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Pharmacological Characterisation of Positive Allosteric Modulators Acting on the Human Metabotropic Glutamate Receptor 2

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Introduction

There are eight subtypes of metabotropic glutamate (mGlu) receptors which bind the neurotransmitter glutamate. The mGlu2 and mGlu3 receptors are expressed pre-synaptically in the cortex, thalamus, striatum, amygdala and hippocampus. Hyperactivity in glutamatergic transmission in these regions is associated with anxiety disorders and psychosis and may be ameliorated by pharmacological activation of mGlu2. The selectivity and tolerance issues faced by orthosteric agonists of mGlu receptors are circumvented by allosteric modulators, which modulate the affinity (α) and/or efficacy (β) of orthosteric ligands. Here we show how reported positive allosteric modulators (PAMs) of mGlu2 (BINA, JNJ-40068782 and LY487379) affect the affinity and efficacy of L-glutamate at human mGlu2 receptors.

Materials and Methods

Binding assay: Studies were established using the orthosteric antagonist [^{3}H]-LY341495 in HEK293 cell membranes transiently expressing mGlu2. A three-way competition binding assay was used to study the interaction between [^{3}H]-LY341495, L-glutamate and the PAMs (BINA, 2 µM – 2 nM; LY487379 and JNJ-40068782, 30 µM – 10 nM) allowing estimation of affinity co-operativity between PAM and L-glutamate (α'). Data was analysed globally using the simple allosteric ternary complex model [1,2].

Functional assays: An inducible mGlu2 expressing Jump-In HEK cell line (Life Technologies) was used to measure $G_{\alpha i/o}$ activity using a commercially available cAMP kit (HTRF; CisBio). Receptor expression was induced with 1 µg/ml doxycycline (16 h) and prior to challenge, 3U/ml glutamate pyruvate transaminase and 5 mM sodium pyruvate was applied to eliminate endogenous glutamate. When necessary, cells were pre-treated with PAM (15 min) before the addition of L-glutamate. Data was analysed globally using the operational model of allosterism [3] to elucidate net affinity/efficacy co-operativity parameters (αβ) and the effect of modulator on orthosteric ligand intrinsic efficacy (β) .

Results

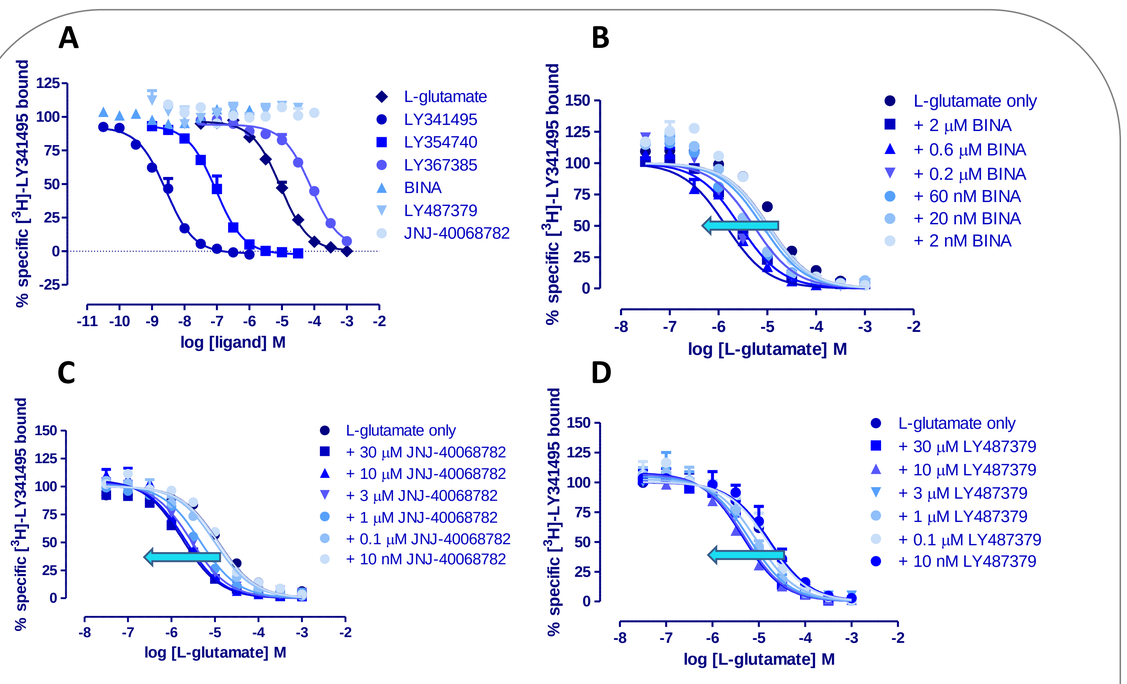

Figure 1: mGlu2 PAMs do not alter equilibrium binding of the orthosteric radioligand [^{3}H]-LY341495 but, upon co-administration, act to increase the affinity of the orthosteric agonist L-glutamate. A. Whilst orthosteric ligands (L-glutamate, LY341495, LY367385 and LY354740) can fully displace [^{3}H]-LY341495 binding, mGlu2 PAMs (BINA, LY487379, JNJ-40068782) have no apparent effect (i.e. they act as neutral modulators; α = 1). Upon co-administration, **B.** BINA, **C.** JNJ-40068782 and **D.** LY487379 cause a concentration-dependent increase in the ability of L-glutamate to inhibit [^{3}H]-LY341495 binding. Using the operational model of allosterism an estimate of allosteric modulator affinity could be calculated (see **Table 1A**-**B**). Representative data from three individual experiments, fitted to the simple allosteric ternary complex model.

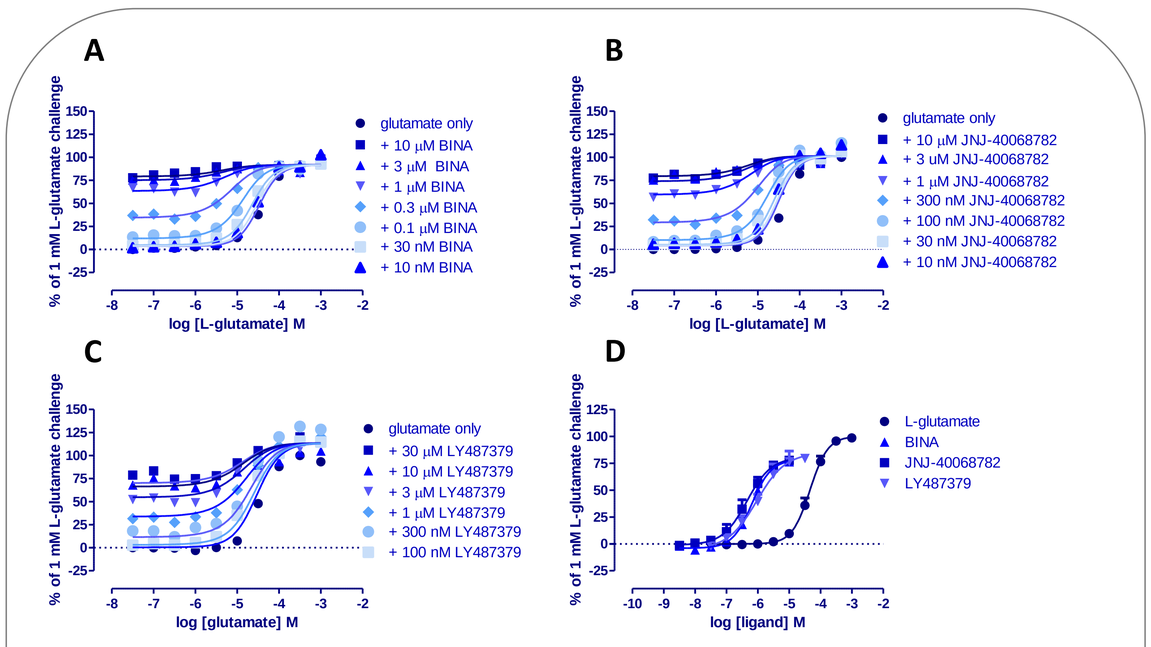

Figure 2: mGlu2 PAMs increase the potency of L-glutamate and, in this system, act as agonists in their own right ('allosteric agonists'). Cells were stimulated with forskolin and concentration-response curves were generated to glutamate in the absence and presence of increasing concentrations of **A.** BINA, **B.** JNJ-40068782 and **C.** LY487379. Each of the PAMs caused a concentration-dependent increase in L-glutamate potency. **D.** In this system each of the PAMs acted as agonists in their own right, although their efficacy was partial compared to L-glutamate (**Table 1A**). Data in **A-C** representative data from three independent experiments, fitted to operational model of allosterism **D.** mean ± S.E.M from n=3. Potency and efficacy values are reported in **Table 1A,** allosteric effects on orthosteric ligand intrinsic efficacy (β) shown in **Table 1B**.

A

Compound	pK_i (mean ± S.E.M; n=3) pK_i of PAMs estimated from binding assay	pEC_{50} (mean ± S.E.M; n=3)	E_{max} (% of L-glutamate response) (mean ± S.E.M; n=3)
L-glutamate	5.69 ± 0.13	4.33 ± 0.04	100 ± 0
BINA	7.40 ± 0.08	6.19 ± 0.06	78 ± 4
JNJ-40068782	5.79 ± 0.21	6.35 ± 0.10	80 ± 6
LY487379	5.91 ± 0.21	6.03 ± 0.17	85 ± 12

B

Compound	Co-operativity of binding between PAMs and L-glutamate (α') (mean ± S.E.M, n=3)	Net co-operativity (αβ; mean ± S.E.M, n=3)	Allosteric effects on orthosteric ligand intrinsic efficacy (β)
BINA	4.81 ± 0.10	23.44 ± 1.48	4.87
JNJ-40068782	2.25 ± 0.25	8.95 ± 1.27	3.98
LY487379	6.65 ± 0.44	5.50 ± 1.39	0.83

Table 1: Properties of mGlu2 PAMs as determined in binding and functional analysis. A. Properties of L-glutamate and mGlu2 PAMs determined from experiments performed in Figure 1 and 2. **B.** Co-operativity of binding (α') between L-glutamate and PAMs were determined from binding assays (**Figure 1**). The net co-operativity (αβ) was determined in functional assays (**Figure 2**), allowing estimation of the allosteric effects on orthosteric ligand intrinsic efficacy (β) to be calculated.

Conclusions

Activation of mGlu2/3 receptors negatively feeds back to reduce glutamate release at glutamatergic synapses. The results from this study highlight subtle differences in the ability of mGlu2 PAMs in modulating the affinity and efficacy of L-glutamate:

- **BINA equally modulated L-glutamate affinity and efficacy**
- **JNJ-40068782 modulated L-glutamate efficacy to a greater extent than affinity**
- **LY487379 acted predominantly to increase affinity of L-glutamate.**

References

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