



mGlu5 receptor functional interactions and addiction

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The idea of “receptor mosaics” is that proteins may form complex and dynamic networks with respect to time and composition. These have the potential to markedly expand the diversity and specificity of G protein-coupled receptors (GPCR) signaling, particularly in neural cells, where a few key receptors have been implicated in many neurological and psychiatric disorders, including addiction. Metabotropic glutamate type 5 receptors (mGlu5) can form complexes with other GPCRs, including adenosine A_{2A} and dopamine D₂ receptors. mGlu5-containing complexes have been reported in the striatum, a brain region critical for mediating the rewarding and incentive motivational properties of drugs of abuse. mGlu5-containing complexes and/or downstream interactions between divergent receptors may play roles in addiction-relevant behaviors. Interactions between mGlu5 receptors and other GPCRs can regulate the rewarding and conditioned effects of drugs as well as drug-seeking behaviors. mGlu5 complexes may influence striatal function, including GABAergic output of striatopallidal neurons and glutamatergic input from corticostriatal afferents. Given their discrete localization, mGlu5-[non-mGlu5] receptor interactions and/or mGlu5-containing complexes may minimize off-target effects and thus provide a novel avenue for drug discovery. The therapeutic targeting of receptor-receptor functional interactions and/or receptor mosaics in a tissue specific or temporal manner (for example, a sub-population of receptors in a “pathological state”) might reduce detrimental side effects that may otherwise impair vital brain functions.

Keywords: metabotropic glutamate receptor, drug addiction, drug-seeking, heteromer, receptor interaction, metabotropic glutamate receptor type 5, hub receptor

DRUG ADDICTION

Drug and alcohol addiction is a major problem in Western society where substance abuse is associated with considerable social, medical, and financial costs (Collins and Lapsley, 2002). It has been estimated that annually around \$7.5 billion is associated with alcohol and alcohol-related problems in Australia and about 5% of the total annual mortality is due to this (Collins and Lapsley, 2002). The Australian Institute of Health and Welfare estimated that in 2004, more than 56,000 people had used opiates in the previous 12 months and around 1% of the population had used cocaine (AIHW 2005). Due to the impact on society and limited success of current therapies to treat addiction, extensive research into the pathophysiology underlying alcoholism and drug abuse is ongoing. In particular, research efforts are focused toward understanding the persistent vulnerability to relapse displayed by drug-addicted individuals despite months or even years of abstinence (Gossop et al., 1989; Dejong, 1994; Jupp and Lawrence, 2009).

Current preclinical research into potential treatments for addiction is focused on gaining a greater understanding of the neurobiological mechanisms underpinning both relapse vulnerability

and the transition from casual to compulsive drug use. This knowledge will enable the identification of potential therapeutic targets for drug development. At present there is a paucity of pharmacotherapies on the market for the treatment of addictions; none of which are particularly effective and involve multiple off-target effects (Jupp and Lawrence, 2009). For this reason it is vital that new therapeutic targets are identified that have potential in this regard. Complexes containing the metabotropic glutamate receptor type 5 (mGlu5), and/or functional interactions between mGlu5 and other G protein-coupled receptors (GPCRs), represent a novel therapeutic option worthy of investigation due to the possibility of limiting off-target effects. mGlu5 has been implicated in numerous central processes, thus targeting mGlu5-containing complexes/functional receptor interactions that are restricted to addiction-relevant brain areas provide an avenue to selectively target those mGlu5 involved in reward and drug-seeking behavior (Bird and Lawrence, 2009b).

mGlu5 AND ADDICTION

A variety of GPCRs have been shown to play critical roles in the reinforcing and motivational properties of drugs, drug-induced

plasticity and drug-seeking behavior (Traynor, 2010). A substantial body of evidence exists which implicates metabotropic glutamate receptors in many of these facets of drug addiction (Bird and Lawrence, 2009a; Olive, 2009) and among them mGlu5 seems to play a significant role. mGlu5 belongs to a distinct family of eight GPCRs that are activated by L-glutamate, the major fast excitatory neurotransmitter in the central nervous system (CNS). At the signaling level, mGlu5 is known to be associated with phosphoinositide hydrolysis and the activation of phospholipase C, stimulation of adenylate cyclase and inhibition of voltage-operated calcium channels (Dhami and Ferguson, 2006). Signaling via mGlu5 is thought to play a role in synaptic plasticity, ultimately affecting learning and memory, neuronal development and neurodegeneration (Lu et al., 1997). It is also thought to have a role in a number of behaviors including stress responses, anxiety-like, and depressive-like behavior (Tatarczynska et al., 2001). mGlu5 is highly expressed in the olfactory bulb and tubercle, dorsal striatum, nucleus accumbens, cerebral cortex, hippocampal formation, and amygdala (Shigemoto et al., 1993; Romano et al., 1995). In the striatum, mGlu5 is located primarily in the perisynaptic ring and at the postsynaptic density (Kennedy, 2000; Smith et al., 2000), including striatal projection neurons containing enkephalin (Testa et al., 1995). Evidence of presynaptic localization of mGlu5 on cortical inputs to the striatum also exists (Romano et al., 1995; Rodrigues et al., 2005). The mGlu5 receptor is structurally linked to the NMDA receptor via a protein scaffold (Naisbitt et al., 1999) and functional interactions have been demonstrated between the two receptors (Attucci et al., 2001). Thus, mGlu5 receptors are perfectly positioned to influence the mesolimbic, corticostriatal, and corticostriatal pathways known to be critical for the actions of drugs of abuse and drug-induced plasticity.

The first evidence to suggest a role for mGlu5 in the rewarding properties of drugs of abuse was provided by Chiamulera et al. (2001) who reported that mice lacking mGlu5 failed to intravenously self-administer a range of doses of cocaine and failed to show any hyperactivity after acute administration of the drug. Subsequent pharmacological studies have supported these initial findings. Antagonism of mGlu5 can reduce the self-administration of multiple drugs of abuse as well as reinstatement of drug-seeking, indicating a facilitatory role for this receptor in these behaviors (Olive, 2009). For example, the mGlu5 antagonists 2-methyl-6-(phenylethynyl)-pyridine (MPEP) and 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP) reduce self-administration of alcohol, cocaine, and nicotine in rats (Kenny et al., 2003; Backstrom et al., 2004; Cowen et al., 2005) as well as alcohol and morphine in mice (Cowen et al., 2007; Brown et al., 2011b). These antagonists can also attenuate reinstatement of alcohol (Backstrom et al., 2004), cocaine- (Backstrom and Hyytia, 2006), nicotine- (Bespalov et al., 2005), opiate- (Brown et al., 2011b), and methamphetamine-seeking (Gass et al., 2009) in animal models of relapse, indicating a clear role for mGlu5 in drug-seeking behavior across a broad range of reinforcers. Genetic mouse models have also implicated mGlu5 in the reinforcing and motivational properties of drugs of abuse. In addition to the original work by Chiamulera and colleagues, deletion of the mGlu5 gene results in altered central reward processing in the case of ethanol

(Bird et al., 2008) and an absence of drug-induced synaptic plasticity in the ventral tegmental area in the case of cocaine (Bird et al., 2010). Thus, the putative involvement of mGlu5 in addictive behavior suggests that selective inhibition of this receptor may provide an exciting new approach for treatment of addictive disorders.

More recently evidence has been forthcoming which implicates mGlu5 in associative reward learning processes more generally. Published reports suggest systemic administration of the selective mGlu5 antagonist MTEP prior to conditioning disrupts the expression of conditioned reinforcement by food delivery (O'Connor et al., 2010). This suggests a critical role for mGlu5 in the acquisition of incentive properties by a conditioned stimulus (O'Connor et al., 2010); an observation which is supported by the finding that mice with knockdown of mGlu5 in striatal D₁-expressing neurons exhibit diminished cue-induced cocaine-seeking, as well as impaired incentive learning (Novak et al., 2010). Hence mGlu5 may play a central role in associative reward learning processes (Novak et al., 2010) in addition to the rewarding properties of drugs of abuse. This possible role in incentive learning is not inconsistent with the involvement of mGlu5 in cue-induced drug-seeking behavior (Backstrom et al., 2004; Backstrom and Hyytia, 2006). Indeed, in the operant conditioning paradigm, the discrete cue is a conditioned stimulus and as such needs to acquire incentive motivational properties in order to elicit drug-seeking upon re-presentation. Collectively, these studies provide further insight into the potentially complex role of mGlu5 in both reward and reward learning.

A role for mGlu5 in extinction learning relevant to drug use and drug-associated cues has also been established by recent research using positive allosteric modulators of mGlu5 (Cleva and Olive, 2011). The first mGlu5 positive allosteric modulators to be characterized were 3-cyano-*N*-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB; Lindsley et al., 2004) and *S*-(4-fluoro-phenyl)[3-[3-(4-fluoro-phenyl)-1,2,4-oxadiazol-5-yl]piperidin-1-yl]methanone (ADX47273; Liu et al., 2008) and were suggested to be beneficial in alleviating the cognitive deficits associated with chronic drug abuse (Reichel et al., 2011). Studies using animal models of addiction have also shown that CDPPB facilitates extinction of both cocaine-conditioned place preference (Gass and Olive, 2009) and operant responding for cocaine (Cleva et al., 2011). This is supported by observations from our laboratory, which demonstrate deficits in extinction learning in mice lacking mGlu5 (Lawrence Laboratory, unpublished observations). Therefore, positive allosteric modulators of mGlu5 have potential use as pharmacological adjuncts to cue exposure therapy in the treatment of drug addiction, with the potential benefit of reversing cognitive deficits associated with chronic drug use (Cleva and Olive, 2011). Recently, a range of compounds that selectively inhibit mGlu5 function have been published and tested in various neurological disorder models and some of them exhibit good tolerability, long-term action, and oral bioavailability (Keywood et al., 2009; Zerbib et al., 2010; Lindemann et al., 2011). Consequently, there is a promising future for the development of new treatments for addiction based on both positive and negative allosteric modulation of mGlu5.

GPCR HETEROMERIZATION AND A_{2A}R–D₂R–mGlu5 COMPLEXES

In order to maintain the speed and subtlety of neuronal signal transmission, intramembrane protein–protein interactions are likely to play a role (Villardaga et al., 2008). Consequently, researchers in the GPCR field are now focusing their efforts on understanding GPCR signaling and function in the context of higher-order structures and not just monomers. Textbooks describing GPCR signaling as a linear cascade, where agonist activation results in the receptor coupling to one G protein and therefore the activation of one signaling pathway, are now being updated to include receptor homomers (macromolecular complexes made up of two or more identical functional receptor units) and receptor heteromers (macromolecular complexes composed of at least two different functional receptor units with biochemical properties that are demonstrably different from those of their individual components; Ferre et al., 2009). Heteromerization not only provides a potential mechanism for increased signaling diversity, but also specificity by, for example, allowing the development of drugs to target in a tissue selective manner and thereby minimize off-target effects (Mustafa et al., 2010). Indeed, there are now numerous examples of physiologically relevant GPCR heteromers (Dalrymple et al., 2008), particularly in the CNS (Albizu et al., 2010), and new methodologies are being developed to investigate such complexes (Mustafa et al., 2010).

Dopaminergic and glutamatergic inputs converge in the dendritic spines of γ -aminobutyric acid (GABA)ergic medium spiny neurons (Totterdell and Smith, 1989; Sesack and Pickel, 1992) which constitute more than 90% of the neuronal population of the striatum (Smith and Bolam, 1990). Dopamine release in the striatum activates two classes of GPCRs that are expressed by distinct subpopulations of medium spiny neurons. Dopamine D₁ receptors (D₁R) are localized on the striatonigral neurons of the direct pathway whereas dopamine D₂ receptors (D₂R) are expressed by the striatopallidal neurons of the indirect pathway (Gerfen, 1992; Le Moine and Bloch, 1995). D₂Rs are coupled to G_{o*i*}, and activation of these receptors triggers a cascade of signaling pathways, reducing cAMP production and protein kinase A (PKA) activity and activating phospholipase C (PLC)-dependent processes as well as the release of Ca²⁺ from internal stores (Missale et al., 1998). D₂R has been linked to a number of behavioral disorders, including Parkinson's disease, obsessive–compulsive disorder, addiction, and schizophrenia (Bonci and Hopf, 2005; Kreitzer and Malenka, 2008). Treatments targeting dopaminergic signaling have utilized dopamine receptor agonists to alleviate symptoms of hypokinesia associated with Parkinson's disease, and dopamine receptor antagonists to treat hallucinations and delusions in schizophrenic patients (Missale et al., 1998). However, side effects have been associated with both therapies, presumably due to the difficulty in re-establishing the balance in dopamine signaling required for appropriate physiological function.

Adenosine is often described as a neuromodulator, which acts on a family of four adenosine receptors: A₁, A_{2A}, A_{2B}, and A₃ receptors (Fredholm et al., 1994, 2001b). Of the four known adenosine receptors, A₁ and A_{2A} receptors are primarily responsible for the central effects of adenosine (Fredholm et al., 2001a). A₁ receptors are widely distributed in the brain (Fastbom et al., 1987; Fredholm

et al., 2001a) whereas A_{2A} receptors are highly enriched in the terminal regions of ascending dopamine projections, concentrated in the dendritic spines of striatopallidal neurons, particularly in the vicinity of glutamatergic synapses (Fink et al., 1992; Svenningsson et al., 1997; Hettlinger et al., 2001; Rosin et al., 2003). Adenosine A_{2A} receptors are also found presynaptically in glutamatergic, but not in dopaminergic, terminals although with lower density (Hettlinger et al., 2001; Rosin et al., 2003). Therefore, it has been suggested that the A_{2A} receptor plays a role in the fine-tuning modulation of glutamatergic neurotransmission onto striatal GABAergic neurons both at the postsynaptic and presynaptic level (Hettlinger et al., 2001; Ciruela et al., 2006; Ferre et al., 2007b). Recently, presynaptic A_{2A} receptors were shown to be preferentially localized in cortical glutamatergic terminals that contact medium spiny neurons of the direct pathway (Quiroz et al., 2009), while their postsynaptic counterparts are located on striatopallidal neurons of the indirect pathway. Thus adenosine acting at A_{2A} receptors has the capacity to modulate neurotransmission of both output pathways of the basal ganglia. Signaling via the A_{2A} receptor has been implicated in the reinforcing properties of multiple drugs of abuse (Brown and Short, 2008). For example, in mice lacking the A_{2A} receptor, a reduction in self-administration of morphine has been reported (Brown et al., 2009). Similarly, A_{2A}R antagonists have also been reported to reduce alcohol-seeking and opiate-seeking behavior in rats (Arolfo et al., 2004; Yao et al., 2006).

A remarkable feature of the two striatal output pathways is the segregation of adenosine and dopamine receptors. GABAergic/enkephalinergic neurons predominantly express adenosine A_{2A} (stimulatory, coupled to G_s/G_{o*f*}) and dopamine D₂ (inhibitory, coupled to G_i) receptors, while GABAergic/dynorphinergic neurons predominantly express adenosine A₁ (inhibitory, coupled to G_i) and dopamine D₁ (stimulatory, coupled to G_s) receptors (Schiffmann et al., 2007). It is not surprising therefore, that evidence has accumulated documenting interactions between these GPCRs in relation to striatal GABAergic efferent neuron function (Ferre et al., 2007c; Schiffmann et al., 2007).

These interactions have been reported to include direct receptor–receptor interactions as well as interactions at the second messenger and behavioral levels, all of which have been reviewed elsewhere (Ferre et al., 1994, 2004; Fredholm and Svenningsson, 2003; Fuxe et al., 2003). The A_{2A}–D₂ interaction is predominantly found to be antagonistic as activation of A_{2A}R reduces D₂R binding, coupling, signaling as well as behavioral effects; thus D₂R exerts a tonic inhibition on striatopallidal neurons which A_{2A}R activation antagonizes (see reviews above). Evidence for the existence of heteromers containing A_{2A} and D₂ receptors in expression systems has been provided by means of co-immunoprecipitation and resonance energy transfer (RET) techniques such as bioluminescence-RET (BRET) and fluorescence-RET (FRET; Canals et al., 2003; Cabello et al., 2009). In terms of their localization in the striatum, ultrastructural and confocal analysis indicate this to be mainly on dendritic spines in the perisynaptic zones of dopamine terminals and glutamatergic synapses in striatopallidal neurons (Fuxe et al., 2010). In addition, interactions involving the A_{2A}R have been described for other receptors, including adenosine A₁, cannabinoid CB₁ and, most

relevant to this review, mGlu5 (Ferre et al., 2007a; Schiffmann et al., 2007).

In the striatum mGlu5 is expressed postsynaptically on both types of striatal GABAergic efferent neuron and potentially presynaptically on glutamatergic terminals (Romano et al., 1995; Tallaksen-Greene et al., 1998; Smith et al., 2000). In striatopallidal neurons, similar to A_{2A}R, mGlu5 is predominantly found postsynaptically and perisynaptically to asymmetric synapses (Smith et al., 2000). This similarity in anatomical location led Ferre et al. (2002) to investigate the possibility of the existence of heteromers containing these two receptors. Thus, co-localization of A_{2A}R and mGlu5 at the membrane level was demonstrated by confocal laser microscopy in co-transfected human embryonic kidney (HEK)293 cells. In addition, western blotting has provided evidence for complexes containing A_{2A}R and mGlu5 in membrane preparations from both co-transfected HEK293 cells and rat striatal tissue (Ferre et al., 2002). Binding of the A_{2A} receptor antagonist [¹²⁵I]ZM241385 in mouse striatum is reduced as a result of pretreatment with mGlu5 antagonist MTEP (Brown et al., 2011a), providing a neurochemical correlate for a possible allosteric interaction between these receptors. Potential for cross-talk also exists between the receptors' signal transduction pathways (Agnati et al., 2003), and there is functional evidence of a relationship between mGlu5 and A_{2A}R to this effect. Firstly, an apparent interdependence exists between the actions of A_{2A}R and mGlu5 in the phosphorylation of dopamine and cAMP-regulated phosphoprotein of 32,000 kDa (DARPP-32) at Thr-34 (Nishi et al., 2003). Thus, A_{2A}R antagonism attenuates the ability of mGlu5 activation to phosphorylate DARPP-32 at Thr-34 in mouse striatal slices and vice versa. In addition, co-activation of mGlu5 and A_{2A}R synergistically increases phosphorylation of DARPP-32 at Thr-34, apparently via an extracellular signal-regulated kinase (ERK)1/2-dependent mechanism involving increases in cAMP formation (Nishi et al., 2003). These findings are consistent with an ERK1/2-dependent synergistic interaction between A_{2A}R and mGlu5 with respect to *c-fos* expression in HEK293 cells (Ferre et al., 2002). Collectively, these data have led some researchers in the field to suggest that the existence of this A_{2A}R–mGlu5 heteromer may provide a point of temporal and spatial convergence of G_s and G_q signaling onto the MAPK cascade, thereby providing the capacity to influence striatal plasticity (Agnati et al., 2010a), thus raising the possibility of the involvement of this heteromer in plasticity resulting from repeated exposure to drugs of abuse. Of interest is the observation that both A_{2A}R and mGlu5 have been shown to play a role in corticostriatal plasticity (d'Alcantara et al., 2001; Sung et al., 2001).

In vivo microdialysis experiments have shown that perfusion with the A_{2A}R agonist 2-[4-(2-carboxy ethyl)phenethylamine-50-*n*-ethylcarboxamidoadenosine (CGS21680) potentiates the facilitation of GABA release from striatopallidal neurons by the mGlu5 agonist (RS)-2-chloro-5-hydroxyphenylglycine (CHPG; Diaz-Cabiale et al., 2002). In addition, A_{2A}R and mGlu5 have been shown to interact synergistically to modulate glutamate release from corticostriatal afferents (Rodrigues et al., 2005). Submaximal concentrations of the A_{2A}R agonist CGS21680 and mGlu5 agonist CHPG have been shown to synergistically facilitate glutamate release, with the CGS21680-facilitated release prevented by

MPEP. Conversely, facilitation of glutamate release by CHPG is prevented by the A_{2A}R antagonist SCH58261 (Rodrigues et al., 2005). These results demonstrate that in addition to modulating striatal output, mGlu5–A_{2A}R interactions also play a role in regulating cortical input into the striatum, providing an additional avenue to influence striatal function and ultimately behavior.

Indeed, behavioral interactions between A_{2A}R and mGlu5 have been reported, particularly with respect to motor behaviors due to the strong interest in these receptors for their potential in the treatment of Parkinson's disease. For example, central co-administration of CGS21680 and CHPG has been found to reduce the motor activity induced by phencyclidine at doses that have no effect alone (Ferre et al., 2002). Furthermore, in both normal and reserpinized mice, locomotion is enhanced in a synergistic manner with combined treatment of MPEP and the A_{2A}R antagonist KW-6002 (Kachroo et al., 2005). Similarly, akinetic symptoms of 6-hydroxydopamine-lesioned rats are improved with combinations of sub-threshold doses of mGlu5 and A_{2A}R antagonists (Coccorello et al., 2004). More recently, evidence has been forthcoming which suggests the A_{2A}R–mGlu5 interaction is also relevant to reward and drug-seeking behavior (discussed in subsequent section).

Recently, co-immunoprecipitation experiments in native striatal tissue provided evidence for the existence of a higher-order oligomeric complex containing mGlu5, D₂R, and A_{2A}R, localized to the dendritic spines of striatopallidal neurons (Cabello et al., 2009). These results supported earlier experiments which demonstrated functional interactions between these three GPCRs. Early studies reported synergy between A_{2A}R and mGlu5 in reducing dopamine D₂R-mediated behaviors such as phencyclidine-induced hyperactivity (Ferre et al., 2002) and dopamine signaling through DARPP-32 (Nishi et al., 2003). In rat striatal membranes, the A_{2A}R agonist CGS21680 potentiates the effect of mGlu5 agonist CHPG in reducing the affinity of D₂ receptors for the agonist (Popoli et al., 2001). It therefore appears that A_{2A}R and mGlu5 work together to antagonize D₂R-mediated signaling in the striatum. Under baseline conditions, there would be strong tonic activation of D₂R in the striatum which would impair the ability of the A_{2A}R to signal through adenylate cyclase/cAMP/PKA. Co-stimulation of mGlu5 thus allows A_{2A}R stimulation to override the inhibitory tone imposed by endogenous dopamine acting at D₂ receptors (Ferre et al., 2002). This is supported by behavioral data as activation of A_{2A}R and mGlu5 causes attenuation of D₂R agonist quinpirole-induced contralateral turning in 6-hydroxydopamine-lesioned rats (Popoli et al., 2001).

Studies performed in heterologous expression systems utilizing a range of biophysical and biochemical techniques have provided evidence for the existence of this unique "biochemical fingerprint" as a result of the specific receptor interaction (Agnati et al., 2010b). For example, in the case of A_{2A}R–D₂R and mGlu5–D₂R complexes, heteromerization appears to result in an antagonistic allosteric effect where A_{2A}R and mGlu5 agonists, respectively, reduced the affinity of the D₂ agonist-binding sites (Ferre et al., 1999; Popoli et al., 2001; Torvinen et al., 2004). In the case of the mGlu5–A_{2A}R heteromer however, co-stimulation of both receptors appears to result in a synergistic effect on downstream signaling (Ferre et al., 2002). mGlu5 and A_{2A}R antagonists

have been reported to reduce alcohol-seeking behavior in rats; interestingly, co-administration of antagonists for these receptors has a synergistic effect in rats, supporting the existence and functional importance of the heteromer in addiction-associated behaviors (Adams et al., 2008). We have consequently suggested exploiting the limited overlapping expression of mGlu5 and A_{2A}R for developing potential therapies, which target a sub-population of receptors (Bird and Lawrence, 2009b). By developing putative heteromer-selective drugs, the problematic issue of modulating the widely distributed and multifunctional mGlu5 receptor could be potentially overcome.

CONCEPTS OF “RECEPTOR MOSAIC” AND “HUB RECEPTOR”

The idea of “receptor mosaics” suggests that proteins can form even more complex and dynamic networks, with respect to time and protein make up, which has the potential to make significant contributions to the diversity and specificity of GPCR signaling, particularly in neuropharmacology, where a few key receptors have been implicated in multiple neurological and psychiatric disorders (Agnati et al., 2009). Agnati and Fuxe first introduced this concept of receptor mosaics in 1982 in the context of learning and memory (Agnati et al., 1982). As suggested by Fuxe et al. (2008) this “receptor mosaic” term not only allows the interactions between the different receptors (stoichiometry) to be taken into account but also their spatial organization (topography) and their order of activation. It has been proposed that by changing the order of activation, functionally distinct receptor mosaics can transduce signals resulting in distinct biological responses. This may be of particular importance for neuropharmacology where different agonist concentrations direct activation of one receptor mosaic activity over another. This can be exemplified by considering a receptor mosaic which consists of three different receptors; A, B, and C and therefore can potentially result in six functional outcomes (Fuxe et al., 2008). For reviews on receptor mosaics and their implications see (Fuxe et al., 2007, 2008; Agnati et al., 2010a,b; Fuxe and Kenakin, 2010).

Furthermore, it has been suggested that receptor mosaics are composed of at least one so called “hub receptor” – a receptor, capable of forming multiple connections, which can result in differing pharmacology depending on these connections (Agnati et al., 2010a). Hub receptors have been classified into three distinct groups; passive hubs which allow the flow of information; intelligent hubs modify the information as it flows through; and switching hubs, which direct the information to the correct destination (Agnati et al., 2010a). It has been postulated that the D₂ receptor acts as a “hub receptor,” with evidence in the literature for its interaction with the A_{2A} and mGlu5 receptors (Cabello et al., 2009) as well as A_{2A} and CB₁ (Agnati et al., 2009) to form trimeric receptor mosaics. Cabello et al. (2009) have reported the existence of these receptor mosaics in the perisynaptic and extrasynaptic parts of the neuronal plasma membrane. As mentioned above, it is thought that this trimeric receptor mosaic plays a role on the extrasynaptic striatopallidal GABA neurons and possibly in corticostriatal glutamate terminals. As only a small population of these receptors are expected to form complexes at any given time or location, this may allow even more selectivity when designing drug therapies. Modulation of specific interactions and pathways

has the potential to reduce side effects, of paramount importance in the treatment of neurological disorders, where lack of specificity could impair vital brain function.

mGlu5 RECEPTOR FUNCTIONAL INTERACTIONS: EVIDENCE FOR A ROLE IN ADDICTION

Given the specific localization of apparent mGlu5 receptor complexes in the striatum, as well as the established involvement of plasticity in glutamatergic corticostriatal neurons in the reinstatement of drug-seeking in animal models of relapse (Kalivas, 2009), one may hypothesize that interactions between mGlu5 and A_{2A}R have the potential to regulate drug-seeking or the conditioned effects of drugs of abuse. Indeed, two recent studies have provided evidence as such. Firstly, as mentioned previously, functional interactions between mGlu5 and A_{2A} receptors were shown to regulate operant self-administration of alcohol and cue-induced reinstatement of alcohol-seeking (Adams et al., 2008). Sub-threshold doses of SCH58261, a selective A_{2A} receptor antagonist and MTEP, an mGlu5 antagonist, were administered in combination and were found to act synergistically to decrease operant self-administration of alcohol and cue-induced reinstatement of alcohol-seeking (Adams et al., 2008). These doses had no effect when given alone. In addition, no support was found for interactions between adenosine A₁ and A_{2A}R, or A₁ and mGlu5, suggesting this reduction in alcohol self-administration and alcohol-seeking behavior was specific to a functional interaction between A_{2A} and mGlu5 receptors (Adams et al., 2008, 2010).

It is now recognized that reinstatement of drug-seeking involves corticostriatal glutamatergic input into the striatum (Kalivas and McFarland, 2003; Kalivas et al., 2005). Prior co-administration of SCH58261 and MTEP may have prevented cue-elicited release of glutamate in this context, thus preventing drug-seeking behavior. This fits nicely with the role discussed earlier for A_{2A}R and mGlu5 in regulating glutamate release from corticostriatal afferent neurons (Rodrigues et al., 2005). In rats, stimulation of corticostriatal afferents has been shown to result in phosphorylation of ERK which can be prevented by A_{2A}R antagonism (Quiroz et al., 2009), thus providing one possible mechanism for this observation. Though no direct evidence exists as yet, it is also possible that specialized receptor complexes containing multiple GPCRs are involved in this behavior, and that plasticity may occur with repeated drug use which alters the arrangement of or signaling through this receptor complex. Though the exact nature of the interactions between mGlu5 and A_{2A} receptors are yet to be determined and involvement of potential “hub” receptors such as the D₂R remains to be explored, it is clear that a biologically relevant functional interaction between these two GPCRs can act to regulate drug-seeking behavior providing evidence of the therapeutic potential of targeting mGlu5 and A_{2A}R in combination.

A similar functional interaction was found between mGlu5 and A_{2A}R in relation to cocaine-driven behaviors (Brown et al., 2011a) providing further evidence of the potential of this possible drug target. Despite preventing a conditioned place preference to cocaine in wildtype mice, MTEP treatment had no impact on the ability of A_{2A}R knockout mice to develop a robust conditioned place preference to cocaine, suggesting a functional A_{2A}R is required for mGlu5 to regulate the acquisition of incentive

properties in a cocaine-paired context. This finding was mirrored by conditioned hyperactivity data, whereby MTEP completely prevented conditioned hyperactivity in wildtype mice but had no impact in A_{2A}R knockout mice. In contrast, MTEP was able to block the locomotor-activating properties of cocaine in both wildtype and A_{2A}R knockout mice. It should be noted that conditioned hyperactivity was assessed during the test session when all mice were in a drug-free state. Conditioned hyperactivity therefore, as with conditioned place preference, is driven purely by contextual associations and is hence likely to be underpinned by similar neural processes. Hence, it appears that a functional A_{2A}R may be critical for the actions of MTEP in regulating both the conditioned reinforcing and conditioned locomotor-activating properties of cocaine, but not the direct locomotor effects of acute cocaine. These data are in line with recent evidence supporting a role for mGlu5 in incentive learning processes (Novak et al., 2010; O'Connor et al., 2010).

As the ventral striatum is a key component of the neural circuitry underlying Pavlovian conditioning (Robbins et al., 2008), it seems probable that it is a likely site of action for this interaction. However it should be noted that both A_{2A}R and mGlu5 were recently shown to be co-localized in the hippocampus (Tebano et al., 2005) and have been implicated in learning and memory processes (Simonyi et al., 2005; Gimenez-Llort et al., 2007). For example, A_{2A}R have been shown to play a key role in hippocampal-dependent associative learning in a classical eye-blinking paradigm (Fontinha et al., 2009) and mGlu5 is implicated in spatial memory (Lu et al., 1997). Given that conditioned place preference is a paradigm that relies on contextual learning, functional interactions between mGlu5 and A_{2A}R in the hippocampus cannot be ruled out.

CONCLUSION

This review has outlined the evidence supporting a role for functional interactions between mGlu5 and other GPCRs, particularly the adenosine A_{2A}R, in reward, drug-seeking, and drug-induced plasticity. Though much evidence now exists describing the individual contribution that mGlu5, D₂R, and A_{2A}R make to various facets of addictive behavior, only a small number of studies thus

far have investigated interactions between mGlu5 and these other GPCRs in this context. Initial studies suggest that mGlu5 receptor interactions may play a role in the rewarding and incentive motivational properties of drugs of abuse, drug-seeking, as well as the conditioned effects of drugs of abuse. This is consistent with the substantial influence that interactions between mGlu5 and these other receptors appear to have on striatal function, regulating both GABAergic output of striatopallidal neurons and glutamatergic input from corticostriatal afferents. The specific nature of these interactions has not yet been determined and though it is possible that striatal mGlu5-containing complexes are responsible for regulating drug-seeking, future studies should aim to determine whether or not this is the case as well as determine the exact site of action as mGlu5 has recently been shown to co-localize with A_{2A}R in the hippocampus (Tebano et al., 2005). In addition, the role of the D₂ receptor as a potential “hub” receptor should be investigated in this context. Furthermore, in order to overcome the problematic issue of therapeutically modulating the widely distributed and multifunctional mGlu5, the limited overlapping expression of mGlu5, D₂, and A_{2A}R may be exploited when designing drug therapies aimed at exhibiting little or no off-target effects. On the other hand, if such “complexes” do not prove to be relevant *in vivo* in humans, exploitation of specific functional interactions that have biological significance (e.g., downstream mGlu5–A_{2A} receptor functional interactions which are well-established) could provide an equally attractive proposition. Indeed, the therapeutic targeting of receptor mosaics/functional interactions in a tissue specific or temporal manner (for example, a sub-population of receptors in a “pathological state”) has the potential to reduce detrimental side effects that may otherwise impair vital brain function.

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