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Review Article

$mGluR_5$ positive allosteric modulators

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CONTENTS

Abstract

The $mGluR_5$ subtype of metabotropic glutamate receptor (mGluR) plays an important role in the modulation of neuronal excitability and synaptic transmission in a number of brain circuits. These slow synaptic responses involving $mGluR_5$ are mediated by activation of second messenger systems and intracellular signaling pathways. Recent advances suggest that selective activation of $mGluR_5$ may have exciting potential for the treatment of multiple psychiatric and neurological disorders. Thus, it is important to develop $mGluR_5$-selective activators as useful tools to study the roles of $mGluR_5$ in diseases or as novel therapeutic agents. Several useful $mGluR_5$ orthosteric agonists have been discovered and, more recently, several families of $mGluR_5$ positive allosteric modulators (PAMs) were identified. The $mGluR_5$ PAMs do not directly activate the receptor, but rather enhance its sensitivity to agonists by acting through binding to the allosteric sites in the seven-transmembrane-spanning domains. Physiological and behavioral studies demonstrate that $mGluR_5$ PAMs potentiate $mGluR_5$-mediated responses in brain slices and display efficacy in animal models that predict for antipsychotic effects. The unique pharmacological properties of $mGluR_5$ PAMs enable them to be used as novel research tools or potential therapeutic agents with distinct advantages over classic orthosteric agonists.

Introduction

Glutamate, the major excitatory neurotransmitter in the mammalian central nervous system (CNS), elicits synaptic responses by activation of ionotropic glutamate receptors and metabotropic glutamate receptors (mGluRs). The mGluRs belong to the family of G-protein-coupled receptors (GPCRs) and eight subtypes of mGluRs have been identified. Based on sequence homology, pharmacological selectivity and primary G-protein coupling, mGluRs have been divided into three groups: group I mGluRs include $mGluR_1$ and $mGluR_5$, both of which are coupled to $G_{q/11}$ to activate phospholipase C (PLC); group II mGluRs ($mGluR_2$ and $mGluR_3$) and group III mGluRs ($mGluR_4$, $mGluR_6$, $mGluR_7$ and $mGluR_8$) are coupled to $G_{i/o}$ and associated effectors such as ion channels or adenylyl cyclase (1). The mGluRs provide a mechanism by which glutamate can modulate activity at the same synapses at which it elicits fast excitatory synaptic responses. Because of the ubiquitous distribution of glutamatergic synapses, mGluRs participate in a wide variety of functions in the CNS (1-3).

$mGluR_5$ is expressed ubiquitously in the mammalian CNS and is primarily localized postsynaptically, although it also displays some presynaptic localization. Activation of $mGluR_5$ elicits slow synaptic responses and modulates neuronal excitability through downstream signaling pathways. Previous studies have led to the hypothesis that $mGluR_5$-selective ligands may have potential utility as novel therapeutic agents for multiple psychiatric or neurological disorders, including schizophrenia (4, 5), depression (6, 7), anxiety disorders (7, 8), substance abuse (9, 10), Parkinson's disease (11), epilepsy (12), Alzheimer's disease (13) and pain (14). Many of these exciting potential therapeutic uses of $mGluR_5$ ligands call for subtype-selective $mGluR_5$ agonists. For instance, cellular and behavioral studies suggest that selective activation of $mGluR_5$ may have potential in the treatment of psychosis associated with schizophrenia and certain neurodegenerative disorders such as Alzheimer's disease (13, 15-20). However, it has been extremely difficult to develop highly selective agonists of most mGluR subtypes with suitable properties for use as drugs. The glutamate binding site is highly conserved across mGluR subtypes (1), making it

difficult to develop highly selective glutamate-site ligands. Also, most glutamate-site agonists are structural analogues of glutamate and do not possess pharmacokinetic properties and sufficient brain penetration to allow them to be useful as drugs. In addition to the unique challenges associated with targeting the glutamate binding site, there are a number of problems associated with the use of direct-acting agonists as drugs. These include adverse effects associated with excessive activation of the receptor, greater receptor desensitization than occurs with more indirect approaches, and loss of activity dependence of receptor activation.

mGluR$_5$ contains three major domains, a large extracellular *N*-terminal domain, a heptahelical domain containing seven-transmembrane regions linked by short loops, and an intracellular *C*-terminal domain. Glutamate binds to the *N*-terminal extracellular domain of mGluRs. This orthosteric binding site is highly conserved throughout all the mGluR subtypes, which is thought to be the cause for the limited subtype selectivity of orthosteric mGluR$_5$ agonists. Recently, the success of subtype-selective mGluR negative allosteric modulators (NAMs) and the discovery of positive allosteric modulators (PAMs) for other families of receptors encouraged efforts to develop mGluR$_5$-selective PAMs (21, 22).

Discovery of mGluR$_5$ PAMs

Three distinct series of mGluR$_5$ PAMs have been discovered, represented by difluorobenzaldazine (DFB), *N*-[4-chloro-2-[(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl]phenyl]-2-hydroxybenzamide (CPPHA) and 3-cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide (CDPPB) (Fig. 1) (23-25). DFB was the first mGluR$_5$ PAM identified (24). It does not activate mGluR$_5$ when added alone (Fig. 2A), but increases the sensitivity of mGluR$_5$ to orthosteric agonists, and thereby shifts the concentration-response curve of orthosteric agonists to the left (Fig. 2B). DFB is highly selective for mGluR$_5$ and has little activity at other mGluR subtypes. The discovery of DFB provided a major breakthrough in demonstrating the possibility of developing mGluR$_5$-selective PAMs. However, the poor potency, efficacy and solubility of this compound prevented its further study in native tissue preparations (24).

A major advance came with the discovery of CPPHA, the second published mGluR$_5$ PAM. Similar to DFB, CPPHA alone has no agonist activity but potentiates mGluR$_5$ activation by glutamate with EC_{50} values in the 400-800 nM range. At a maximally effective concentration (10 μM), CPPHA shifts mGluR$_5$ agonist concentration-response curves of multiple orthosteric agonists 4-7-fold to the left. Importantly, in electrophysiological studies of brain slice preparations, CPPHA potentiates dihydroxyphenylglycine (DHPG)-induced enhancement of NMDA receptor currents in hippocampal slices, while having no effect on these currents by itself (23). Similarly, CPPHA also potentiated mGluR$_5$-mediated DHPG-induced depolarization of rat subthalamic nucleus neurons (23). These results demonstrate that mGluR$_5$ PAMs have similar activities in native tissue preparations.

Effects of mGluR$_5$ PAMs in animal models of schizophrenia

As mentioned above, the major motivation for the discovery of mGluR$_5$-selective PAMs came from previous anatomic, electrophysiological and behavioral studies with mGluR$_5$ antagonists suggesting that activation of

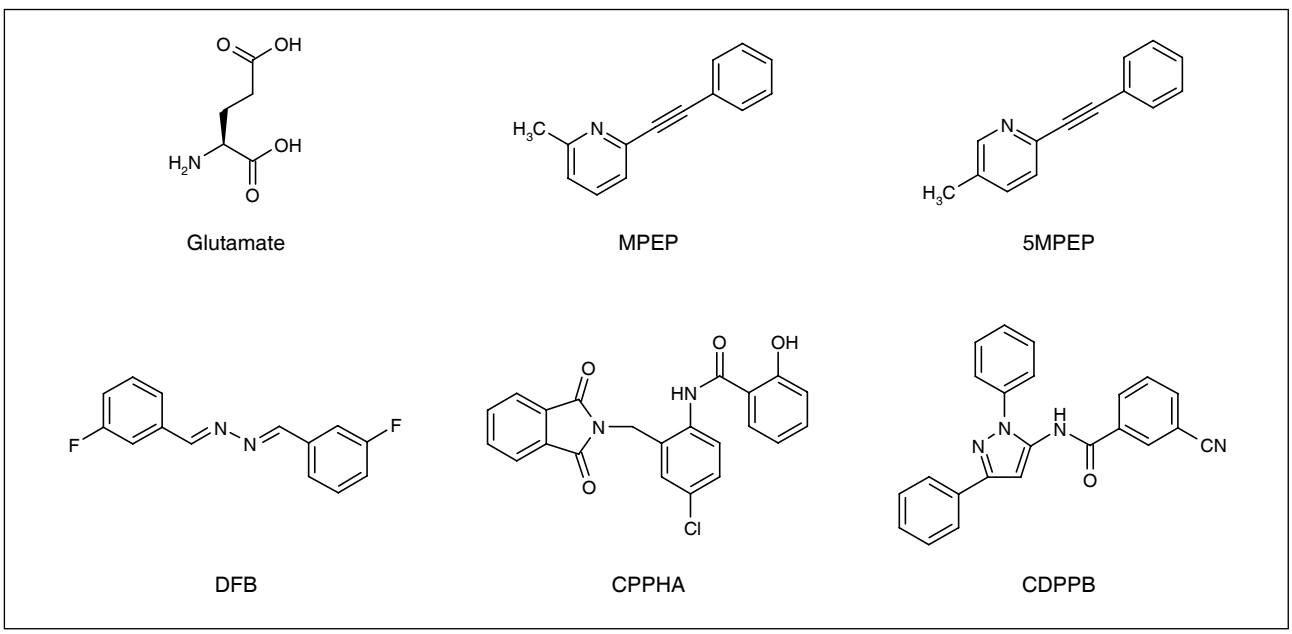

Fig. 1. Structures of mGluR$_5$ ligands. Glutamate, an endogenous orthosteric agonist; MPEP, a prototypical negative allosteric modulator (NAM); 5MPEP, a neutral allosteric modulator; DFB, CPPHA and CDPPB, three families of positive allosteric modulators (PAMs).

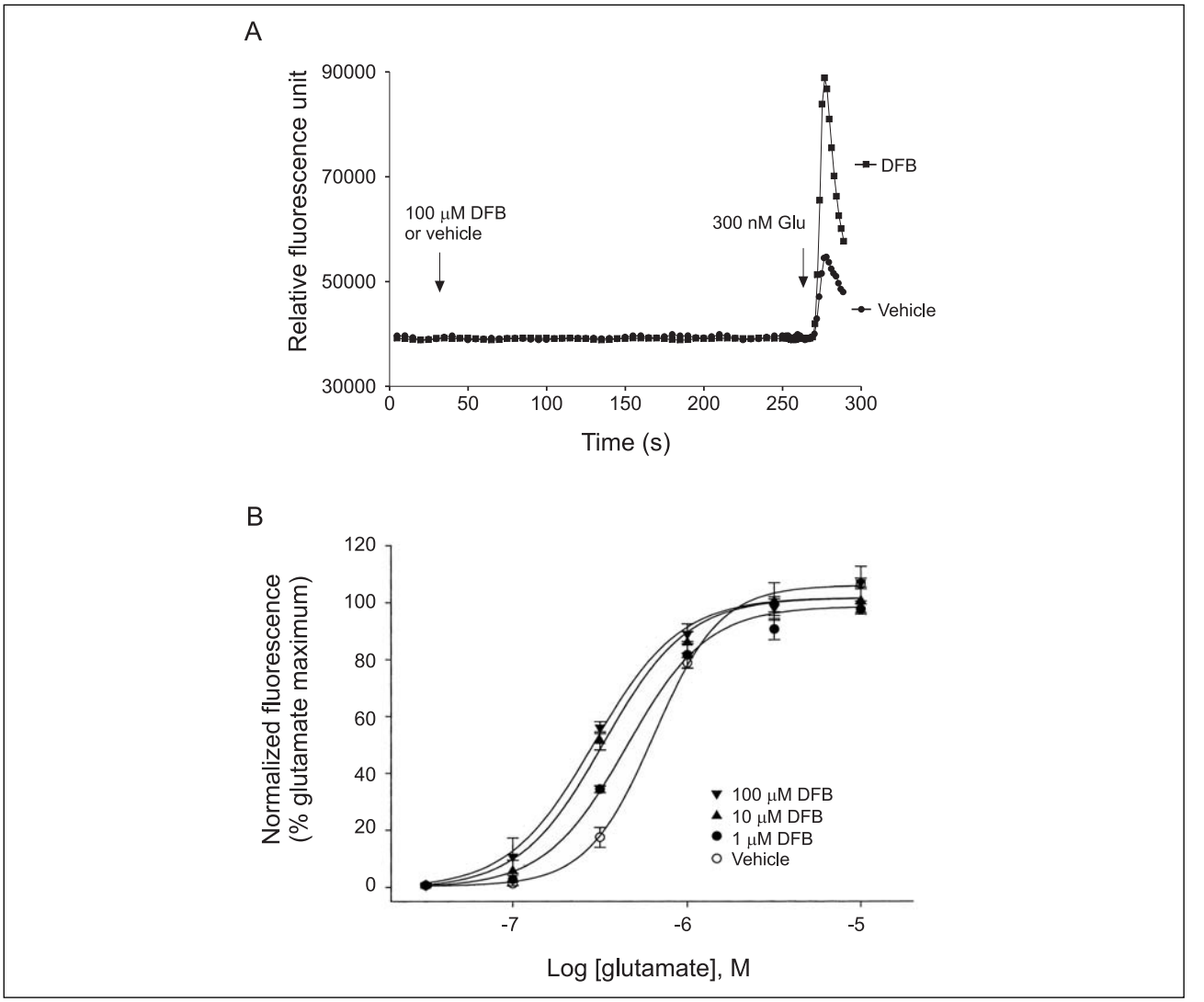

Fig. 2. DFB potentiates $mGluR_5$-mediated calcium mobilization in a recombinant system. **A**, assay traces show DFB potentiates $mGluR_5$-mediated calcium mobilization in a recombinant cell line. **B**, 100 μM DFB does not induce calcium flux by itself, but enhances the glutamate-induced increase in calcium flux.

$mGluR_5$ had potential as a novel approach to the treatment of schizophrenia and other disorders involving psychosis and impaired cognitive function. Based on this, there was a need to discover highly selective activators of this receptor that could be used in animal models predictive of efficacy in these disorders. The discovery of DFB and CPPHA represented a major advance in establishing the potential for developing PAMs selective at the cellular and molecular level. However, these compounds have relatively low potencies and inadequate pharmacokinetic properties for physiological and behavioral studies.

The next major advance came with the discovery of a third series of $mGluR_5$ PAMs that have properties making them more suitable for studies both in rat brain slices and animal models (25-29). These compounds are represented by CDPPB. CDPPB has higher potency and solubility compared to DFB and CPPHA, as well as other critical properties that make it more suitable for *in vitro* studies in rat brain slices and *in vivo* studies to test the hypothesis that $mGluR_5$ PAMs will have antipsychotic-like activity in animal models (25, 26). CDPPB induces a robust potentiation of $mGluR_5$-mediated responses, with an EC_{50} value of about 25 nM. At 1 μM, CDPPB shifts $mGluR_5$ agonist concentration-response curves 9-fold to the left. Furthermore, when the activity of CDPPB was tested against a panel of 175 receptors, transporters, ion channels and enzymes, it showed no activity at any of these targets at submicromolar concentrations (25). Finally, pharmacokinetic studies in Sprague-Dawley rats revealed that CDPPB (2 mg/kg in DMSO) has a plasma half-life of 4.4 h and readily crossed the blood-brain barrier (BBB). Thus, while CDPPB behaves in a manner similar to DFB and CPPHA at the cellular level, this compound represents a major advance relative to the previous compounds in that its properties make it more useful for electrophysiological studies in brain slices and

for determining the behavioral effects of mGluR$_5$ potentiators *in vivo*. However, it is important to note that CDPPB is still suboptimal for *in vivo* studies in that it is not readily soluble in vehicles most useful for animal dosing.

mGluR$_5$ is primarily localized postsynaptically, where it potentiates NMDA receptor currents in a wide range of neuronal populations. This effect, together with the NMDA hypofunction hypothesis of schizophrenia, provided the major basis for considering mGluR$_5$ PAMs as compounds that may have antipsychotic-like effects (4, 5). Interestingly, CDPPB is able to penetrate the brain and reverses amphetamine-induced increases in locomotor activity and amphetamine-induced disruption of prepulse inhibition in rats, two models sensitive to antipsychotic drug treatment (25). These results demonstrate that mGluR$_5$ PAMs exert significant behavioral effects, suggesting that such modulation serves as a viable approach to increasing mGluR$_5$ activity *in vivo*. These effects are consistent with the hypothesis that allosteric potentiation of mGluR$_5$ might be a novel approach for the development of antipsychotic agents.

Neutral allosteric modulator of mGluR$_5$ as a useful tool to study PAMs

The discovery of mGluR$_5$ PAMs has led to a number of important insights that have increased our understanding of the pharmacological properties of allosteric sites and allosteric ligands at mGluR$_5$. For instance, one of the early insights gained by the discovery of DFB was that allosteric modulators can interact with a single allosteric site to have a range of activities (24). Thus, the synthesis and testing of a series of analogues of DFP revealed that DFB, dichlorobenzaldazine (DCB) and dimethoxybenzaldazine (DMeOB) all bind to the allosteric site occupied by the prototypical mGluR$_5$ NAM MPEP (2-methyl-6-[phenylethyl]pyridine). However, only DFB is a PAM, whereas DMeOB is a NAM and DCB neither potentiates nor inhibits the response to glutamate. Interestingly, DCB blocks the inhibitory effects of DMeOB and the potentiating effects of DFB. These exciting results suggest that structurally related compounds can bind to a single allosteric site to exert effects ranging from antagonist to agonist and including neutral compounds. This is directly analogous to the activities of agonists, inverse agonists and neutral antagonists at orthosteric binding sites on a broad range of receptors.

More recently, three novel MPEP analogues were found to bind to the allosteric MPEP site on mGluR$_5$ but had only partial inhibition or no functional effects on the mGluR$_5$ response (27). Two of these compounds, 2-[2-(3-methoxyphenyl)ethynyl]-5-methylpyridine (M-5MPEP) and 2-[2-(5-bromopyridin-3-yl)ethynyl]-5-methylpyridine (Br-5MPEPy), act as partial antagonists at the mGluR$_5$ receptor because they only partially inhibit the response of this receptor to glutamate. The third compound, 5-methyl-6-(phenylethynyl)pyridine (5MPEP), has no effect on mGluR$_5$-mediated responses alone but still fully displaces MPEP site binding. Interestingly, 5MPEP blocks the effects of both the allosteric antagonist MPEP and the allosteric potentiators CDPPB and CPPHA. Schild analysis shows that 5MPEP inhibits MPEP antagonism in a competitive manner. Thus, 5MPEP is another example of a neutral mGluR$_5$ allosteric modulator at the MPEP site (27). Interestingly, electrophysiological studies reveal that 5MPEP is also active in brain slices, where it blocks the effects of mGluR$_5$ PAMs. This provides a unique tool to study the pharmacological properties and physiological roles of mGluR$_5$ allosteric modulators in both recombinant and native systems (27, 28).

mGluR$_5$ PAMs bind at distinct allosteric sites on the receptor

None of the three major families of mGluR$_5$ PAMs alter the binding of [^{3}H]-quisqualate to the orthosteric glutamate binding site, suggesting that these compounds do not act by increasing the affinity of orthosteric agonists. Both DFB and CDPPB displace radioligand binding to the MPEP site, suggesting that these compounds might require the same site of action to the previously identified NAM MPEP (24, 25). We synthesized a series of CDPPB analogues and reported that these compounds bind to the MPEP site with affinities that are closely related to their potencies as mGluR$_5$ potentiators. Furthermore, allosteric potentiation by these PAMs is antagonized by the neutral ligand at the MPEP site in a competitive manner, as determined by Schild analysis (28, 30). Finally, mGluR$_5$ potentiation by CDPPB and related compounds is reduced by a mutation in mGluR$_5$ that eliminates MPEP binding (28, 30). Taken together, these results support the hypothesis that interaction of the CDPPB family of mGluR$_5$ PAMs with the MPEP site is required for potentiation of the receptor response. Analogous results have been reported for DFB (28, 31), suggesting that these two mGluR$_5$ PAMs act at a common site that is shared with MPEP and related mGluR$_5$ NAMs.

Interestingly, CPPHA does not reduce the binding of ligands to the MPEP site (23). Based on this, it has been proposed that CPPHA acts at a distinct allosteric site on mGluR$_5$. Consistent with this, the neutral MPEP-site ligand 5MPEP inhibits CPPHA potentiation in a noncompetitive manner (30). Additionally, the mutation A809V/mGluR$_5$ that reduces the binding of ligands to the MPEP site eliminates the effect of CDPPB and its analogue VU-29, but has no effect on the potentiation by CPPHA. Conversely, another mutation, F585I/mGluR$_5$, eliminates the effect of CPPHA but does not alter the response to CDPPB and VU-29 (30). These data suggest that CPPHA likely acts at a second allosteric site distinct from the site of action for CDPPB, DFB, MPEP and related compounds.

mGluR$_5$ PAMs differentially regulate coupling of mGluR$_5$ to different signaling pathways

Increasing evidence suggests that different agonists can differentially activate different signaling pathways of a

single GPCR, a phenomenon termed agonist receptor trafficking (32-34). Based on this, it is possible that $mGluR_5$ PAMs could differentially regulate different signaling pathways coupled to a single mGluR subtype. $mGluR_5$ has been shown to couple to multiple signaling pathways and physiological responses. For example, in secondary cultured rat cortical astrocytes, $mGluR_5$ activates phosphatidylinositol hydrolysis and extracellular signal-regulated kinase (ERK2) phosphorylation by independent mechanisms (35, 36). Both DFB and CPPHA induce parallel leftward shifts of the concentration-response curves of DHPG- and glutamate-induced calcium transients in secondary cultured rat cortical astrocytes. DFB induced a similar shift of the concentration-response curve of DHPG-induced ERK1/2 phosphorylation (37). However, CPPHA induces an increase in basal $mGluR_5$-mediated ERK1/2 phosphorylation and potentiates the effect of low concentrations of agonists. In contrast, CPPHA significantly decreases ERK1/2 phosphorylation induced by high concentrations of DHPG. Thus, CPPHA has qualitatively different effects on $mGluR_5$-mediated calcium responses and ERK1/2 phosphorylation (37). Together, these data suggest that different PAMs could differentially modulate different signaling pathways coupled to a single receptor. This finding has important implications for the development of $mGluR_5$ PAMs that selectively potentiate certain signaling pathways. These selective effects could inadvertently lead to the discovery of compounds that do not modulate pathways important for a given therapeutic response and could also be used to reduce unexpected side effects in cases where modulation of a single signaling pathway offers advantages.

Advantages of $mGluR_5$ PAMs relative to orthosteric agonists

$mGluR_5$ PAMs act at distinct sites compared to classic orthosteric ligands and possess several unique properties that may provide advantages as novel pharmacological tools or therapeutic agents. Firstly, allosteric modulators could display better subtype selectivity among mGluR subtypes. All three families of $mGluR_5$ PAMs have improved subtype selectivity compared with orthosteric agonists. Additionally, classic orthosteric agonists of mGluRs are amino acid analogues and have difficulty crossing the BBB. Thus, their use is limited to *in vitro* studies. In contrast, the mGluR allosteric modulators are usually hydrophobic and theoretically can exhibit better penetration of the BBB (Fig. 1). Moreover, departure from amino acid scaffolds allows optimization of a number of other properties that are critical for therapeutic agents, such as favorable pharmacokinetics. Lastly, there are many potential advantages of $mGluR_5$ PAMs based on the fact that they do not directly activate the receptor but only amplify the response to endogenous agonists. When used *in vivo*, this has the potential of maintaining the activity dependence of $mGluR_5$ activation and selectively enhancing physiologically relevant activation of the receptor by synaptically released glutamate. This could reduce adverse effects or tolerance that can be associated with orthosteric agonists. As the discovery and development of $mGluR_5$ PAMs continues to progress, it will be exciting to determine whether these unique properties provide advantages as therapeutic agents.

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