Commentary

Orthosteric *versus* allosteric GPCR activation: The great challenge of group-III mGluRs

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ARTICLE INFO

Article history:
Received 9 March 2012
Received in revised form 17 April 2012
Accepted 17 April 2012
Available online xxx

Keywords:
GPCR
Metabotropic glutamate
LSP1-2111
LSP4-2022
PHCCC
AMN082

ABSTRACT

Group-III metabotropic glutamate receptors (mGluRs) comprise four structurally related brain and retinal G protein-coupled receptors (GPCRs), mGluR4, mGluR6, mGluR7 and mGluR8, which receive much attention as promising targets for nervous system drugs. In particular, activation of mGluR4 is a major focus for the development of new therapeutics in Parkinson's disease, while mGluR7 activation is considered a potential approach for future treatments of specific psychiatric conditions. The first generation group-III mGluR agonists, *e.g.* L-AP4 and L-SOP, are characterized by an essential phosphonate functional group, which became a major limitation for the development of systemically active, potent and receptor subtype-selective drugs. Recently however, two approaches emerged in parallel providing resolution to this constraint: *in silico* high-throughput screening of chemical libraries against a 3D-model of the mGluR4 extracellular domain identified a hit that was optimized into a series of potent and subtype-selective orthosteric agonists with drug-like properties and novel chemotype structures; secondly, high-throughput random screening of chemical libraries against recombinantly expressed group-III receptors identified diverse chemical sets of allosteric agonists and positive modulators, which are drug-like, display selectivity for mGluR4, mGluR7, or mGluR8 and act *via* novel pharmacological sites.

Here, we illustrate new scientific insights obtained *via* the use of those strategies. Also, we compare advantages and disadvantages of both approaches to identify the desired group-III mGluR activators and we conclude with suggestions how to employ those discovery strategies with success for the identification, optimization, and development of clinical drug candidates; this may have important implications for the entire field of GPCR research.

Abbreviations: ACPT-I, (1*S*,3*R*,4*S*)-1-aminocyclopentane-1,3,4-tricarboxylic acid; ADMET, absorption, distribution, metabolism, excretion, toxicity; ADX88178, 5-methyl-*N*-(4-methylpyrimidin-2-yl)-4-(1H-pyrazol-4-yl)thiazol-2-amine; AMN082, *N*,*N*′-dibenzhydryl-ethane-1,2-diamine dihydrochloride; AMPA, 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid; AP4, 2-amino-4-phosphono-butyric acid; APCPr, 1-amino-2-(phosphonomethyl)cyclopropanecarboxylic acid; APDC, 4-Aminopyrrolidine-2,4-dicarboxylic acid; AZ12216052, 2-(4-bromobenzylthio)-*N*-(4-sec-butylphenyl)acetamide; BBB, blood-brain barrier; CNS, central nervous system; CPCCOEt, 7-(hydroxyimino)cyclopropa[*b*]chromen-1a-carboxylate ethyl ester; CPPG, α-cyclopropyl-4-phosphonophenylglycine; L-DOPA, L-3,4-dihydroxyphenylalanine; EC_{50}, half maximal effective concentration; GPCR, G protein-coupled receptor; HTS, high-throughput screening; iGluR, ionotropic glutamate receptor; LSP1-2111, [((3*S*)-3-Amino-3-carboxy)propyl][(4-hydroxy-5-methoxy-3-nitrophenyl)hydroxymethyl]phosphinic acid; LSP1-3081, [(3*S*)-3-(3-amino-3-carboxypropyl(hydroxy)phosphinyl)-hydroxy-methyl]-5-nitrothiophene; LSP4-2022, [((3*S*)-3-Amino-3-carboxy)propyl][(4-(carboxymethoxy)phenyl)hydroxymethyl]phosphinic acid; mGluR, metabotropic glutamate receptor; Lu AF21934, (1*S*,2*R*)-*N*1-(3,4-dichlorophenyl)-cyclohexane-1,2-dicarboxamide); LY2140023, *N*-methionine amide of LY404039; LY354740, (1*S*,2*S*,5*R*,6*S*)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid; LY404039, (1*R*,4*S*,5*S*,6*S*)-4-amino-2-sulfonylbicyclo[3.1.0]hexane-4,6-dicarboxylic acid; MAP4, 2-amino-2-methyl-4-phosphonobutanoic acid; MMPIP, 6-(4-methoxyphenyl)-5-methyl-3-(4-pyridinyl)-isoxazolo[4,5-*c*]pyridin-4(5*H*)-one; MPEP, 2-methyl-6-(phenylethynyl)pyridine; MPPG, α-methyl-4-phosphonophenylglycine; MSOP, α-methylserine-O-phosphate; NAM, negative allosteric modulator; NMDA, *N*-methyl-D-aspartate; PAM, positive allosteric modulator; PCEP, 3-amino-3-carboxypropyl-2′-carboxyethyl phosphinic acid; PEPT1, peptide transporter 1; PHCCC, *N*-phenyl-7-(hydroxylimino)cyclopropa[b]chromen-1a-carboxyamide; PK, pharmacokinetics; PPG, 4-phosphonophenylglycine; PTSD, post-traumatic stress disorder; siRNA, small interfering RNA; SOP, serine-O-phosphate; TM, transmembrane; VFT, Venus flytrap; VU0155041, *cis*-2-(3,5-dichlorphenylcarbamoyl)cyclohexanecarboxylic acid; VU0364770, *N*-(3-chlorophenyl)picolinamide.

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http://dx.doi.org/10.1016/j.bcp.2012.04.013

1. Introduction

L-Glutamate serves as neurotransmitter at the majority of excitatory synapses in the mammalian CNS and its circuits are crucial for many basic brain functions such as control of motor activity, learning and memory, neuroendocrine regulation and emotional homeostasis. At the synaptic level, L-glutamatergic neurotransmission is ensured by glutamate transporters and two families of glutamate receptors: ligand-gated ionotropic glutamate receptors (iGluRs) that are sub-divided into NMDA, AMPA and kainate receptors [1] and secondly, the G protein-coupled mGluR subtypes 1 through 8. Fast excitatory transmission is mediated by iGluRs, which open upon agonist binding to induce an influx of cations within milliseconds. The mGluR family resembles seven transmembrane (7TM)-receptors that modulate L-glutamatergic neurotransmission in a much slower fashion *via* the coupling to heterotrimeric G proteins. As a consequence, mGluR activation regulates the levels of the second messengers cAMP, diacylglycerol, inositol phosphates and Ca^{++} as well as the activity state of various voltage-sensitive ion channels [1–3]. Due to their modulatory nature, the mGluR family receives much attention as targets for medication drugs of the future. For historic and technical reasons, major industrial and academic drug discovery programs have so far largely focused on group-I (mGluR1 and -5) and group-II (mGluR2 and -3) receptors, and several of their ligands are currently in clinical testing [3–7]. The four group-III receptors (mGluR4, -6, -7, and -8) are less investigated, mainly due to technical difficulties to discover suitable compounds for advanced preclinical and clinical investigation. Advances however in the identification of early tool drugs selective for mGluR4, mGluR7, or mGluR8 and the characterization of transgenic mice or siRNAs for those receptors revealed important insights into the potential role of group-III receptors in specific CNS disorders. In particular, activating ligands for mGluR4, mGluR7, or mGluR8 appear to be very promising. For instance the selective activation of mGluR4 shows benefits for the treatment of Parkinson-like motor symptoms in several animal models, but it also reduces nigrostriatal neurodegeneration in rodents. Therefore, in addition to correcting motor symptoms of Parkinson's disease, those drugs carry also the potential to modify the cause of chronic neurodegenerative disease, in this case, preventing the progressive death of *substantia nigra pars compacta* dopaminergic neurons [8–13]. In addition, a potential role for mGluR4-activating drugs in the treatment of pain, mood and anxiety disorders is slowly emerging [14–17]. Selective activation of mGluR7 has been shown to facilitate extinction of conditioned fear and aversion and it also reduced the rewarding effects of the addictive drugs cocaine and ethanol [18–21]. These behavioural effects of mGluR7 activation are consistent with the phenotype of mGluR7-deficient mice in extinction tests of conditioned emotional behaviours and in an ethanol-drinking paradigm [22,23]. Thus, mGluR7 activation is of great interest for the development of future treatments of drug addiction and the fact that activation of mGluR7 facilitates extinction of learned fear and aversions is of potential therapeutic application in human anxiety conditions including post-traumatic stress disorder (PTSD). In particular, we suggested recently that mGluR7 allosteric activators could be used as add-on drugs following exposure-based psychotherapy sessions and may thus find application for longer-term treatment of incompletely extinguished/persistent fear and aversion memories [18,21]. Similarly, relatively selective activation of mGluR8 with different drugs induces anxiolytic effects in several rodent animal models [24–26]. In combination with the anxiogenic phenotype of mGluR8 deficient mice [27,28], an involvement of this receptor in specific physiological parameters of anxiety disorders seems very likely. In particular, mGluR8 activation provides a powerful inhibitory control of synaptic transmission within the lateral amygdala, with the ability to reduce activity in such a way that the expression and the acquisition of cue-conditioned fear in rodents become impaired [24]. On the other hand, mGluR8-deficient mice show a strong deficit in context-conditioned fear, which also suggests a specific role for mGluR8 in anxiety conditions involving exaggerated contextual fear, such as observed in generalized anxiety disorders [29].

In addition, the potential application of group-III activators as novel seizure treatments in epilepsy is supported by numerous studies, specifically, the activation of mGluR7 and mGluR8, possibly in combination, seems most promising [30–35]. And finally, early evidence from a spectrum of rodent depression and despair models provides support for the potential use of mGluR4- and mGluR7-selective activators to treat mood disorders [15,36–38].

The least promising group-III receptor for drug discovery seems to be mGluR6 as it appears restricted mainly to ON-bipolar retinal cells where it stimulates cGMP phosphodiesterase in order to control vision under dim light conditions, termed scotopic vision. Consequently, mGluR6 mutations that abolish receptor trafficking lead to congenital stationary night blindness in man [39,40]. In this commentary, we will not discuss mGluR6 in much detail, but it is important to keep in mind that group-III mGluR ligands with a potent mGluR6 component may possess the risk of undesired ocular side effects. However, this potential drawback is purely speculative at present and awaits experimental assessment.

Taken together, although there is quite little published evidence for the therapeutic potential of group-III mGluR blockers or antagonists (see Section 2.2, below), broad evidence exists for the selective activation of mGluR4, -7, and -8 as possible future therapeutic strategies providing both symptomatic as well as causal relief in disorders of chronic neurodegeneration, anxiety, mood, epilepsy, pain and addictive states.

The three group-III brain receptors are predominantly localized in the presynapses of L-glutamatergic and GABAergic neuron terminals and their activation leads to up- or down-regulation of presynaptic neurotransmitter release. The exact signal transduction mechanisms how group-III mGluRs regulate transmitter exocytosis are still under investigation. However, the modulation of several second messengers (cAMP, diacylglycerol, and Ca^{++}) *via* different heterotrimeric G proteins (Gi, Go, and possibly Gq) and the interaction with presynaptic Ca^{++}- and K^{+}-channels are likely all involved [2,41–43].

As discussed so far, activation of group-III mGluRs has application potential in psychiatry and neurology, but it is important to distinguish conceptually between different modes of GPCR activation: Firstly, classical agonist are chemicals that binds to the receptor of a cell, trigger a specific physiological response, and thus mimic the action of a natural ligand, *e.g.* of the neurotransmitter L-glutamate. The binding site for the natural ligand is termed orthosteric, and classical agonists are therefore also referred to as orthosteric agonists. Secondly, positive allosteric modulators (PAMs) are drugs that increase the activity of a receptor indirectly *via* their interaction with an allosteric site, a location on the receptor protein which is topographically distinct form the orthosteric site. PAMs are similar to agonists in that they contribute to overall receptor activation, but they are different because their binding primarily enhances the potency and/or efficacy of a bound neurotransmitter or orthosteric agonist; thus, PAMs are also called allosteric enhancers. Allosteric agonists represent a third mode of GPCR activation or a special case of PAMs as they activate the receptor also *via* an allosteric site, but in contrast to regular PAMs, no ligand occupancy at the orthosteric site is required. Over the past ten years many novel compounds with good potential of all three pharmacological activation modes

Please cite this article in press as: Flor PJ, Acher FC. Orthosteric *versus* allosteric GPCR activation: The great challenge of group-III mGluRs. Biochem Pharmacol (2012), http://dx.doi.org/10.1016/j.bcp.2012.04.013

have been described for group-III mGluRs. Here, we describe the different routes of discovery for the currently most prominent activator types of group-III mGluRs, and we focus on advantages and drawbacks of the individual pharmaceutical classes, which allows suggestions for future drug discovery in this challenging field of G protein-coupled glutamate receptors.

2. Limitations of first generation group-III mGluR-selective agonists and antagonists

2.1. Agonists

Structurally, mGluRs are believed to form predominantly homodimers, each subunit is composed of a large N-terminal extracellular domain of approximately 590 amino acids, a 7TM region of around 260 amino acids and an intracellular C-terminus, which consists of 30–80 amino acids in the case of group-III mGluRs. The extracellular N-terminal region which folds in two lobes connected by a hinge, is termed the Venus flytrap (VFT) domain and contains the L-glutamate-binding site ([2,44–47]; Fig. 1). L-glutamate or orthosteric agonist binding to the VFT domain induces conformational changes (*i.e.* closing of the two lobes) which are propagated *via* an extracellular cystein-rich region to the 7TM and cytoplasmic domains, which results in G protein activation, second messenger formation and ion channel modulations [48]. The orthosteric binding site within the VFT domain of mGluRs shows a high degree of evolutionary conservation between the individual receptor subtypes [49,50], and as a consequence, L-glutamate-like diacidic derivatives with a distant phosphonate group were described as first generation group-III agonists. L-AP4, a ω-phosphonic acid isostere of L-glutamate, became the prototypic group-III mGluR agonist. The compound shows low micromolar potencies at mGluR4, -6, and -8 (EC_{50}-values are 0.5–5 μM), while the affinity at mGluR7 is about 100-fold lower (EC_{50} = 100 to 500 μM; [32,51,52]). This striking pattern of agonist potencies between the group-III subtypes is virtually identical for L-SOP, an endogenous phosphonic acid in mammals, and (*S*)-PPG, a phosphono-derivative within the class of phenylglycine compounds that has been used in many *in vivo* studies, like L-AP4, to define physiological functions of group-III receptors [32,34,47,53,54]. (*S*)-PPG, L-AP4, L-SOP, and related compounds show very little agonist or antagonist activity at group-I and -II mGluRs or iGluRs, which qualifies them as useful tools to address the overall roles of group-III receptors in brain physiology and in animal correlates of CNS disease. However, their lack of receptor subtype-selectivity within group-III is a major limitation in terms of interpretation of physiological results with such drugs. Further problematic issues associated with first generation group-III mGluR agonists are low oral bioavailability and extremely low potential to cross the blood-brain barrier (BBB). Those negative parameters are intrinsically linked to the drug's amino acid-like structure and more importantly to the ω-phosphonate group, which is considered to be the main factor that limits the utility of L-AP4, L-SOP and (*S*)-PPG for *in vivo* studies [32]. While those phosphonates do not penetrate the blood-brain barrier, more recent drugs with a second carboxylic function instead, such as ACPT-I and (*S*)-DCPG, do ([55–58], Fig. 2). In general, drug screening and chemical optimization at group-III mGluRs during the last decade aimed primarily to circumvent the phosphonate group, to gain selectivity and potency for the individual receptors mGluR4, mGluR7, or mGluR8, to obtain good pharmacokinetic properties, and finally, to enter broader chemotype spaces that

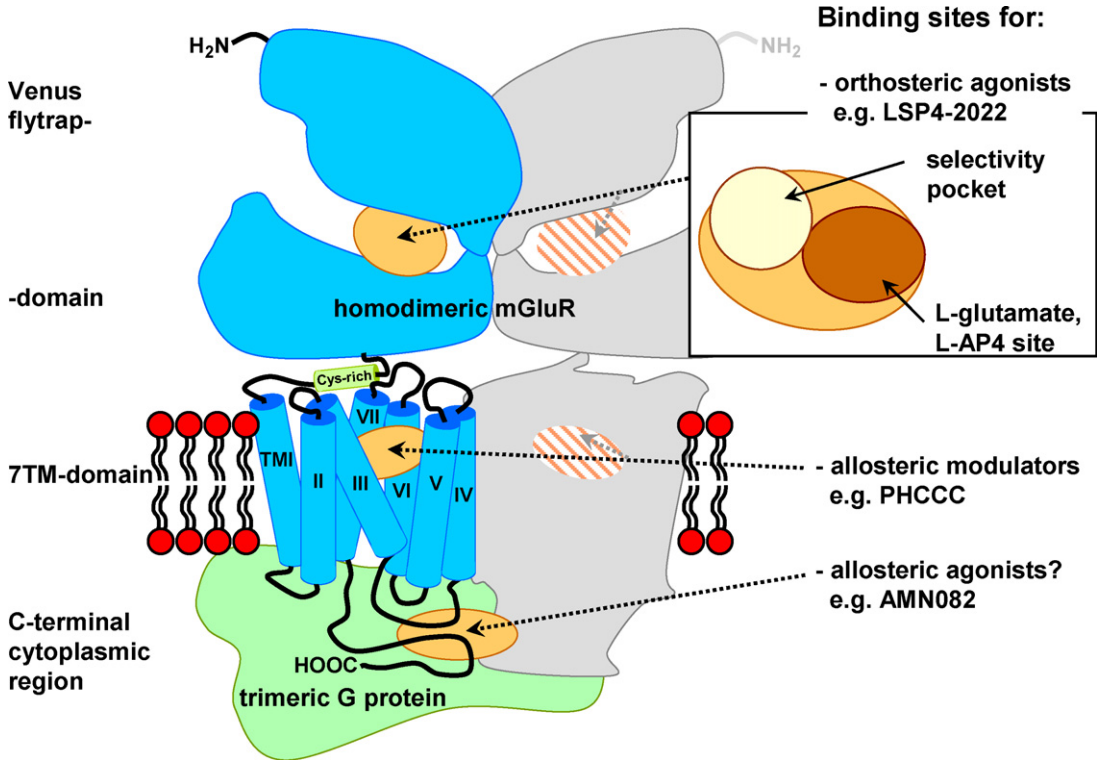

Fig. 1. Schematic representation of a group-III mGluR homodimer with orthosteric and allosteric drug binding sites indicated. An mGluR dimer contains two large extracellular domains called the Venus flytrap (VFT) domains, which bind L-glutamate, L-AP4 and other competitive ligands. Last generation orthosteric agonists, such as LSP4-2022, contain two structural moieties, an α-amino acid group that binds to the L-glutamate site and a distally extended substituent that reaches into a selectivity pocket that is unique for each receptor subtype (see boxed insert). The cysteine-rich domains link the VFT domains to the 7TM-spanning domains that carry the binding sites for allosteric modulators, *e.g.* for PHCCC, which may form direct contacts with TM-III and -VII, as described in the text. Intracellular loops and C-terminal cytoplasmic regions are responsible for trimeric G protein activation and may represent target sites for further allosteric drugs. Here we speculate that allosteric agonists, such as AMN082, bind to and bridge both subunits of the mGluR7 homodimer, possibly at the C-terminal cytoplasmic region and/or intracellular loops (see Section 3.2).

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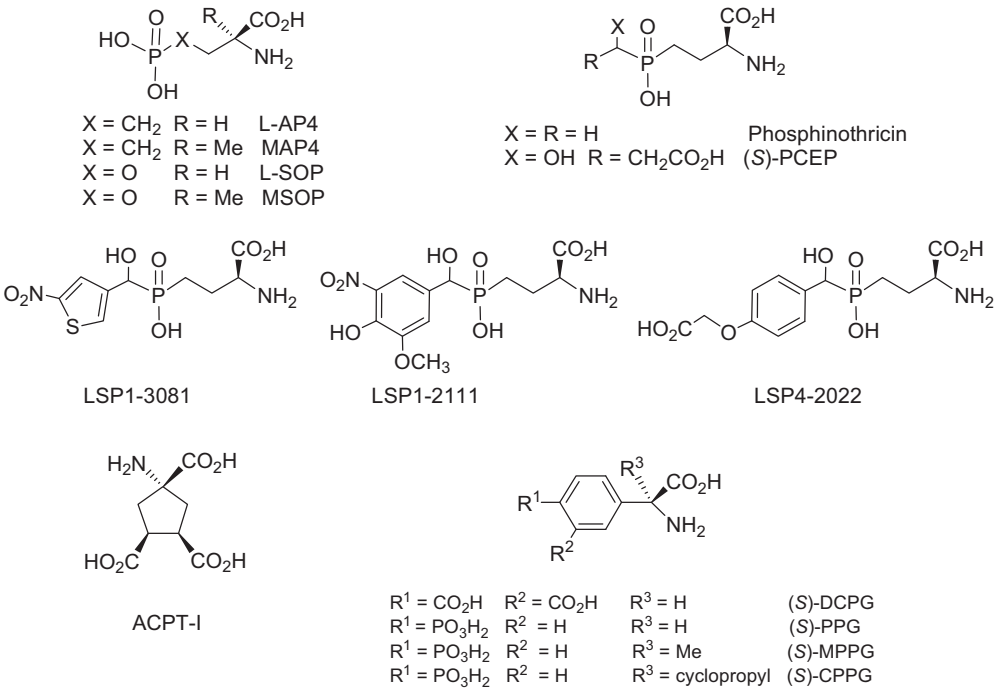

Fig. 2. Chemical structures of selected orthosteric group-III mGluR agonists and antagonists. All those molecules are further explained in the text.

allows better patenting opportunities than with classical amino acid derivatives.

2.2. Antagonists

Almost all group-III mGluR-selective antagonists described to date bind to the orthosteric site and prevent the VFT domain closure [48]. The α-methyl analogues of L-AP4 and L-SOP, named MAP4 and MSOP, were the first relatively selective antagonists for group-III (Fig. 2). Phosphono-substituted phenylglycine derivatives such as CPPG and MPPG (Fig. 2) were described a few years later and showed slightly improved potency compared to the first molecules [47,51]. All current group-III mGluR orthosteric antagonist display very poor BBB penetration, therefore, *in vivo* studies need to be performed *via* intracerebral administration, and none of those molecules discriminates significantly between the four group-III receptor subtypes. Several researchers performed intracerebral administration studies with group-III antagonists but unfortunately, MAP4, MPPG as well as MSOP produced considerable proconvulsant activity in rodent tests [47,59,60]; up to date it remains unclear, which of the three group-III mGluRs expressed in seizure-relevant brain nuclei is/are responsible for the observed convulsions. In this context, it is quite interesting to mention that MMPIP, the only commercially available and *in vivo* characterized mGluR7-selective antagonist, does not affect the seizure threshold for electrically- nor chemically-induced convulsions; on the other hand, surprisingly little positive activity in rodent CNS disease models has been seen with MMPIP so far [61], which is in contrast to multiple benefits of genetic antagonism in mGluR7-deficient mice [62]. A possible explanation may be that MMPIP is only a context-dependent antagonist of mGluR7, *i.e.* it antagonizes certain physiological pathways downstream of mGluR7 but leaves others untouched, in other words, such compounds have different actions depending on coupling in different cell types [20,63,64].

Taken together, the proconvulsive action of broad spectrum group-III antagonists is prohibitive for further consideration as therapeutic principle. Furthermore, too little is still known about group-III subtype-selective blockers, *e.g.* the mGluR7-selective

Table 1
Pharmacological properties of 12 important drugs depicted in Figs. 2 and 3.

Compound	Potency (EC_{50} μM)				Selectivity	References
	mGluR4	mGluR6	mGluR7	mGluR8		
L-AP4	0.10	1.0	330	0.25	group-III selective agonist	[74]
ACPT-I	1.7	10.6	280	5.1	group-III selective agonist	[55,59,66–68,74]
(*S*)-DCPG	8.8	3.6	>100	0.03	mGluR8 selective agonist	[56,57]
LSP1-2111	2.2	1.7	53	66	mGluR4 preferring agonist (in brain)	[16,17,74,78]
LSP4-2022	0.11	4.4	11.6	29.2	mGluR4 selective agonist	[75]
(−)-PHCCC	3–4	>30	>100	>10	mGluR4 preferring PAM	[8,85]
VU0155041	0.7	n.r.	>15	>15	mGluR4 selective agonist/PAM	[89,90]
VU0364770	0.3	7	>10	>10	mGluR4 selective PAM	[13,90]
ADX88178	0.004	>3	>3	>3	mGluR4 selective PAM	[90]
AMN082	>10	>10	0.09	>10	mGluR7 selective allosteric agonist; a metabolite of AMN082 shows additional pharmacological activities	[10,18,64,98]
AZ12216052	>30	n.r.	n.r.	1	mGluR8 preferring PAM, but with additional pharmacological activities	[25,26]

n.r.: Not reported.

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negative allosteric modulators (NAMs; [61,63,64]), in order to discuss their potential for specific clinical conditions, at present.

3. Path of discovery and pharmacology of new generation orthosteric and allosteric activators of group-III mGluRs

3.1. Discovery and state-of-the-art of new generation group-III orthosteric agonists

As discussed above, the first group-III orthosteric agonists displayed no subtype selectivity and poor brain exposure. A first improvement was achieved with the discovery of dicarboxylic acid derivatives such as ACPT-I [55] and (*S*)-DCPG [56]. They were both able to penetrate into the brain and (*S*)-DCPG was selective for mGluR8 while constraining L-AP4 into (1*S*,2*R*)-APCPr did not provide significant benefits compared to L-AP4 [65]. Although ACPT-I did not discriminate between mGluR4 and mGluR8 (Table 1), positive effects were observed in animal models of convulsions [59], anxiety [66], Parkinson's disease [67], and pain [68]. (*S*)-DCPG was also shown to be anticonvulsant and neuroprotective [30] as well as anxiolytic [25]. The major breakthrough was gained with the discovery of PCEP [69] resulting from a virtual high throughput screening (vHTS) of a homology model of the mGluR4 VFT domain [70]. The mGluR4 homology model was generated and validated [44,48] and a vHTS workflow set up to screen data bases of commercially available compounds [70]. Out of about 720,000 molecules, 38 were selected after the docking-scoring process, purchased and tested on HEK293 cells expressing mGluR4 [70]. Activities at 100 μM were compared to the response of 1 mM L-glutamate. One compound, (*R*)-PCEP, gave a full agonist response and its molecular structure suggested numerous chemical modulations [69]. (*S*)-PCEP is a phosphinate derivative of L-AP4 yet such compounds had not been discovered previously by rational design since phosphinates were thought to be inactive because phosphinothricin, a well known herbicide (Fig. 2), was found to be poorly active at group-III mGluRs [71,72]. Potency was restored thanks to the carboxyethyl chain of the elongated PCEP that allowed the discovery of a new binding pocket adjacent to the L-glutamate binding site in lobe 1 of the VFT domain [69]. This new pocket is lined with residues that differ among the mGluR subtypes, and it should prompt the discovery of selective ligands that bear an elongated chain reaching into this site. Varying the PCEP chemical structure revealed that its L-AP4-like part may not be easily modified while numerous substitutions are tolerated in the distal side chain [69]. Indeed, the optimization of (*S*)-PCEP led to LSP1-3081 [73], LSP1-2111 [74] and LSP4-2022 [75] that displayed increased potency and/or selectivity for the mGluR4 subtype (Fig. 2, Table 1). LSP4-2022 is the first subtype-selective orthosteric agonist to be discovered, with EC_{50}'s of 0.11 μM, 4.4 μM, 11.6 μM and 29.2 μM at mGluR4, -6, -7 and -8 and no effect at group-I and II receptors when tested at 100 μM. A detailed mutagenesis study confirmed that the selectivity of LSP4-2022 originates from the size of the new binding pocket which can fit the phenoxyacetic side chain in mGluR4 but not in mGluR8 [75]. This pocket is thought to be a chloride binding cavity [76], thus, substituents taking the place of this ion should increase the affinity of ligands by allowing stabilizing interactions. Indeed, the significantly improved potency of LSP4-2022 at mGluR4 results from the distal carboxylic function that binds to the cavity through the same hydrogen bonding network as the presumed chloride ion [75,76]. Interestingly, such an interchange between a chloride ion and the carboxylate of a glutamyl residue has been shown to be the core of the ClC chloride channel function [77]. In terms of perspective, other selectivity sites that can be reached with extended ligands may possibly be discovered within the family of mGluRs. Such sites will take advantage of receptor regions which underwent relatively little evolutionary conservation pressure and that are situated outside of the highly conserved L-glutamate binding site. In this context, LSP4-2022 has opened the way.

The new orthosteric drugs described here were demonstrated to activate presynaptic receptors that inhibit the release of neurotransmitters [73–75] and to produce beneficial effects in animal models of Parkinson's disease [74,75] and other neuropathologies [16,17]. Finally, although it was not expected due to the very polar structure of these compounds, they are able to cross rapidly the blood-brain barrier [74,75] and display good pharmacokinetic properties with high plasma and brain free-fractions (>10%) and little, if any, off-target interactions [78] in rats. The brain exposure to LSP1-2111 and LSP4-2022 was detected after systemic injections of the compounds which resulted in anticataleptic effects in rats treated with haloperidol [74,75]. These effects were similar to those observed after i.c.v. injection, attesting the drug's penetration into the brain [74,75]. Other mGluR agonists that are also negatively charged amino acids such as ACPT-I [58] and DCPG [57] (see above) as well as group-II mGluR agonists (*e.g.* APDC [79] and LY354740 [80]) are also brain penetrant. It is assumed that they are all actively transported [81] in contrast to allosteric modulators that presumably transit the BBB by passive diffusion. When tested on group-I and -II mGluR subtypes at 100 μM [74] and in a profiling platform that included a large panel of GPCRs, ion channels and enzymes at 10 μM (Cerep, Poitiers, France), LSP1-2111 exhibited no off-target interaction [78] as expected with such charged molecules. However this property may also result in rapid clearance of the drugs: when injected i.p. at a dose of 15 mg/kg, the effect in a PD model was seen during 2 h with LSP1-2111 [74] and was reduced to a duration of about 1 h with the more polar (but also more potent) LSP4-2022 compound at 0.75 mg/kg [75]. Regarding the oral bioavailability of orthosteric mGluR agonists, this is limited by the gastro-intestinal barrier. However, this restriction may be overcome by dipeptide prodrugs that are transported to the blood by PEPT1 (peptide transporter 1). This is well illustrated with LY2140023, the prodrug of LY404039, which is presently in advanced clinical trials [82].

In conclusion, new orthosteric agonists of group-III mGluRs may be found that meet the various criteria that are expected for drug development: (i) subtype selectivity, (ii) high potency, (iii) transport through the BBB, (iv) good pharmacokinetic parameters and (v) high aqueous solubility. The historic dogma that L-glutamate analogues may not be suitable for drug development may need to be reassessed and moreover, such compounds could even provide distinct advantages, *e.g.* very little interaction with other brain proteins and high soluble free-fraction. Future development of this type of orthosteric ligands will tell whether our optimistic prospectives are valid.

3.2. Discovery and current status of group-III allosteric agonists and PAMs

Allosteric modulation recently emerged as a novel and very promising concept of target receptor intervention [2,47,83–85] and this approach experienced expansive utilization since the discovery of the fist mGluR subtype-selective allosteric modulators CPCCOEt and SIB-1757 (as well as its chemical derivative MPEP, Novartis Pharma AG, Switzerland) that were found to be negative allosteric modulators (NAMs) at mGluR1 and mGluR5, respectively. Pharmacological binding activity of those drugs was found to be topographically distant from the mGluRs' VFT domain; instead, a direct interaction with specific amino acids of the TM-III, and -VII domains was demonstrated [83,84,86,87]. The discovery of those early group-I NAMs and the development of the most recent follow-up drugs that progressed into preclinical and clinical development [3] is tightly linked to progressive utilization of high-throughput

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screening (HTS, [88]) of large, random libraries of chemicals *via* the use of second messenger functional assays with recombinant cell lines expressing individual mGluR-subtypes on their membrane surface. Recently, HTS was also successfully applied for mGluR4, mGluR7, and mGluR8; and PHCCC was the first allosteric modulator described for a group-III receptor. The molecule was identified in an HTS screen at Novartis Pharma AG (Basel, Switzerland) with a CHO cell line expressing human mGluR4, the drug is a quite selective PAM for mGluR4, at least within group-III, but it is chemically related to CPCCOEt and therefore displays also some mGluR1 NAM-activity [8,85]. Since its discovery as mGluR4 PAM, PHCCC proved to be instrumental to demonstrate the benefits of selective mGluR4 activation for the experimental treatment of Parkinson-like motor symptoms and nigrostriatal neurodegeneration in rodents [8,9,12,13,67,85]. However there is no information available, to our knowledge, how this efficacy of PHCCC or of more recent mGluR4 PAMs translates to primates, *e.g.* to MPTP-treated monkeys, which would be important to know in order to predict the translational value for human Parkinson patients. We are also not aware of many beneficial results of mGluR4-activating drugs in rat rotation models, which are often used as first line tests in the pharmaceutical industry to predict antiparkinsonian-like activity. However very recently, Jones et al. reported additive effects of the novel mGluR4 PAM VU0364770 and L-DOPA on 6-hydroxydopamine induced rotation and forelimb asymmetry in rats [13]. Positive results in rotation and/or MPTP monkey models are generally desired before clinical development. A further lack of knowledge relating to PHCCC and follow-up PAMs is how their effect size of activity in animal models of Parkinson's disease compares to L-DOPA or bromocriptine, which are registered drugs for this disorder. This lack of knowledge may result from the possibility that mGluR4-activating drugs showed lower efficacy in direct comparisons with the registered drugs. But it is important to mention here that mGluR-interfering drugs in general, and group-III agonists/PAMs in particular, are not primarily meant to replace L-DOPA or bromocriptine therapy in man, but instead to serve as possible efficacy augmentation drugs and maybe for neuroprotective therapy. In addition and unfortunately, PHCCC does not show good bioavailability and chemical optimization into more potent molecules with better pharmacokinetic properties failed until today. Consequently, extensive further random screening of chemical libraries was conducted at several University and industrial sites and multiple mGluR4 PAMs of novel and diverse chemical scaffolds were identified and further optimized. Several of those drugs, *e.g.* VU0155041, Lu AF21934, VU0364770 and ADX88178 (Fig. 3), already progressed into animal models of Parkinson's disease and the beneficial effects previously observed with PHCCC were convincingly replicated and extended [13,89,90]. Moreover, several of those recent mGluR4 PAMs show nanomolar affinity while PHCCC has an EC_{50} at mGluR4 of approximately 3–4 μM (Table 1); particularly VU0364770 and ADX88178 combine high potency with good bioavailability and convincing *in vivo* efficacy [8,13,85,89,90]. In contrast to group-I mGluR modulators, the exact localization of mGluR4 PAM binding at the amino acid level has not been addressed until now. But at least in the case of PHCCC, chimeric receptor studies were employed and the bindings site was localized C-terminal to the cystein-rich domain of mGluR4, most likely within the transmembrane domain [85]. It is interesting to speculate that PHCCC may directly interact with specific TMVII amino acids of mGluR4 (and/or with TMIII; see Fig. 1), as this drug represents a very close chemical derivative of CPCCOEt, which binds to the TMVII amino acids Thr815 and Ala818 of mGluR1; the mGluR5 modulator MPEP also binds to an overlapping site of TMVII, with TMIII amino acids also being involved [83,84].

Like PHCCC, the first mGluR7-selective activator AMN082 was also directly identified by HTS at Novartis Pharma AG (Switzerland), and no further and successful chemical optimization was reported. In both cases, a radioactive GTPγS functional-binding assay with recombinant CHO cell membranes expressing human mGluR-subtypes was used for primary screening [10,85]. AMN082 is selective for mGluR7, it shows an EC_{50} of approximately 60–90 nM, acts as an allosteric agonist that does not require the presence of any orthosteric ligand, which was shown in several *in vitro* assays [10,18,64]. In terms of molecular pharmacological mechanism, AMN082 governs context-dependent receptor activation, stimulating selectively certain mGluR7 pathways in specific brain regions and recombinant cells, but leaving other mGluR7 pathways untouched. For instance, cAMP metabolism in clonal cell lines, HPA axis-driven stress hormone release to the blood and synaptic plasticity in the amygdala are all modulated by AMN082, while certain mGluR7-regulated hippocampal ion channel functions and promiscuous G protein-coupling of mGluR7 stay unaffected [2,10,18,64,91]. Although the biochemical basis for this discrepancy remains unknown, it seems likely that AMN082 targets mGluR7 such that only a subset of the receptor's signal transduction pathways is activated. This may be explained by a novel pharmacological site at the receptor-G protein interface, which functions as binding site for allosteric agonists such as AMN082 (see Fig. 1) and possibly NAMs, too. Interestingly, MMPIP selectively antagonizes AMN082 *in vitro* and *in vivo* [19,20,64], but also acts in a context-dependent manner affecting similar physiological and behavioural parameters as AMN082, but leaving

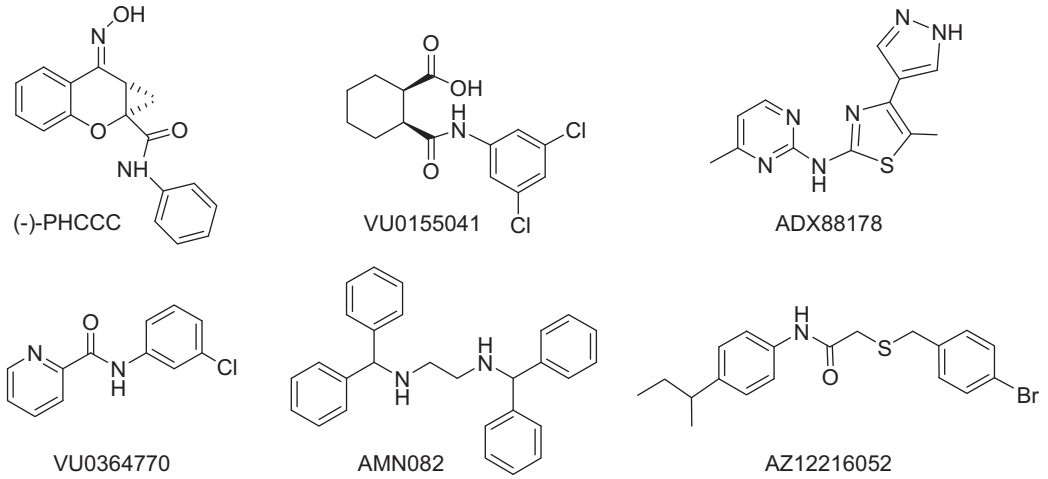

Fig. 3. Chemical structures of selected allosteric group-III mGluR activators. All those molecules are further explained in the text.

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other mGluR7 functions unaffected [20,63]. Furthermore, the perfect bilateral symmetry of AMN082 is a quite unusual feature for an allosteric drug, but it may suggest that the binding site for AMN082 bridges both subunits of the mGluR7 homodimer, possibly at the C-terminal cytoplasmic region, thus in close vicinity or even direct contact to the heterotrimeric G protein (Fig. 1).

The receptor mGluR8 shows a broad CNS distribution and is possibly involved in many disease-relevant physiological processes, including anxiety and epilepsy. The best characterized mGluR8-selective PAM is AZ12216052, which is a chemical derivative of an HTS compound, it has an EC_{50} of 1 μM, and in contrast to AMN082 has little, if any, intrinsic agonist activity [25,26]. Like AMN082 and the more recent mGluR4 PAMs, AZ12216052 is systemically active in rodent CNS disease models upon intraperitoneal administration, but relatively few *in vivo* studies were published to date [25,26].

Taken together, selective allosteric activators for mGluR4, -7, and -8 were discovered by HTS of random chemical libraries, using recombinant cell lines for cloned mGluRs in combination with functional receptor assays. Several examples of nanomolar-potent allosteric compounds were already identified, which are likely to target different hydrophobic regions on their receptors' surface. In general, the lipophilic nature of current group-III allosteric potentiators has the advantage of good bioavailability and systemic *in vivo* activity, but there is also the potential drawback that such compounds interact unspecifically with hydrophobic regions of several other CNS membrane proteins, such as ion channels or transporters, too (see below, Section 4).

4. Advantages and disadvantages of current group-III orthosteric agonists *versus* current allosteric activators

The potential future therapeutic value of group-III mGluR activating ligands is supported by numerous rodent studies, *e.g.* available preclinical data supports an antiparkinsonian profile of mGluR4 and possibly also of mGluR7 activators [8–13]. This justifies the numerous discovery programs aiming to find novel chemotypes of such ligands. Two types of activating ligands, orthosteric and allosteric, are described in this review. Until very recently, most efforts were devoted to allosteric modulators; however the discovery of potent and selective mGluR4 orthosteric agonists reopened the debate on which mode of pharmacological activation to prefer [90].

The desirable attributes of a new chemical entity selected for drug development include optimal pharmacological activity, that is potency and selectivity, but also good solubility, good ADMET properties and patenting opportunities [92].

Because allosteric modulators generally bind to sites that have not been under much pressure of evolutionary conservation, it will be easier to discover subtype-selective compounds in comparison to orthosteric ligands that bind to highly conserved sites. This is one of the major advantages of allosteric modulators across all mGluR-subtypes. However with the recent discovery of a selectivity pocket next to the L-glutamate binding site and the subtype selective mGluR4 agonist LSP4-2022, it is now conceivable that selectivity may be also found with orthosteric agonists. Similarly, nanomolar affinities may be reached more easily with allosteric modulators than with analogues of L-glutamate for which potency is typically found in the micromolar range. Yet the new selectivity pocket may also provide additional interactions and a substantial increase in potency of orthosteric agonists [75].

The highly polar structure of L-glutamate derivatives results obviously in a large aqueous solubility while the hydrophobicity of many allosteric ligands limits their solubility. On the opposite, high hydrophobicity is favourable for passive diffusion across the blood-brain barrier and amino acids often do not get into the brain by this way. Nevertheless, this limitation may be by-passed *via* active transport as described above [93]. The polarity of the compounds may also affect their free fraction in the plasma and in the brain. The more hydrophobic the drug, the lower the free fraction, which provides an advantage for orthosteric agonists.

Off-target pharmacological activity, metabolism and excretion are other important parameters to consider. Lipophilic compounds are more prone to non-specific/off-target binding, potentially resulting in undesired side effects, and to CYP450 metabolism, which often limits their plasma half-life; highly polar drugs like orthosteric group-III agonists, on the other hand, are more susceptible to rapid renal clearance. Regarding oral absorption, allosteric modulators are usually better at getting through the gastric intestine tract but dipeptidic prodrugs with the help of transporters, *e.g.* through PEPT1, may circumvent this drawback of orthosteric agonists [82].

Finally, positive allosteric modulators (PAMs) do not activate directly the receptors but potentiate the response to endogenously released agonists (phasic activation). PAMs are thus often preferred as it is feared that continuous direct activation (tonic activation) by orthosteric agonists would desensitize and/or internalize the receptors. This reservation is somehow depending on the type of signalling pathway activated by agonists. Agonist-induced internalization and desensitization is indeed observed when mGluRs are Gαq-coupled but less likely when coupled to the Gαi/o-pathway. Fortunately, group-III mGluRs are mostly of the second type and may be activated without internalization and desensitization [94] except for mGluR7 which seems to internalize rapidly upon orthosteric and allosteric agonist exposure [95]. Furthermore, certain pharmacological applications, *e.g.* obtaining long lasting and permanent anti-neurodegenerative activity, may require tonically acting group-III receptor agonists to obtain best efficacy. In contrast, if aversive side-effects of tonic activation emerge as the major concern during the development of a certain agonist compound class, phasic activation using a PAM may provide the desired solution.

In general, it is difficult at present to evaluate whether orthosteric or allosteric activators of group-III mGluRs will be superior. This uncertainty includes patent protection opportunities. Clearly, in the field of group-II mGluR orthosteric agonists a multitude of chemical structures is already broadly protected by the pioneering chemistry of Eli Lilly and competing drug companies. Here, it becomes increasingly difficult to find patent niches for mGluR2/3 agonists. In contrast, obtaining patent protection for novel group-III mGluR orthosteric agonists will be easier, as there is little chemical space covered until today. Moreover, future utilization of the novel selectivity pocket that is located adjacent to the L-glutamate site, as described in this text, provides the opportunity to design and patent chemically very diverse group-III agonists. Patenting opportunities for allosteric GPCR drugs are generally considered favorable. This is still very true for group-III mGluR PAMs and allosteric agonists, because relatively few discovery efforts were successful so far, for instance when compared to mGluR5 NAM discovery.

The common assumption that future drugs should meet the Lipinski rules for drug-like features is currently reappraised [96]. In particular chemical compounds which demonstrated favourable ADMET properties, but do not show drug-like chemical structures according to Lipinski, may be reassessed as drug-development candidates. Altogether, it emerges that considering the list of requirements for a drug to proceed into development, both allosteric modulators as well as orthosteric ligands may be selected. Allosteric modulators will probably keep the favour of a large part of the research community; however we show in this commentary that orthosteric ligands will also offer valuable opportunities.

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5. Conclusions and perspectives: future drug discovery directions for the activation of group-III mGluRs

A diverse panel of subtype-selective mGluR ligands is currently under clinical development for the treatment of a variety of nervous system dysfunctions including schizophrenia, Parkinson's disease and L-DOPA-induced dyskinesias, Fragile-X syndrome, generalized anxiety disorder, gastroesophageal reflux disorder, and chronic pain. However, all those drugs selectively target group-I or group-II mGluRs, primarily mGluR2/3 and mGluR5 [3–7]. As described here and elsewhere, the selective activation of the group-III mGluR subtypes mGluR4, -7 and -8 holds equally great promise for future development of clinical drugs [11–14,16,17,20,39,47,67,74,75]. Most notably, functional enhancement of individual group-III receptors, using various modes of pharmacological activation, has been preclinically validated for convulsive disorders (mGluR7 and -8), mood and anxiety disorders (mGluR4, -7, and -8), Parkinson's disease and pain (mGluR4 and possibly -7), as well as drug addiction (mGluR7). Besides this still continuing validation in animal CNS disease models, high and widespread brain expression of group-III mGluRs, most notably of mGluR4 and mGluR7, provides further support for their therapeutic utility [2,47].

Moving forward with group-III mGluR ligands into clinical development was so far limited by insufficient pharmacological suitability of early compounds, *i.e.* low potency and selectivity, poor bioavailability and/or metabolic stability as well as patenting issues. Recent advances with two approaches, *i.e. in silico* HTS against a 3D-model of the mGluR4 extracellular domain and functional receptor assay HTS against recombinantly expressed group-III receptors identified diverse chemical sets of orthosteric agonists and allosteric enhancers, respectively. As described here, both strategies led to the discovery of chemical molecules with drug-like properties and novel chemotype structures. Clearly, both approaches to discover group-III activators hold great promise and need to be continued, but with distinctly different application possibilities. For instance in the case of convulsive disorders, the activation of mGluR7 and mGluR8 might result in optimal efficacy (in contrast to the activation of just a single receptor; [30–35]). As dual or even triple agonist activity is rather characteristic for orthosteric agonist, this pharmacological mode of action could be best suited to develop new treatments for human epilepsy disorders. Recently, new evidence emerged that the activation of mGluR4 and -7 could be favorable to obtain good efficacy across several pain models, with inhibition of inflammatory pain *via* the activation of mGluR7 and relief of neuropathic pain *via* mGluR4 activation ([14] and references therein). This may again provide an excellent direction for the development of orthosteric agonists with dual receptor agonist activity.

Allosteric agonists and PAMs, on the other hand, are normally very specific for a single receptor subtype within group-III mGluRs. This feature of very high specificity has been also observed for group-I NAMs and PAMs [2,86,97]. Therefore, allosteric compounds are best suited for therapeutic indications where interference with a single receptor target is sufficient and activation or blockade of closely related receptors carries the risk of prohibitive side-effects. Although no such adverse examples have been clearly demonstrated in the case of group-III mGluRs, it is of interest to mention that ligands with a potent mGluR6 component may possess the risk of undesired ocular side effects, as this receptor controls vision under dim light conditions and as mGluR6 mutations that abolish receptor trafficking lead to congenital stationary night blindness [39,40]. The best characterized allosteric activators for mGluR4, -7, and -8 seem to show little, if any, interaction with mGluR6 [10,13,25,85], which definitely reduces their chances of undesired ocular effects. Most orthosteric agonists, on the other hand, do not discriminate strictly between the four subtypes of group-III mGluRs, presumably due to the high evolutionary conservation of the L-glutamate binding pocket. However the recent discovery of LSP4-2022 describes a 40-fold selectivity of this drug for mGluR4 over mGluR6 and even better discrimination against mGluR7 and mGluR8 [75], which provides confidence that the selectivity disadvantage of orthosteric group-III agonists can be overcome *via* recruitment of additional binding pockets that lie adjacent to the L-glutamate site, but are subject to less evolutionary conservation pressure (see Fig. 1).

The mGluR7-subtype confronts orthosteric drug discovery with two serious problems: firstly, no single orthosteric agonist has been found so far that prefers mGluR7 over the other group-III receptors; and secondly, the potencies of orthosteric agonists at mGluR7 are roughly 100-fold weaker than at other group-III mGluRs (Table 1) and thus, discovery of nanomolar-active orthosteric agonists for mGluR7 seems to be extraordinarily difficult. However, incremental increases in agonist potencies at mGluR7 have been obtained from L-glutamate (EC_{50} = 1–3 mM) to phosphonate analogues as L-AP4 ($EC_{50} \approx 300$ μM) and (*S*)-PPG (EC_{50} = 185 μM), to LSP1-2111 (EC_{50} = 53 μM) and LSP4-2022 (EC_{50} = 12 μM) [74,75]. These improvements result from additional binding contacts within the VFT domain [44] and in particular to the selectivity pocket for LSP4-2022 [75]. Indeed, two of the critical residues of this pocket, S157 and G158, are conserved between mGluR4 and mGluR7 allowing the phenoxyacetic side chain of LSP4-2022 to fit and provide additional stabilizing interactions with that pocket as described with mGluR4 [75]. It will be interesting to see whether further improvements could be gained by optimizing the recent series of agonists or by *in silico* screening of chemical libraries against 3D-models of the mGluR7 extracellular region. The high physiological and potentially therapeutic relevance of mGluR7 (see above) provides great motivation to further develop and utilize extracellular mGluR7 3D-models and *in silico* screening against yet undiscovered mGluR7 binding pockets within its VFT domain. There is great hope that this could be successful within the next 3–5 years. Meanwhile and since the discovery of AMN082, the mGluR7 allosteric agonist approach provides an attractive alternative; AMN082 shows high potency (EC_{50} at mGluR7 = 60–90 nM) with no mGluR4, -6, or -8 interaction up to 10 μM [10,18]. Although this approach holds great promise, much better follow-up compounds need to be identified, due to several reasons: (i) AMN082 binds not only to mGluR7 but with weaker affinity also to other neural proteins including monoamine receptors and transporters, (ii) a fast *in vivo* metabolite of AMN082, termed Met-1, inhibits the brain serotonin transporter with a physiologically relevant affinity of 320 nM [98], (iii) the mGluR7-selective dose-range for AMN082 is very narrow and 2-fold elevated doses already induce side-effects like body tremor and ataxia [20]; these adverse effects seem to be off-target (*i.e.* not mGluR7-mediated) as they also arise in mGluR7-deficient mice (P.J.F., unpublished observations). Similar off-target effects were also observed with the mGluR8-PAM AZ12216052, presumably involving other CNS receptors [26].

Such off-target effects as described in this report for AMN082 and AZ12216052 are unfortunately a frequent feature of highly hydrophobic compounds that are most often identified by HTS with functional CNS receptor assays. Chemical derivation cannot easily eliminate this undesired compound activity as the molecular targets for off-target effects are rarely known. Quite likely, this issue has been the reason for several failures of recent mGluR compound development efforts in the pharmaceutical industry. There are certainly multiple further reasons why so many L-glutamatergic receptor ligands failed in development: importantly, the L-glutamate neurotransmitter system is intimately involved in learning and memory, emotional homeostasis, and, in conjunction with the

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GABA-system, in providing a balance between neural excitation and inhibition, the latter serving as a control over brain seizure activity. Thus, it is fair to speculate that L-glutamatergic agents may always have the danger of failure in preclinical or clinical development due to proconvulsive, neurotoxic, or cognition-impairing side effects, especially in long-term mammalian studies with drug-induced neural circuitry adaptations being possible.

Taken those considerations into account, validation of the mGluR4- and mGluR7-activators' therapeutic utility still advanced over the last years, at least in part due to the discovery of selective drug tools such as LSP4-2022, PHCCC, VU0364770, Lu AF21934, ADX88178 and AMN082, as well as *via* the utilization of transgenic mouse- and siRNA-approaches [9,10,18,54,62] involving those receptors. Little however is currently known about the mGluR4- and mGluR7-activators' potential side effects in long-term preclinical studies. An understanding of mGluR6 and mGluR8 in CNS/brain physiology and pathology starts to emerge but their pharmacological utility, or their roles in adverse side-effects, remain largely unclear until present.

In addition to the L-glutamate related orthosteric mGluR2/3 agonists which show early positive signals in clinical studies across various disorders [3,5,7], group-III mGluR-subtype selective orthosteric agonists are also likely to follow a similarly successful route, but an important prerequisite is that suitable dipeptidic prodrugs of those agonists (*e.g.* of LSP4-2022) become available; this will be an important next step for medicinal chemistry. Such orthosteric agonists with mGluR4 selectivity have a good chance of success in human Parkinson's trials. Interestingly and in addition, recent evidence suggests that *in vivo* efficacy of the orthosteric group-III mGluR-selective agonist ACPT-I in animal depression models can be enhanced with an mGluR4 PAM [36], which provides a potential rational for combining PAMs and orthosteric agonists in human mood disorder clinical trials.

The good oral bioavailability, blood-brain-barrier penetration, *in vivo* efficacy in rodents, and high potency at individual group-III receptors qualifies the recent allosteric modulators/agonists as excellent starting points for medicinal chemistry and to consider clinical CNS trials in the near future. Several allosteric compounds however, *e.g.* AMN082 and AZ12216052, shall be considered only as tool drugs to probe mGluR7 and mGluR8 *in vivo* function, respectively. Here, focused chemical derivation and optimization will be required to improve the molecules' metabolic parameters and to address, and possibly eliminate, their off-target interaction with hydrophobic regions of multiple other CNS proteins (see above). In contrast, several PAMs for mGluR4 are much further advanced (Table 1) and the scientific community awaits with eagerness initiation of clinical trials in Parkinson's disease and possibly for further human neurodegenerative disorders.

Acknowledgements

P.J.F. is supported by the grant FL 729/2-1 from the German Research Foundation (DFG). F.C.A. acknowledges the Agence Nationale de la Recherche Neuro and EraNet Neuron programs, the Fondation de France Parkinson's committee and the Centre National de la Recherche Scientifique.

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