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COMMENTARY

Two new non-competitive mGlu1 receptor antagonists are potent tools to unravel functions of this mGlu receptor subtype

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The validation of the selective, potent and systemically active non-competitive mGlu1 antagonists YM-298198 and JNJ16259685 in a physiological functional assay will facilitate elucidation of this receptor's role in brain function and as a potential drug target.
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The vast majority of mammalian excitatory synapses use glutamate (Glu) as transmitter. Fast glutamatergic transmission is mediated by receptors that form ion channels conducting synaptic ionic currents. In addition to these ionotropic Glu receptors, Glu can also activate receptors with seven transmembrane spanning domains that couple to G-proteins and that mediate their effects via intracellular chemical signalling cascades. These metabotropic Glu receptors (mGluR) allow Glu to function both as an excitatory and an inhibitory neurotransmitter (Coutinho and Knöpfel, 2002).

The pioneering molecular approach to the identification of mGlu receptors took place in early 1990s before the development of specific pharmacological tools (Masu *et al*., 1991). Eight genes coding for different subtypes of mGluRs were identified and the naming of mGlu1 to 8 receptors follows the historical progression of their cDNA cloning. In view of sequence similarities, principal signal transduction capabilities in recombinant expression systems and pharmacological properties, the family of mGluR subtypes is divided into three groups. In recombinant expression systems, such as human embryonic kidney (HEK 293) and Chinese hamster ovary cells, group I mGluRs (consisting of mGluR1 and 5) couple to phospholipase C and thereby activate the inositol 1,4,5-trisphosphate (IP3)/Ca^{2+} signalling pathway. The subtypes of group II (mGluR2 and 3) and group III (mGluR4, 6, 7 and 8) inhibit adenylate cyclase and thereby inhibit production of cyclic AMP (Knöpfel *et al*., 1995).

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Soon after their discovery, mGlu receptors were recognized as potential drug targets for a variety of pathological states ranging from epilepsy and neurodegeneration to neuropsychiatric diseases (Knöpfel *et al*., 1995; Nicoletti *et al*., 1996). This high expectation coincided with an emerging trend in pharmaceutical industry to develop high-throughput screening technologies based on recombinant human receptors in functional cellular assays. The fruits of these efforts are now becoming freely available as pharmacological tools. Here I comment on two new potent non-competitive antagonists at mGlu1 receptors. The new compounds are JNJ16259686 ((3,4-dihydro-2*H*-pyrano[2,3-*b*]quinolin-7-yl)-(*cis*-4-methoxycyclohexyl)-methanone) and YM-298198 (6-amino-*N*-cyclohexyl-*N*,3-dimethylthiazolo[3,2-*a*]benzimidazole-2-carboxamide hydrochloride). In this issue of the *British Journal of Pharmacology*, Batchelor and co-workers have used sophisticated electrophysiological methods to characterize these compounds (Fukunaga *et al*., 2007b). Remarkably, their assay is close to the physiological function of mGlu1 receptors as they use synaptic responses to test the antagonists (complemented by conventional 'bath-applied agonist in the presence of antagonist' experiments). This approach is to be applauded since signal transduction mechanisms of mGlu1 receptors are notoriously different when expressed in recombinant systems, as compared with endogenous expression (Charpak *et al*., 1990) and, at least for allosteric ligands, should be validated in a natural receptor environment.

Activation of mGlu1 induces an excitatory current (and slow synaptic potential) in cerebellar Purkinje neurons (PNs) (Staub *et al*., 1992; Batchelor *et al*., 1994). PNs are a good choice for testing mGlu1 receptor pharmacology because they express high levels of mGlu1 but not the structurally and pharmacologically related mGlu5 receptors. Indeed, PNs have been successfully used as a model to characterize the

first mGlu1 antagonists (Lingenhohl *et al.*, 1993; Batchelor *et al.*, 1997), including the first non-competitive mGlu antagonist CPCCOEt (7-(hydroxyimino) cyclopropa[*b*]chromen-1*a*-carboxylate ethyl ester) (Annoura *et al.*, 1996; Casabona *et al.*, 1997). This compound has since been proven as a useful tool. However, CPCCOEt is of modest potency (IC50 ~ 40 μM at PN mGlu1 responses) and therefore a recent report of some unexpected side effects, again using PN synaptic responses, should not have been surprising (Fukunaga *et al.*, 2007a). The two new compounds, YM-298198 and JNJ16259685, withstand such scrutiny. In the functional physiological assay, YM-298198 and JNJ16259685 exhibited IC_{50} values of 24 and 19 nM, respectively. These values are very close to those obtained in recombinant expression systems and brain membrane preparations (YM-298198: 16–20 nM; Kohara *et al.*, 2005; JNJ16259685: 1.2–3.2 nM; Lavreysen *et al.*, 2004). The lesson here is that despite quite massive efforts (at the industrial level), development of potent and selective compounds can take quite some time and, after initial success, there is always space for further improvement; in particular, better selectivity against mGlu5 receptors should be considered. JNJ16259685 exhibits antagonistic effects at mGlu5 albeit with a roughly 1000-fold lower potency compared to mGlu1 (Lavreysen *et al.*, 2004) and YM-298198 binds to mGluR5 with more than 100-fold less potent antagonism at mGlu5 compared to mGlu1 (Kohara *et al.*, 2005). The selectivity of both compounds against mGlu5 is sufficient to secure convincing mGlu1 specificity when local concentrations (or receptor occupancies) are known and overdosing is avoided.

Both compounds are systemically active (that is, pass the normal blood–brain barrier) and are therefore extremely valuable tools for system pharmacology and physiology. The mGlu1 receptor has received attention as potential target for anxiolytic, antidepressant, antipsychotic and antinociceptive drugs. mGlu1 receptors have also been suggested to play a role in the modulation of cognitive processes. In line with at least some of these ideas, JNJ16259685 reportedly induces some anxiolytic-like effects but also impairs spatial learning (Steckler *et al.*, 2005a, b) and YM-298198 was analgesic at very high doses (Kohara *et al.*, 2005). Unequivocal allocation of these effects to antagonism at mGlu1 is limited by the above-mentioned good, but not excellent, selectivity against the structurally and functionally-related mGlu5 receptor.

The physiological and pathophysiological roles of mGlu1 receptors at the behavioural level were previously deduced from alterations seen in mGlu1 receptor knock-out (KO) mice, with all the difficulties of separating developmental and adaptive effects from the acute effect of receptor blockade (and hence the acute and long-term physiological actions). First results that may lead to a revision of the KO conclusions are already emerging. While mGlu1 receptor KO mice are dramatically ataxic, systemic chronic delivery of either YM-298198 and JNJ16259685 at effective doses did not produce such dramatic alterations in motor coordination (Lavreysen *et al.*, 2004; Kohara *et al.*, 2005; Steckler *et al.*, 2005b). This certainly reinforces the cautious view that pharmacological approaches using high-specificity compounds can prevail over KO models.

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