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Review

Does conventional anti-bipolar and antidepressant drug therapy reduce NMDA-mediated *neuronal* excitation by downregulating *astrocytic* GluK2 function?

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ABSTRACT

Chronic treatment with anti-bipolar drugs (lithium, carbamazepine, and valproic acid) down-regulates mRNA and protein expression of kainate receptor GluK2 in mouse brain and cultured astrocytes. It also abolishes glutamate-mediated, Ca^{2+}-dependent $ERK_{1/2}$ phosphorylation in the astrocytes. Chronic treatment with the SSRI fluoxetine enhances astrocytic GluK2 expression, but increases mRNA editing, abolishing glutamate-mediated $ERK_{1/2}$ phosphorylation and $[Ca^{2+}]_i$ increase, which are shown to be GluK2-mediated. Neither drug group affects Glu4/Glu5 expression necessary for GluK2's ionotropic effect. Consistent with a metabotropic effect, the PKC inhibitor GF 109203X and the IP_3 inhibitor xestospongin C abolish glutamate stimulation in cultured astrocytes. In CA1/CA3 pyramidal cells in hippocampal slices, *activation* of extrasynaptic GluK2 receptors, presumably including astrocytic, metabotropic GluK2 receptors, causes long-lasting inhibition of slow neuronal afterhyperpolarization mediated by Ca^{2+}-dependent K^+ flux. This may be secondary to the induced astrocytic $[Ca^{2+}]_i$ increase, causing release of 'gliotransmitter' glutamate. Neuronal NMDA receptors respond to astrocytic glutamate release with *enhancement* of excitatory glutamatergic activity. Since *reduction* of NMDA receptor activity is known to have antidepressant effect in bipolar depression and major depression, these observations suggest that the *inactivation* of astrocytic GluK2 activity by antidepressant/anti-bipolar therapy ameliorates depression by inhibiting astrocytic glutamate release. A resultant strengthening of neuronal afterhyperpolarization may cause reduced NMDA-mediated activity.

Contents

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1. Introduction

Both phases of bipolar disorder are treated with lithium, carbamazepine or valproic acid. These drugs chemically and functionally have very few common effects, most of which are exerted on astrocytes (see below). The conventional treatment of major depression is the use of drugs that interact with monoaminergic signaling, i.e., i) tricyclic antidepresssants, which inhibit noradrenaline reuptake; ii) the so-called 'serotonin specific reuptake inhibitors' (SSRIs); and iii) monoamine oxidase inhibitors that reduce noradrenaline and serotonin degradation. However, presently available drug therapy of either disorder is not always successful, and even when it is effective, there is a latency phase of about 3 weeks (or more for treatment of an initial depression during bipolar disorder). For this reason alternative drug treatment is being studied, and emphasis has been placed on down-regulation of the glutamatergic system. Glutamate is the major excitatory transmitter in the brain, and astrocytic-neuronal interactions are pivotal for its production and degradation (Fig. 1). Both neurons and astrocytes are on one hand capable of glutamate release and on the other hand they express a variety of receptors for glutamate (NMDA receptors, AMPA receptors, kainate receptors, or metabotropic glutamate receptors). Based on our recent observations that both treatment with a conventional anti-bipolar drug and treatment with an SSRI lead to a reduced function of an *astrocytic* kainate receptor, GluK2, the present paper presents a hypothesis how this effect can reduce excitatory glutamatergic activity *in neurons*. This is not meant to suggest that this effect should be the only relevant action of conventional anti-bipolar or antidepressant drug therapy, but it does provide a link between traditional medications and drugs acting directly on glutamatergic activity.

2. Glutamate and depression

2.1. Glutamate homeostasis in bipolar depression and major depression

2.1.1. Effect of drugs affecting glutamatergic activity

Both the depressive phase of bipolar disorder (Brennan et al., 2010) and major depression (Zarate et al., 2004; Sanacora et al., 2008) respond to riluzole, a drug that supposedly decreases excitatory glutamatergic activity by facilitating astrocytic glutamate uptake (Fumagalli et al., 2008) and reducing its release (Doble, 1996). The rate of improvement is virtually identical in the two disorders with a small apparent or clinical response after 1 week, a maximum effect after 3–4 weeks, and some residual pathology at that time. However, no evidence suggests that riluzole acts more rapidly than classical antidepressants in major depression (Zarate et al., 2010). Whether it does so in bipolar-depressed patients is difficult to establish from the results by Brennan et al. (2010), because most of their patients were already on different medication. However, conventional anti-bipolar therapy acts slowly and unreliably when initiated during a depressive period, although it may prevent both manic and depressive episodes during maintenance therapy.

A much faster response of major depression (Berman et al., 2000; Zarate et al., 2006), and of depression in bipolar disorder (Diazgranados et al., 2010), has been found using sub-anesthetic doses of ketamine (Machado-Vieira et al., 2009a). A single intravenous dose resulted in significant antidepressant effects in patients with treatment-resistant major depression within 2 h, and 29% of the patients achieved

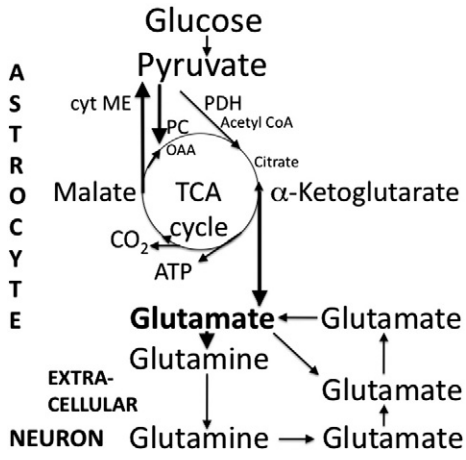

Fig. 1. Glucose metabolism produces energy (ATP) and glutamate. During glycolysis pyruvate is formed. Its metabolism exclusively by pyruvate dehydrogenase (PDH) occurs in virtually all mammalian cell types and produces acetyl coenzyme A (Acetyl CoA), from which the acetate part (two carbon atoms [2-C]) condenses with pre-existing 4-C oxaloacetate (OAA) in the tricarboxylic acid (TCA) cycle to form the 6-C intermediate citrate. One turn of the cycle leads to the oxidation of two carbon atoms (C) to CO_2 (only one shown) and ATP production and the re-generation of OAA [4-C]. The two released C are replenished by PDH activity, generating acetyl CoA at the beginning of the next turn of the TCA cycle. It enables continued ATP production *but no net production of any TCA cycle intermediate or its metabolites, including glutamate/glutamine*. Pyruvate metabolism by pyruvate carboxylase (PC), an astrocyte-specific enzyme (heavy arrow), produces a *new* TCA cycle intermediate, initially OAA, which after condensation with acetate from newly formed astrocytic acetyl CoA generates a new molecule of citrate and eventually α-ketoglutarate, a direct precursor of glutamate. After glutamate's net synthesis, exclusively in astrocytes (heavy arrow), it can be transferred via the extracellular space to neurons in a glutamate–glutamine cycle, with glutamine synthesis (by glutamine synthetase) being astrocyte-specific (heavy arrow). After neuronal uptake of glutamine it is hydrolyzed to glutamate, accumulated into vesicles and released as transmitter glutamate. The glutamate–glutamine cycle in addition carries previously released transmitter glutamate, which has been taken up by astrocytes, the major fate of released glutamate. Astrocytes also oxidize glutamate, via α-ketoglutarate and malate, which can leave the TCA cycle and, catalyzed by the astrocyte specific cytosolic malic enzyme (cME) (heavy arrow), form pyruvate, ready for renewed utilization in the TCA cycle.

remission 24 h following the infusion of ketamine (Zarate et al., 2006). Very similar results were seen in depressed patients suffering from bipolar disorder (Diazgranados et al., 2010). However, the therapeutic effect was transient, and sedation, potential psychotomimetic effects, cognitive problems, and abuse potential prevent chronic treatment. Notably, the time to relapse is not extended by subsequent riluzole treatment (Mathew et al., 2010). Ketamine is a noncompetitive NMDA receptor antagonist, and a microdialysis study in conscious rats indicated that low doses of ketamine increase glutamate outflow in the prefrontal cortex. In contrast, an anesthetic dose of ketamine decreases release, and an intermediate dose does not affect glutamate levels (Maeng et al., 2008). The increased synaptic glutamate level at low doses may be due to an inhibitory effect on NMDA receptors on GABA-ergic neurons and a resulting increase in enhanced AMPA-mediated stimulation, which may have anti-depressant effects (Sanacora et al., 2008; Machado-Vieira et al., 2009b). Thus, a difference between the effect of drugs like riluzole and ketamine could be that riluzole inhibits all glutamatergic signaling, whereas ketamine selectively inhibits NMDA-mediated signaling and may enhance AMPA-mediated signaling. However, a different specific NMDA blocker, memantine, is without similar dramatic antidepressant effect as ketamine (Teng and Demetrio, 2006), although it did produce some cognitive gain in 2 depressed patients. Moreover, a double-blind trial showed it to be without effect in major depression (Zarate et al., 2006). This may raise the question if the reason for ketamine's almost immediate antidepressant effect is its blockade of NMDA receptors. Blockade of NMDA channels is also generally assumed to mediate the *anesthetic* action of ketamine (Kohrs and Durieux, 1998). However, recently Chen et al. (2009) provided evidence that inhibition of the hyperpolarization- and cyclic nucleotide-gated HCN ion channels (Wahl-Schott and Biel, 2009), with a resulting better preserved post-spike neuronal hyperpolarization, may account for ketamine's anesthetic effects.

2.1.2. Brain glutamate turnover and content in depressed states

Glutamate and the related amino acid glutamine (which is both a glutamate precursor and a product of glutamate metabolism — Fig. 1) do not easily cross the blood-brain barrier. Accordingly, glutamate used for neurotransmission must be produced from glucose within the brain, and a corresponding amount must eventually also be disposed of. Both processes are prominent in astrocytes but absent in neurons, which do not express the enzymes required. Glutamate synthesis depends on the combined actions of i) the ubiquitous pyruvate dehydrogenase (PDH), which converts pyruvate to acetyl coenzyme A, and ii) pyruvate carboxylase (PC), which catalyzes formation of oxaloacetate (OAA) from pyruvate plus CO_2, and is an astrocyte-specific enzyme (Fig. 1). In the astrocytic tricarboxylic cycle, located in the mitochondria, oxaloacetate and acetyl CoA-derived acetate condense to form citrate. α-Ketoglutarate, a direct precursor of glutamate, is generated from citrate by a partial turn of the cycle. Astrocytes also selectively express the enzyme glutamine synthetase (GS) (Norenberg and Martinez-Hernandez, 1979), which converts glutamate to glutamine, and whose mRNA expression is reduced in post-mortem brain tissue from patients having suffered from major depression (Choudary et al., 2005). Newly synthesized glutamine is released from astrocytes to the extracellular space and taken up in neurons in a glutamate–glutamine cycle. Most accumulation is in glutamatergic neurons, where glutamine after hydrolysis to glutamate is concentrated in synaptic vesicles by vesicular glutamate transporters. However, glutamine is also, albeit to a lesser degree, accumulated into GABA-ergic neurons, where it is converted via glutamate to GABA. After their release as transmitters, most glutamate and some GABA are accumulated into astrocytes. Approximately two thirds of the transmitters accumulated in astrocytes are returned to neurons in the glutamate–glutamine cycle, while the remaining one third is oxidized, balancing the one third of glutamine trafficking which represents newly synthesized glutamate (Lieth et al., 2001; Lebon et al., 2002; Hertz et al., 2007; Hertz, 2011). mRNA expression of the astrocytic glutamate transporters is reduced in brains, including locus coeruleus, from patients having suffered from major depression (Choudary et al., 2005; Bernard et al., 2010). Glutamate oxidation is initiated by its re-conversion to α-ketoglutarate, which is metabolized in the TCA cycle to malate, generating ATP in the process. Malate can exit the mitochondria and in the cytosol be oxidatively decarboxylated by cytosolic malic enzyme (cyt ME), a third astrocyte-specific enzyme (Kurz et al., 1993), to pyruvate (Fig. 1). However, besides oxidizing glutamate or transferring it to neurons, *astrocytes also release glutamate (Fig. 1), as a 'gliotransmitter'* (Parpura et al., 2010), which can act on neuronal glutamate receptors. Moreover, expression of glutamate receptors is not limited to neurons, but these receptors are also found on glial cells, including astrocytes (Hansson and Rönnbäck, 2004; Domingues et al., 2010). Accordingly, presynaptically released glutamate can activate not only postsynaptic (and presynaptic) neuronal receptors, but also glutamate receptors on perisynaptic astrocytes. A resulting release of glutamate from the astrocytes subsequently can act on neuronal receptors, modifying their response to glutamate.

Contents of glutamate and glutamine can be determined non-invasively in the human brain by ^{1}H-based magnetic resonance spectroscopy (MRS), although many studies rather than determining each amino acid separately determine 'Glx', which includes both glutamate and glutamine, occasionally also with some GABA. This can complicate interpretations, since glutamate and glutamine concentrations during riluzole treatment of depressed bipolar patients have been found to change in opposite directions (Brennan et al., 2010). Moreover, glutamate changes in the brains of patients suffering from major depression or a bi-polar depressive phase are likely to be region-specific. This probably explains the difference between a distinct increase in glutamate in the occipital cortex of non-medicated major depression patients found by Sanacora et al. (2004) and a decrease in the pregenual anterior cingulate cortex observed by the Walter group (Walter et al., 2009 [non-medicated patients]; Horn et al., 2010 [medicated patients]) in patients with the highest scores on the Hamilton depression scale.

In most studies an increase in glutamate or in Glx has been reported in depressed bipolar patients (Yüksel and Öngür, 2010). During treatment of bipolar depressed patients with riluzole a non-significant decrease in Glx occurred after 2 days of treatment, coupled with a similarly non-significant increase in glutamine, which together resulted in a significant increase in glutamine/glutamate ratio (Brennan et al., 2010). This increase was interpreted as an increase in glutamatergic activity. However, ^{1}H-based MRS does not allow this conclusion. This is because the reason for the change could either be an increased astrocytic uptake of previously released transmitter glutamate and its conversion to glutamine (Fig. 1), which would suggest an increase in glutamatergic activity, or it could be a decreased utilization of astrocytically generated glutamine as precursor for neuronal glutamate, which would not allow such a conclusion. A distinction between these two possibilities would be possible by MRS following administration of [^{13}C]glucose, which would label glutamine generated in astrocytes by net synthesis differently from glutamate and glutamine labeled as result of a bidirectional exchange between glutamate and α-ketoglutarate, which mainly occurs in neurons (because they have the highest content of glutamate) and allows isotope exchange but no net glutamate synthesis (Hertz, 2011).

2.1.3. Summary

The findings discussed above suggest glutamatergic pathology and increased NMDA-mediated activity in depression. There is consistency between observations in patients suffering from major depression and from depression in bipolar disorder. Inhibition of excess excitatory

NMDA activity may be therapeutic in depression, regardless of its origin. Astrocytes are essential for net synthesis and degradation of glutamate. Released neurotransmitter glutamate acts not only on neuronal receptors, but also on astrocytic receptors, triggering release from glutamate at perisynaptic sites.

2.2. Glutamate receptors in major depression and bipolar disorder

2.2.1. Glutamate receptors

Glutamate receptors are traditionally divided into the ionotropic, ion channel-associated AMPA, NMDA and kainate receptors and the G protein-coupled metabotropic receptors. They are tetrameric (consisting of 4 subunits) and often heteromeric (containing different subunits). The glutamate receptors comprise i) the AMPA receptors GluA1-4; ii) the NMDA receptors (GluN1, GluN2A, GluN2B, GluN2C, GluN2D, GluN3A, and GluN3B); iii) the metabotropic glutamate receptors mGlu1-8 or groups I (mGlu1 and mGlu5), group II (mGlu3–4) and group III (remaining mGlus); and iv) the kainate receptors GluK1–5 (Collingridge et al., 2009). Postsynaptic AMPA and NMDA receptors are responsible for most excitatory synaptic transmission. Kainate receptors are in part expressed postsynaptically (Castillo et al., 1997; Mulle et al., 1998; Cossart et al., 2002; Pinheiro and Mulle, 2006), but also play important modulatory roles presynaptically (Contractor et al., 2000; Schmitz et al., 2000; Frerking et al., 2001; Fernandes et al., 2009). Either location could include astrocytic receptors, and astrocytic glutamate receptor sites might also be perisynaptic. GluK1–GluK3 can be expressed as homomeric (containing only one subunit) or heteromeric channels. GluK4 and GluK5 do not by themselves form functional receptors, but induce pharmacological changes when forming heteromeric assemblies with GluK1 or GluK2 (Mott et al., 2010). In hippocampal slices GluK2 is mainly associated with pyramidal cells and dentate granule neurons. Conversely, GluK1 is prominently expressed in nonpyramidal GABA-ergic interneurons, where they are important for neuronal plasticity (Paternain et al., 2000; Lerma, 2006). However, there is also a significant population of GABA-ergic cells that co-express the two glutamate receptor subunits (Paternain et al., 2000; Sun et al., 2009). Astrocytic expression of GluK receptors will be discussed later.

2.2.2. Alterations of glutamate receptors in depression and mania

Abnormalities in glutamate receptors and their genes have recently been authoritatively reviewed by Sanacora et al. (2008) and Machado-Vieira et al. (2009b). Although interpretations of altered receptor expression may be hampered by interfering drug therapy, these reviews present convincing evidence that both AMPA and NMDA receptors are down-regulated in major depression and bipolar depression (which could be a response to elevated glutamate concentrations). There are also indications that enhanced AMPA receptor signaling may have antidepressant effect(s). In contrast, NMDA receptor activation, and perhaps especially that of GluN2A may play a pivotal role in the etiology of major depression. This conclusion is supported by the observation that GluN2A knock-out mice exhibit decreased anxiety-like behavior. In contrast GluA1 knock-outs show symptoms of both depression and mania (Fitzgerald et al., 2010). Polymorphisms in *GRIN1*, *GRIN2A*, and *GRIN2B* (coding for GluN1, GluN2A and GluN2B) confer susceptibility to bipolar depression. Preclinical studies have provided information about depression-relevant properties of the 3 groups of metabotropic glutamate receptors (see also Pilc et al., 2008). Recently a plausible biological association between depressive traits and single nucleotide polymorphisms within *Grm8* (coding for mGlu8) has been established (Terracciano et al., 2010). Knock-outs have been developed for all 8 metabotropic receptors and their characteristics have been described by Niswender and Conn (2010). These animals have been very useful, although the potential compensatory mechanisms following deletion of a gene must be kept in mind.

GluK2 knockout mice show behavioral symptoms related to mania that could be prevented by lithium (Shaltiel et al., 2008). Membrane-associated hippocampal and prefrontal cortical membrane levels of GluK1 and GluK4 receptors were also substantially decreased in the GluK2 knockout mice. However, GluK1 and GluK4 receptors in the cytosol were unaffected. The membrane expression of other glutamatergic receptors was not significantly altered. The GluK2 knockout mice were more active in multiple tests than wild-type animals and showed enhanced increase in locomotor activity in response to amphetamine. They exhibited more risk-taking type of behavior and less manifestation of despair. These observations may be of special interest, since the GluK2 gene *(GRIK2)* resides in a genetic linkage region (6q21) associated with bipolar disorder (Schulze et al., 2004). In addition, GluK2 expression (and expression of some AMPA receptors) was reduced in the entorhinal and perirhinal cortices from medicated patients with bipolar disorder, without concomitant reduction of GluK4 or GluK5 (Beneyto et al., 2007). However, Benes et al. (2001) found no significant difference in GluK2 expression in the hippocampus of bipolar patients. A recent family-based association study evaluating *GRIK3* also described a significant linkage disequilibrium in major depression but not in bipolar depression (Schiffer and Heinemann, 2007). Nevertheless, an increased *GRIK3* DNA-copy number was shown in bipolar depressed patients, and a common variant in the 3′ untranslated region of the *GRIK4* gene protected against bipolar disorder (Machado-Vieira et al., 2009b).

We have recently reported that mRNA and protein expression of GluK2 is down-regulated in the cerebral cortex of mice treated for 2 weeks with daily intraperitoneal injection of carbamazepine at therapeutically relevant concentration but up-regulated by similar treatment for 1 week with the SSRI fluoxetine at 10 mg/kg (Fig. 2a and b). The down-regulation of GluK2 occurred without effect on other kainate receptors, including GluK4 and GluK5 (Li et al., 2009), although all 5 kainate receptors are expressed in the brain. Down-regulation after anti-bipolar drug treatment may explain the observations by Beneyto et al. (2007) in medicated patients and both data sets suggest a specific

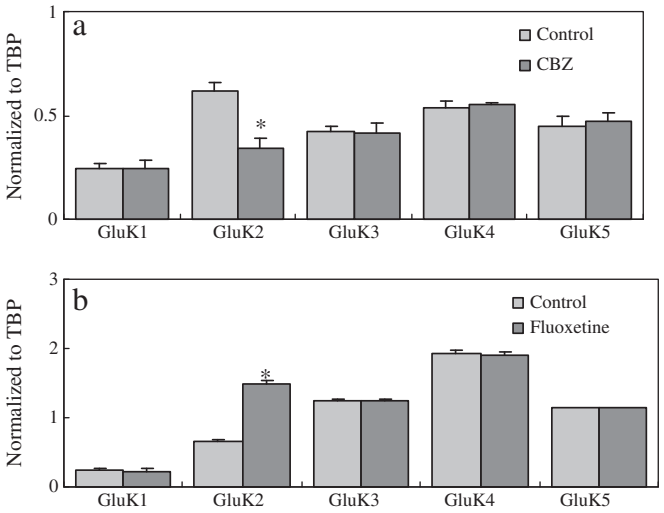

Fig. 2. Chronic treatment with carbamazepine (CBZ) (a) or fluoxetine (b) on mRNA expression of GluK1, GluK2, GluK3, GluK4 and GluK5 in brain in vivo. (a) Adult mice were daily injected intraperitoneally with phosphate-buffered saline (PBS) or with 25 mg/kg CBZ in PBS for 2 weeks. (b) Adult mice were treated with either PBS or fluoxetine (10 mg/kg/day) in a similar manner for seven days. (a and b) Average mRNA expression was quantitated as ratios between, GluK1, GluK2, GluK3, GluK4 or GluK5 and tata-binding protein (TBP), used as a house-keeping gene. SEM values are indicated by vertical bars. *Indicates statistically significant difference from control group ($P<0.05$).
Modified from Li et al. (2009, 2011).

effect on homomeric GluK2 receptors. The up-regulation of the gene by fluoxetine is accompanied with an increased mRNA editing, bringing the edited form close to 90% of the total and reducing the non-edited form by 50% (Li et al., 2011).

2.2.3. Summary

Expression of genes for glutamatergic receptors in depression and the effect of both antidepressant medications (SSRIs) and anti-bipolar medications (lithium, carbamazepine and valproic acid) support the concept that deviations in glutamatergic function may be important in depressive illness and they draw attention to GluK2 receptors. Selective effects on GluK2 without the involvement of other kainate receptors suggest that only homomeric GluK2 receptors are affected.

3. GluK2 receptor function

3.1. Receptor characteristics

3.1.1. GluK2 distribution and synaptic function

GluK2 receptors are widely distributed in the central nervous system and have been demonstrated on both astrocytes and neurons in rat hippocampus (Strutz-Seebohm et al., 2005). In the entorhinal cortex GluK2 receptors are restricted to the somatodendritic region of layer III (Beed et al., 2009). In the spinal cord GluK2 and GluK3 are expressed on astrocyte cell bodies and processes, whereas GluK1 is limited to perivascular processes (Brand-Schieber et al., 2004). GluK2 mRNA expression in an astrocytic cell fraction from intact mouse brain is about one half of that in a corresponding neuronal fraction (Cahoy et al., 2008).

GluK2 expressed in HEK 293 cells or oocytes forms functional ion channels gating Na^+ and, depending upon degree of editing, also Ca^{2+} (Burnashev et al., 1996). However, the affinity toward glutamate is relatively low with a threshold of 0.3 μM and a maximum effect requiring around 10 μM (Mott et al., 2010). These glutamate concentrations are well above the ambient glutamate concentration of 25 nM estimated for a hippocampal slice by Herman and Jahr (2007). Heteromeric GluK2 channels with GluK4 or GluK5 subunits display high agonist affinity, conveyed by the GluK4 and/or GluK5 subunit(s), as well as a lower affinity agonist site, attributed to the GluK2 subunit (Mott et al., 2010). The high-affinity site shows little or no desensitization, whereas there is considerable desensitization of the low-affinity site.

3.1.2. Metabotropic function

In addition to functioning as ionotropic receptors kainate receptors have metabotropic actions, which involve the activation of G proteins and second messenger cascades (Rodríguez-Moreno and Sihra, 2007a). The first established metabotropic effect of kainate receptor stimulation was depression of GABA release from interneurons in the hippocampal stratum oriens, caused by presynaptic activation of a $G_{i/o}$ protein and of protein kinase C (PKC) (Rodríguez-Moreno and Lerma, 1998). The effect was due to GluK1 activation, and this receptor subtype seems to be involved in many cases of kainate-mediated depression of GABA-ergic activity, often in heteromeric receptors together with GluK2 (Rodríguez-Moreno and Sihra, 2007b; Sun et al., 2009). Similar signaling also plays a major role in the amygdala (Braga et al., 2004). However, since GluK1 is not affected in brain by either anti-bipolar or antidepressant treatment, modulation of GABA release will not be further discussed. This is in spite of the fact that GABA-ergic abnormalities are found in major depression (Sanacora et al., 2004).

Activation of GluK2 receptors in both the CA1 and the CA3 region of hippocampal brain slices causes an inhibition of a post-spike K^+ current in pyramidal cells, I_{sAHP}, which represents the longest lasting phase (5–10 s) of a normally occurring neuronal afterhyperpolarization following excitation (Melyan et al., 2002, 2004; Fisahn et al., 2004). I_{sAHP} is a voltage-independent K^+ current, which follows short bursts of action potentials. It is activated by Ca^{2+} influx associated with chains of action potentials, large depolarizations or receptor activation, and it restrains repetitive firing in hippocampal pyramidal neurons (Lancaster et al., 2001). It should not be confused with I_h, which is the current associated with opening of hyperpolarization- and cyclic nucleotide-activated HCN ion channels that causes the opposite effect and is antagonized by ketamine (Chen et al., 2009). I_{sAHP} is also inhibited by noradrenaline, isoproterenol, cAMP and protein kinase A (PKA) (Madison and Nicoll, 1982; Lancaster et al., 2001; Grabauskas et al., 2007). Downstream of PKA, protein kinase PKC activity is required for the inhibition, which is dependent upon $ERK_{1/2}$ phosphorylation (Grabauskas et al., 2007). It is well established that stimulation of β-adrenergic receptors can lead to PKA-dependent "G_s/G_i switching", which in turn induces $ERK_{1/2}$ phosphorylation (Martin et al., 2004). Although the measured effect on hyperpolarization and I_{sAHP} is undoubtedly neuronal, the activation of both GluK2 receptors and β-adrenergic receptors could equally well occur on adjacent astrocytes, and trigger them to release glutamate acting on perisynaptic neuronal receptors.

It is not known to what extent homomeric GluK2 receptors suffice for metabotropic activation of GluK2 receptors, or whether GluK4 and/or GluK5 expression is also required (Contractor et al., 2001; Ruiz et al., 2005; Fernandes et al., 2009). Fernandes et al. (2009) concluded from results obtained in double knock-out mice that at least two populations of presynaptic GluK2 receptors exist: GluK2 receptors, which co-express GluK4 and GluK5, are positioned close enough to the synapse to respond to single release events during paired high-frequency stimulation, whereas GluK2 homomeric receptors mediate facilitation during longer, lower-frequency stimulation and possibly may be located further away from glutamate release sites. This would be consistent with an astrocytic localization. It may be an indication of an association between the inhibited hyperpolarization and depressive disorders that slow hyperpolarization in amygdala in vivo is inhibited by chronic, but not acute restraint stress, leading to hyperexcitability (Rosenkranz et al., 2010). In spinal cord slices GluK2 activation leads to induction of long-term potentiation (LTP) of excitatory postsynaptic potentials in substantia gelatinosa neurons (Youn et al., 2005).

3.1.3. Summary

Many GluK2 receptors are extrasynaptic. Both GluK1 and GluK2 have not only ionotropic but also metabotropic effects. GluK1 stimulation is generally associated with enhanced GABA-ergic function. In brain slices GluK2 activation inhibits post-spike slow hyperpolarization of neurons carried by the K^+ current I_{sAHP} and mediated by opening of Ca^{2+}-dependent channels, and it enhances excitatory activity.

3.2. K^+ channel and protein associated with I_{sAHP}

3.2.1. K^+ channels

Neuronal depolarization resulting from action potentials opens voltage-gated Ca^{2+} channels, causing a rapid entry of Ca^{2+} into neurons, which activates K^+ channels and leads to a post-spike hyperpolarization, reducing excitability. This afterhyperpolarization is divided into 3 phases: i) fast (fAHP); ii) intermediate (mAHP); and iii) slow (sAHP) afterhyperpolarization (Tzingounis and Nicoll, 2008). In CA1 pyramidal neurons sAHP is associated with a substantial decrease in synaptic efficacy (Borde et al., 1999). As discussed above, sAHP is inhibited by activation of metabotropic GluK2 receptors or β-adrenergic receptors. Such an inhibition of GluK2 activity can therefore be expected to reduce glutamatergic neurotransmission.

The identity of K^+ channels mediating sAHP long remained elusive. There is consensus that Ca^{2+} is required for sAHP, but the current mediating it, I_{sAHP}, is not dependent on small or large conductance Ca^{2+}-activated K^+ channels (BK and SK channels). This is in contrast to those carrying the preceding fAHP and mAHP (Storm, 1987; Bond et al., 2004;

Pedarzani et al., 2005). Recently Nicoll and coworkers (Tzingounis and Nicoll, 2008; Tzingounis et al., 2010) obtained evidence in knockout mice that at least part of the K^+ current responsible for sAHP (I_{sAHP}) may be carried by one or more of the KCNQ (Kv7) channels in a cell type-specific manner. Four of the 5 KNCQ channels are expressed in the brain (Brown and Passmore, 2009). As described in detail by this group the channels are closed by receptors coupled to G_q, such as M1 and M3 muscarinic receptors; this closure underlies some forms of cholinergic excitation. Brown and Passmore (2009) showed that the channels also can be closed by many other receptors coupled to G_q or G_{11} protein, including group I metabotropic glutamate receptors, histamine H1, $5HT_{2C}$ serotonergic and P2Y purinergic receptors as well as several peptide receptors. It has not been established if the inhibition of I_{sAHP} by GluK2 activation is caused by a similar channel closure. Effects of channel closure include facilitation of repetitive discharges and burst-firing and induction of spontaneous firing in hippocampal neurons, whereas the drugs flupirtine and retigabine enhance channel activity and reduce neural excitability (Brown and Passmore, 2009).

3.2.2. Ca^{2+}-binding proteins

A problem with the conclusion that KCNQ channels may mediate sAHP is that I_{sAHP} generally is considered to be independent of voltage-sensitive channels, whereas KCNQ channels are voltage-activated (Vogalis et al., 2003; Brown and Passmore, 2009). Moreover, cAMP stimulates KCNQ channels (Schroeder et al., 2000) but inhibits I_{sAHP} (Madison and Nicoll, 1982). Tzingounis et al. (2010) suggested that dependence on the diffusible neuronal Ca^{2+} sensor protein hippocalcin might explain the delayed expression and apparent voltage independence of I_{sAHP}, because hippocalcin must bind cytosolic Ca^{2+} and translocate to the membrane before gating I_{sAHP}. Moreover, hippocalcin may be necessary for noradrenaline's inhibitory effect on I_{sAHP} (Tzingounis et al., 2007). Translocation of yellow fluorescein protein-tagged hippocalcin to the membranes has been demonstrated in hippocampal neurons and increases with the number of preceding action potentials (Markova et al., 2008). Hippocalcin and a related protein are also recruited in the cerebral cortex in spite of a lower hippocalcin concentration (Villalobos and Andrade, 2010). Periodic bursting in autonomously firing striatal cholinergic interneurons is driven by a delayed and slowly decaying I_{sAHP} effectuated by opening of L-channels for Ca^{2+} and a time constant for the decay of somatic $[Ca^{2+}]_i$ approaching 2 s (Goldberg et al., 2009). Gonadotropin-releasing neurons in the basal forebrain also exhibit long-duration (~10 s) calcium transients that are synchronized with their burst firing. They are initiated by opening of L-channels but supplemented by capacitative Ca^{2+} release from intracellular stores, driven by the Ca^{2+} entry (Lee et al., 2010).

3.2.3. Summary

Slow after-hyperpolarization results from opening of Ca^{2+}-dependent K^+ channels, possibly KCNQ channels. Many of its characteristics may be explained by the involvement of hippocalcin or related Ca^{2+}-binding protein(s).

4. Relevance of astrocytes

4.1. Glutamate receptors and glutamate release

4.1.1. Effects of anti-bipolar/antidepressant medication

GluK2–GluK5 kainate receptor subunits have been demonstrated in cultured mouse astrocytes from cerebral cortex, but GluK1 is absent (Li et al., 2009, 2011). mRNA (Fig. 3a) and protein expressions *specifically* of GluK2 are downregulated by chronic, but not acute treatment with either carbamazepine, lithium, or valproic acid at therapeutically relevant concentrations (Li et al., 2009). In addition, a normally occurring rise in $[Ca^{2+}]_i$ in response to glutamate acting on the GluK2 receptor (see below), and indicated by an increase in 340/380 nm fluorescence ratio in fura-2-treated astrocytes (Fig. 3b), is abolished after chronic treatment with any of the 3 anti-bipolar drugs at similar concentrations. Cultured neurons have not been studied to nearly the same extent (because they do not survive sufficiently long after maturation to allow longtime chronic studies). However, neither cerebellar granule neurons, nor hippocampal neurons show any down-regulation of GluK2 expression in response to the treatment with carbamazepine that down-regulated astrocytic GluK2 expression (Fig. 3c). This is in spite of GluK2 being the main kainate receptor expressed on cultured hippocampal neurons (Ruano et al., 1995).

In cerebral cortical astrocytes GluK2 receptor activation is essential for glutamate-induced increase in $[Ca^{2+}]_i$ (Fig. 4a) and downstream $ERK_{1/2}$ phosphorylation (Fig. 4b). This has been demonstrated by complete inhibition of both stimulated $[Ca^{2+}]_i$ increase and $ERK_{1/2}$ phosphorylation by NS102 (a specific antagonist of GluK1 and GluK2) and after GluK2 down-regulation by administration of small interfering RNA (siRNA) against GluK2 (Li et al., 2009; B. Li and L. Peng, unpublished experiments). In addition, both the PKC inhibitor GF 109203X (Fig. 5a) and the IP_3 receptor inhibitor xestospongin C (Fig. 5b) abolish $ERK_{1/2}$ phosphorylation in response to glutamate. These findings indicate that GluK2 receptor activation stimulates ERK phosphorylation by operating in its metabotropic mode of action. They also identify the signaling pathway of the metabotropic GluK2 receptor in astrocytes as being linked to phospholipase C (PLC) and activated via either $G_{\alpha q}$ or $G_{\beta\gamma i}$ proteins (Birnbaumer, 2007). Finally, they show that $ERK_{1/2}$ phosphorylation depends on both of the products generated by PLC activity, i.e., inositoltrisphoshate (IP_3), which stimulates release of Ca^{2+} from intracellular stores, and diacylglycerol (DAG), which activates PKC.

Stimulation of the metabotropic glutamate receptor mGlu5 is more generally known to increase $[Ca^{2+}]_i$ in astrocytes (Nakahara et al., 1997; Fellin et al., 2007), but mGlu5 activation has been reported to stimulate ERK phosphorylation in cultured astrocytes independently of PLC activation (Peavy et al., 2001). This does, however, *not* mean that the observed dependence of GluK2 receptor-stimulated $ERK_{1/2}$ phosphorylation on PLC activity proves that mGlu5 receptor activation could not *also* be required for phsphorylation of ERK in the present system. We therefore tested whether glutamate-induced $ERK_{1/2}$ phosphorylation could be selectively inhibited by MPEP, an inhibitor of the mGlu5 receptor, and found that MPEP completely inhibited $ERK_{1/2}$ phosphorylation by glutamate (Fig. 5c). Accordingly, simultaneous GluK2 and mGlu5 receptor activations seem to be necessary for astrocytic ERK phosphorylation by glutamate. Additional experiments will be required to confirm or refute this concept, but synergism between activation of different glutamate receptors has previously been established in hippocampal astrocytes (Porter and McCarthy, 1996).

The SSRIs fluoxetine, paroxetine and citalopram at low micromolar concentrations act on $5\text{-}HT_{2B}$ serotonergic receptors to stimulate $ERK_{1/2}$ phosphorylation in cultured astrocytes (Li et al., 2008; Zhang et al., 2010). Chronic effects of SSRIs are even more potent. Concentrations of any of the five conventional SSRIs (the 3 mentioned above plus fluvoxamine and sertraline), which are similar to or lower than those found clinically in plasma (e.g., 0.1 μM citalopram), induce a $5\text{-}HT_{2B}$ receptor-mediated up-regulation of calcium-dependent phospolipase A_2 ($cPLA_2$) after 2–4 weeks of drug treatment (Zhang et al., 2010). Chronic treatment with fluoxetine also upregulates mRNA (Fig. 6a) and protein expression of GluK2 and of the mRNA-editing enzyme ADAR2 (Fig. 6b). The resulting enhanced GluK2 editing abrogates glutamate-mediated increase in intracellular Ca^{2+} (Fig. 6c) and $ERK_{1/2}$ phosphorylation (Li et al., 2011). *Thus, anti-bipolar and antidepressant drug treatments have opposite effect on GluK2 expression in astrocytes but, due to increased editing of GluK2 after fluoxetine treatment, the effects on the metabotropic activity of GluK2 are identical.* These observations call for consideration of the manners in which *astrocytic* GluK2 activity may be linked to excitatory *neuronal* signaling. This is especially important, since upregulation of GluK2 expression and editing recently has been confirmed in an astrocytic cell fraction isolated from fluoxetine-treated mice, whereas no corresponding

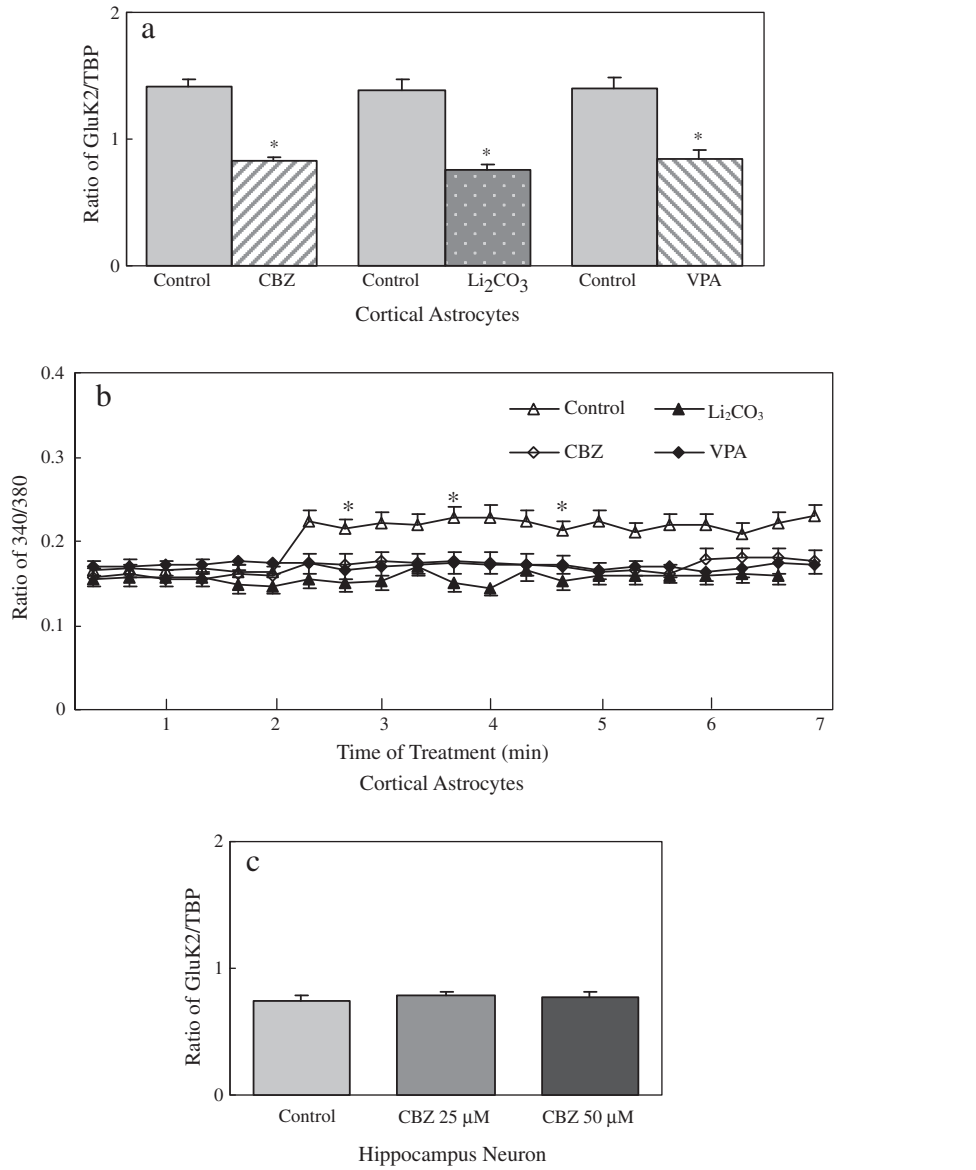

Fig. 3. Anti-bipolar drugs down-regulate expression of GluK2 and its effect on intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in cultured astrocytes (a and b), but do not affect GluK2 expression in cultured neurons (c). (a) Down-regulation of mRNA expression of GluK2 by chronic treatment with CBZ, Li_2CO_3 or valproic acid (VPA) in primary cultures of astrocytes. Cells were treated with PBS (Control) or with 50 μM CBZ, 1 mM Li_2CO_3 or 1 mM VPA in PBS for 1 week. Average mRNA expression was quantitated as ratios between GluK2 and TBP, used as a house-keeping gene. SEM values are indicated by vertical bars. *Indicates statistically significant ($P<0.05$) difference from control. Data for CBZ are from Li et al., 2009, and data for Li^+ and VPA from unpublished results by B. Li and L. Peng. (b) Down-regulation of glutamate-induced increase of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) by chronic treatment with CBZ, Li_2CO_3 or VPA in astrocytes. Cells were untreated controls (open triangles) or treated with 50 μM CBZ (open diamonds), 1 mM Li_2CO_3 (filled triangles) or 1 mM VPA (filled diamonds) for 1 week. After the cells had been loaded with fura-2, they were incubated for 2 min in 300 μl saline solution in the absence of any drug. Subsequently, 30 μl glutamate solution to a final concentration of 100 μM was added to each well, and the incubation was continued for another 5 min. Fura-2 fluorescence ratios at 340/380 nM, an indicator of $[Ca^{2+}]_i$, was recorded at 20 s intervals, and means ± SEM were calculated for three individual experiments from the fluorescence ratios at selected times (0, 160, 220, and 280 s after addition of glutamate solution). *Statistically significant ($P<0.05$) difference from all other groups at the same selected recording time. Unpublished results by B. Li and L. Peng. (c) Hippocampal neurons were treated with PBS (Control) or with 25 μM or 50 μM CBZ in PBS for 1 week. Average mRNA expression was quantitated as ratios between GluK2 and TBP, used as a house-keeping gene.
From Li et al. (2009).

upregulations occurred in the neuronal fraction (B. Li and L. Peng, unpublished experiments).

4.1.2. Summary

In astrocytes anti-bipolar and antidepressant drug treatment inhibit GluK2's metabotropic activity by down-regulation of GluK2 expression and by increased editing of an upregulated receptor, respectively. Inhibitor experiments indicate that the astrocytic GluK2 receptor must be PLC-linked.

4.2. Astrocytic glutamate release enhances glutamatergic transmission in neurons

4.2.1. Increase in $[Ca^{2+}]_i$ activates release of 'gliotransmitters'

The glutamate-induced increase in $[Ca^{2+}]_i$ shown in Figs. 3c, 4a, and 6c and its abolishment after treatment with anti-bipolar or antidepressant drugs are important, because the astrocytic Ca^{2+} response plays an essential role in the bidirectional communication between neurons and astrocytes in the tripartite synapse (Araque et al., 1999). Astrocytes contribute to the modulation of synaptic

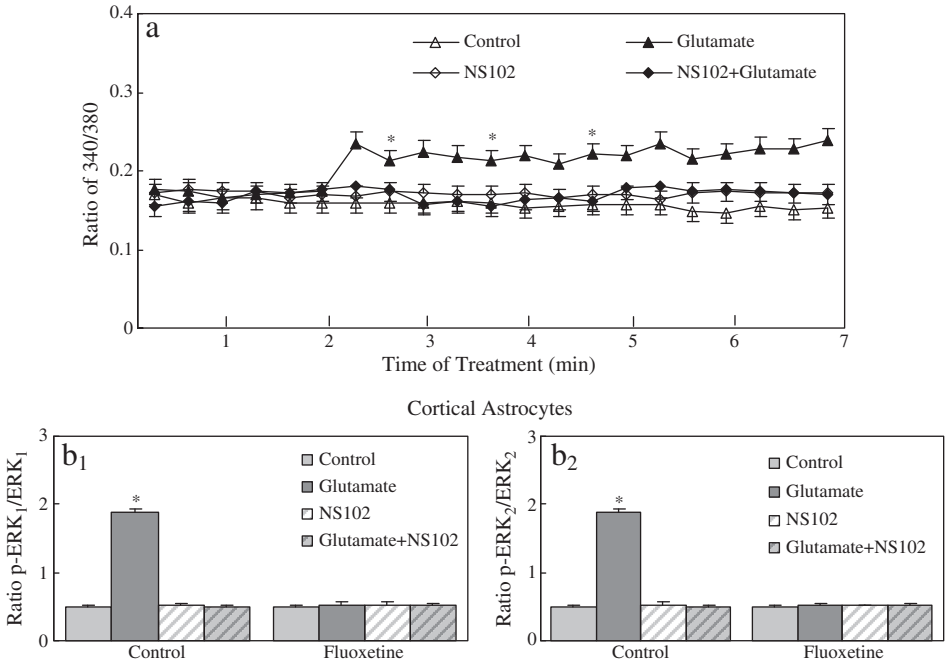

Fig. 4. GluK2 activity mediates glutamate-induced increase of intracellular Ca^{2+} concentration and $ERK_{1/2}$ phosphorylation in astrocytes, and both effects are reduced by chronic treatment with fluoxetine. (a) Glutamate-induced increase of intracellular Ca^{2+} concentration is inhibited by NS 102. After the cells had been loaded with fura-2, cells were incubated for 2 min in the absence of any drug (Control; open triangles) or in the presence of 100 μM glutamate (filled triangles), of 10 μM NS102 (open diamonds), an inhibitor of GluK1 and GluK2, or of glutamate plus NS 102 (filled diamonds), and the incubation was continued for another 5 min. Fluorescence was recorded at 20 s interval. Means ± SEM were calculated for three individual experiments from the fluorescence ratios 0, 160, 220, and 280 s after addition of glutamate solution. *Statistically significant ($P<0.05$) difference from all other groups at the same recording time. From unpublished results by B. Li and L. Peng. (b) Cells were untreated controls or treated with 10 μM fluoxetine for three days. Thereafter, cells were incubated for 20 min in the absence of any drug (Control) or in the presence of 100 μM glutamate, of 10 μM NS102, an inhibitor of GluK1 and GluK2, or of glutamate plus NS 102. Average ERK phosphorylation was quantitated as ratios between p-ERK_1 and ERK_1 (b_1) and between p-ERK_2 and ERK_2 (b_2). SEM values are indicated by vertical bars. *Statistically significant ($P<0.05$) difference from other groups for ERK_1 and ERK_2.
From Li et al. (2011).

transmission by reacting to transmitters released during synaptic activity (Araque et al., 1998; Fellin and Carmignoto, 2004; Perea and Araque, 2007, 2010). In brain slices astrocytes react to different transmitters, including glutamate (Porter and McCarthy, 1996; Pasti et al., 1997) with an increase in $[Ca^{2+}]_i$, due to release of Ca^{2+} from intracellular stores in response to IP_3, generated by activation of neurotransmitter receptors linked to PLC. This includes metabotropic glutamate receptors of group I and, as indicated above, also GluK2 in its metabotropic mode. The increase in $[Ca^{2+}]_i$ can be mimicked by photolytically releasing caged Ca^{2+} injected into astrocytes in the neigborhood of a specific synapse (Fig. 7a) and recording the postsynaptic response of a single neuron. The response of a CA1 pyramidal neuron to a specific number of stimuli, with and without astrocytic Ca^{2+} release, is illustrated in Fig. 7. It can be seen that astrocytic Ca^{2+} release increases the number of successful stimuli from 3 to about twice this amount (Fig. 7b). It does so by enhancing synaptic efficacy, determined as *mean* amplitude to *all* applied stimuli, in this case 15 consecutive stimuli (Fig. 7c). The probability that released transmitter is able to cause a stimulation becomes enhanced, shown as a higher probability ratio (Pr), i.e., the ratio between the number of *successful* stimuli and *all* applied stimuli (Fig. 7d), but the amplitude of the successful responses is not changed (Fig. 7e). Although these results were interpreted by Perea and Araque (2007) as a result of increased presynaptic release of glutamate, an increased synaptic efficacy as a result of reduced afterhyperpolarization (Borde et al., 1999) might probably also be able to explain them. ATP-evoked increases in $[Ca^{2+}]_i$ have a similar effect, and the increased efficacy of synaptic transmission is secondary to release of glutamate and its stimulatory effect on presynaptic neuronal metabotropic glutamate receptors of group I. This was shown by applying antagonists of these receptors, which blocked the enhanced synaptic transmission without diminishing the astrocytic response to ATP (Perea and Araque, 2007). The involvement of neuronal group I metabotropic receptors is noteworthy, because an agonists of these receptors, DHPG, repeatedly has been found to decrease sAHP (Zahorodna and Bijak, 1999; Ireland et al., 2004; Pan et al., 2010). A much slower initiation and decline of the increase in $[Ca^{2+}]_i$ by IP_3-mediated Ca^{2+} release from intracellular astrocytic stores than for neuronal Ca^{2+} uptake through NMDA receptor channels accounts for the long timescale of astrocyte-mediated synaptic facilitation (Nadkarni and Jung, 2007; Postnov et al., 2009), which is evident in Fig. 7c and d. There is precedent for an effect of antidepressants on this system, since chronic treatment of rats with imipramine for 14 days (10 mg/kg, twice daily) attenuates the DHPG-induced decrease in sAHP in excised CA1 tissue, although acute treatment with imipramine has no effect (Zahorodna and Bijak, 1999).

Astrocytes also release other 'gliotransmitters', and ATP can be hydrolyzed to adenosine, an inhibitory transmitter. Moreover, release of glutamate also stimulates GABA-ergic neurons, implying that astrocytes can enhance both excitatory and inhibitory signaling. However, in the entorhinal cortex non-synaptic GluK2 receptors, without distinction between astrocytic and neuronal location or ionotropic and metabotropic modes, are essential for kainate-induced gamma oscillations and they might be involved in epileptic activity (Beed et al., 2009).

4.2.2. Does inhibition of astrocytic GluK2 activity mimic neuronal hyperpolarization?

It was described above that activation of extrasynaptic, homomeric GluK2 receptors in hippocampal slices causes long-lasting inhibition of slow afterhyperpolarization, mediated by Ca^{2+}-dependent K^+ flux in CA1 and CA3 pyramidal cells (Melyan et al., 2002; Fernandes et al., 2009). Although it remains to be verified that these GluK2 receptors were astrocytic, it suggests that down-regulation of *astrocytic* GluK2

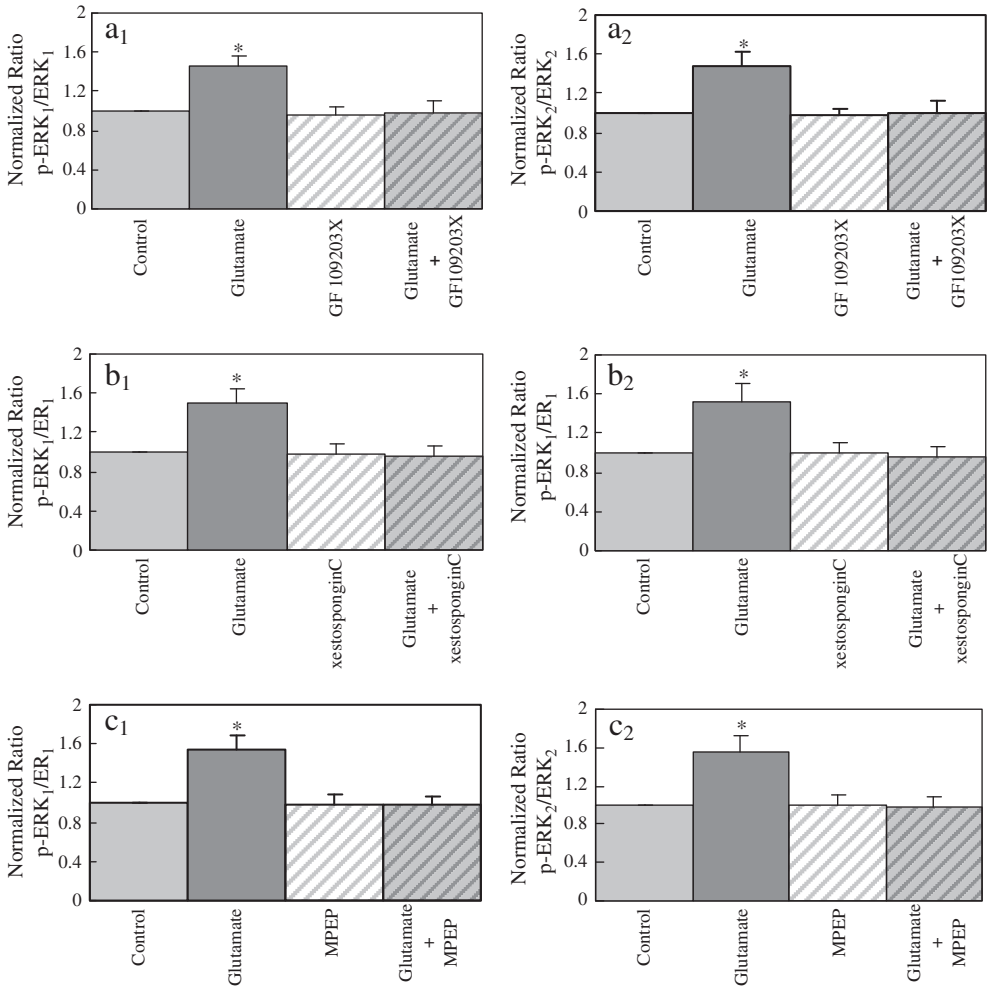

Fig. 5. Phosphorylation of ERK induced by glutamate requires activities of PKC, IP_3 receptor and co-stimulation of mGlu5 in primary cultures of mouse astrocytes. (a) Cells were incubated for 20 min in the absence of any drug (Control) or in the presence of 100 μM glutamate, of 500 nM GF 109203X, an inhibitor of PKC, or of glutamate plus GF 109203X. (b) Cells were incubated for 20 min in the absence of any drug (Control) or in the presence of 100 μM glutamate, of 100 μM xestospongin C, an inhibitor of the IP_3 receptor, or of glutamate plus xestospongin C. (c) Cells were incubated for 20 min in the absence of any drug (Control) or in the presence of 100 μM glutamate, of 25 μM MPEP, an inhibitor of mGlu5, or of glutamate plus MPEP. Average ERK phosphorylation for 2–3 experiments was quantitated as ratios between p-ERK_1 and ERK_1 (a_1, b_1 and c_1) and between p-ERK_2 and ERK_2 (a_2, b_2 and c_2). SEM values are indicated by vertical bars. *Statistically significant ($P<0.05$) difference from other groups for ERK_1 and ERK_2. Unpublished results by T. Du, B. Li and L. Peng.

receptors can reduce *neuronal* excitability, including NMDA-mediated excitatory activity, by preventing GluK2-induced inhibition of neuronal hyperpolarization. In this manner the astrocytic response to chronic treatment with either anti-bipolar or antidepressant drugs mimics the effects of drugs like riluzole, although the glutamatergic target may be more selective by aiming at specific glutamatergic activity, i.e., that mediated by GluK2. The resulting effect may more closely resemble that of neuronal hyperpolarization, especially sAHP. This conclusion raises the question if the rapid anti-depressant effect of ketamine is brought about by its inhibition of HCN channels (Chen et al., 2009), resulting in stabilization of hyperpolarization, rather than by its antagonism of NMDA-mediated glutamatergic activity. The inability of another NMDA receptor blocker, memantine, to replace ketamine (Teng and Demetrio, 2006; Zarate et al., 2006) may support this concept. If that should be the case, it might be worthwhile to test drugs like flupirtine and retigabine for their potential to combat symptoms of depression.

A different question is the etiology of bipolar disorder and major depression. The beneficial effect of correcting a neuronal behavior by drugs acting on astrocytes does not per se indicate whether the primary reason for depression is neuronal or astrocytic malfunction (or both). However, other observations also link astrocytes to depressive illnesses, as will be discussed below.

4.2.3. Summary

Increase in astrocytic $[Ca^{2+}]_i$ is sufficient and necessary to cause release of the 'gliotransmitter' glutamate, which acts on neuronal metabotropic glutamate receptors of group I and increases the efficacy of glutamatergic synaptic activity. Since stimulation of astrocytic GluK2 activity increases $[Ca^{2+}]_i$ in the cells it enhances neuronal excitation. The inhibition of astrocytic GluK2 activity by chronic anti-bipolar or antidepressant drug treatment may therefore accomplish antidepressant effects similar to those, which can be achieved by drugs directly reducing glutamatergic activity.

4.3. Other indications that astrocytes are involved in depressive illness

4.3.1. Post-mortem and in vivo observations

Loss of astrocytes and GFA staining is an accepted feature of major depression (McNally et al., 2008; Hercher et al., 2009; Gosselin et al., 2009; Miguel-Hidalgo et al., 2010; Altshuler et al., 2010), but the number of oligodendrocytes is also reduced (Hamidi et al., 2004). In prefrontal cortex the decrease of glutamine synthetase staining and glutamate transporters is proportional to the lowering of astrocyte density in brains from victims of major depression (Miguel-Hidalgo et al., 2010). Expression of astrocyte specific genes (GFAP and ALDH1L1) is significantly increased in major depression, but their

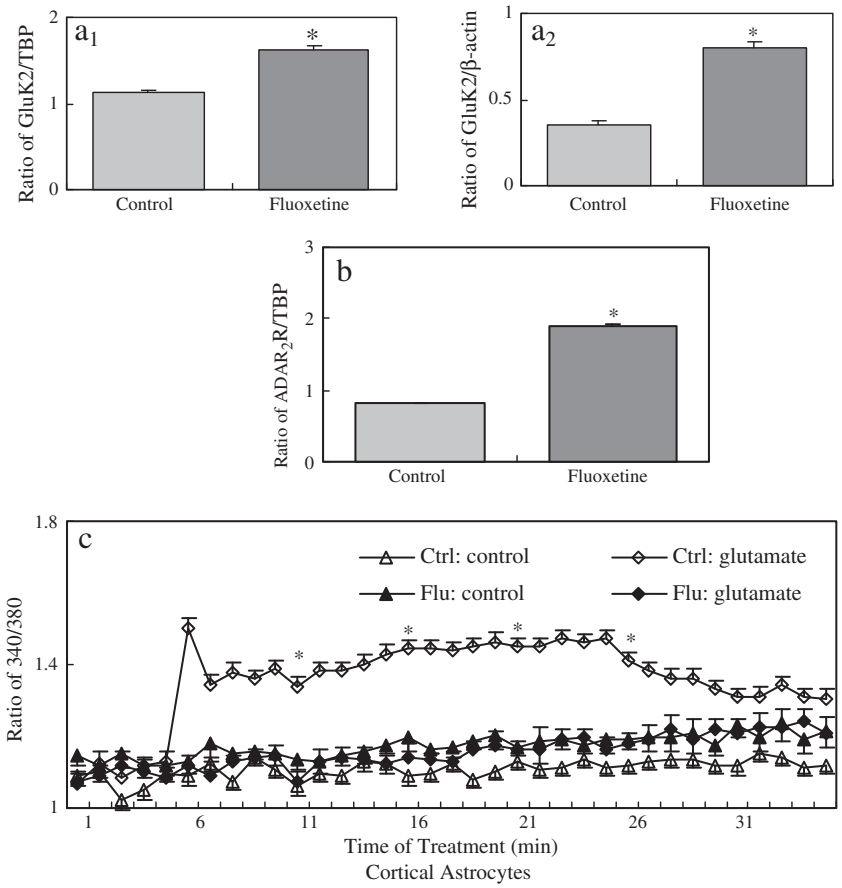

Fig. 6. Chronic treatment of primary cultures of mouse astrocytes with fluoxetine increases the expression of GluK2 and ADAR2, but decreases the increase in $[Ca^{2+}]_i$ in response to glutamate. (a and b) Cells were either untreated controls or treated with 10 μM fluoxetine for three days. (a_1) Average mRNA expression was quantitated as ratios between GluK2 and TBP. (a_2) Average protein expression was quantitated as ratios between GluK2 and β-actin. (b) Average mRNA expression was quantitated as ratios between ADAR2 and TBP. SEM values are indicated by vertical bars. *Statistically significant ($P<0.05$) difference from control group. (c) Down-regulation of glutamate-induced increase of intracellular Ca^{2+} concentration by chronic treatment with fluoxetine in astrocytes. Cells were untreated controls (open triangles or diamonds) or had been treated with 10 μM fluoxetine (filled triangles or diamonds) for three days. After the cells had been loaded with fura-2, they were incubated for 2 min in 300 μl saline solution in the absence of any drug. Subsequently, either 30 μl saline solution (triangles) or 30 μl glutamate solution (diamonds) to a final concentration of 100 μM was added to each well, and the incubation was continued for another 30 min. Fluorescence was recorded at 60 s interval. Means ± SEM were calculated for four individual experiments from the fluorescence ratios 0, 10, 15, 20 and 25 min after addition of 30 μl saline solution or glutamate solution. *Statistically significant ($P<0.05$) difference from all other groups at the same recording time.
From Li et al. (2011).

abundance is correlated with accumulative drug administration (Barley et al., 2009). ALDH1L1 codes for mitochondrial 10-formyltetrahydrofolate dehydrogenase, and it is a highly selective astrocytic marker (Cahoy et al., 2008). It is most highly expressed in pancreas, heart, and brain and may play an essential role in the distribution of one-carbon groups between cytosolic and mitochondrial cell compartments (Krupenko et al., 2010).

The concentrations of astrocytic proteins in blood or blood cells may have clinical significance. The expression of the 18-kDa translocator protein (TSPO), previously known as peripheral type benzodiazepine receptor (PBR), is in the brain restricted to astrocytes, and it is significantly decreased in platelets from anxiety-prone patients with bipolar depression (Abelli et al., 2010). The serum concentration of the protein S100B, which is highly enriched in astrocytes, is elevated in major depression and mania (but also in schizophrenia), and its concentration is reduced by antidepressive treatment (Schroeter et al., 2002; Schroeter and Steiner, 2009). S100B is increased to a greater extent in suicidal adolescents with psychosis or mood disorder than in non-suicidal adolescent patients (Falcone et al., 2010).

4.3.2. Other effects by anti-depressant or anti-bipolar drugs on astrocytes

Very few effects are identical after treatment with lithium, carbamazepine and valproic acid, and except for some effects in very immature neurons the identical changes have been reported in astrocytes. *Myo*-inositol is needed for re-synthesis of the membrane constituent phosphatidylinositide-4,5-bisphosphate (PIP_2) following its neurotransmitter-mediated hydrolysis by PLC to DAG and IP_3. This re-synthesis is essential to maintain the cell's ability to generate the second messengers DAG and IP_3 in response to a multitude of transmitters. Re-supply of *myo*-inositol depends on hydrolysis of IP_3 via inositol bisphosphate and inositol monophosphate to *myo*-inositol (a process which in brain is inhibited by lithium, but not by carbamazepine or valproic acid) and/or uptake of *myo*-inositol from extracellular fluid. Chronic administration of any of the 3 anti-bipolar drugs inhibits astrocytic uptake (Wolfson et al., 1998; Lubrich and van Calker, 1999), except at low *myo*-inositol concentrations, where the uptake may be increased (Wolfson et al., 2000). This dual effect might be explained by the presence of two inositol transporters in astrocytes: i) the high-affinity Na^+-dependent *myo*-inositol transporter (SMIT), which is inhibited at low pH, and ii) the lower-affinity H^+-dependent HMIT, which is inhibited at high pH. Based on the different effect of chronic treatment of astrocyte cultures with anti-bipolar at low and high extracellular concentrations of *myo*-inositol it was hypothesized that chronic anti-bipolar treatment might cause an intracellular alkalinization (Hertz et al., 2004). A gradually developing intracellular alkalinization during chronic exposure of cultured astrocytes to lithium, carbamazepine or valproic acid has, indeed, been established and

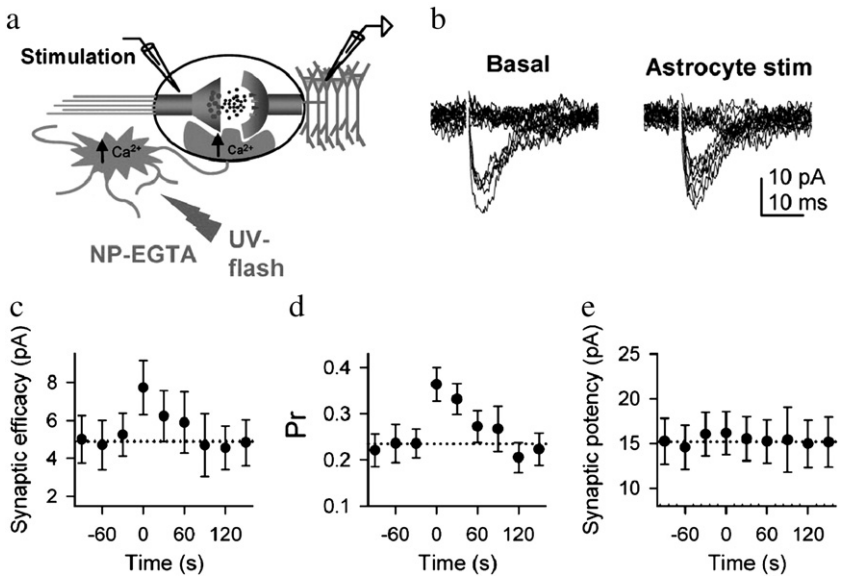

Fig. 7. Regulation of synaptic transmission by astrocytes. (a) Schematic drawing showing recordings from a single CA1 pyramidal neuron in response to stimulation of a Schaffer collateral under control conditions and after Ca^{2+} uncaging in a single neighboring astrocyte from injected *o*-nitrophenyl-EGTA (NP-EGTA) by UV flash photolysis. (b) An example of synaptic response to 15 consecutive stimuli before (Basal) and after astrocytic Ca^{2+} uncaging (Astrocyte Stim). Under basal conditions the successful rate of synaptic responses is 3, but this value is approximately doubled after Ca^{2+} uncaging in the neighboring astrocyte. (c) In 18 neuron-astrocyte pairs, synaptic efficacy (mean amplitude of all stimulations including those without postsynaptic response) increased transiently (but during a prolonged time) after uncaging (at 0 time) from 4.8 pA to ~7 pA immediately after uncaging. (d) This is due to an enhancement of the probability ratio (Pr) for a successful response increasing from 0.24 (corresponding to an average of 3.6 responses to 15 stimuli) to almost 0.40. In contrast, synaptic potency (mean amplitude of *successful* responses) is unaltered at ~15 pA (e). Thus astrocyte stimulation potentiates transmitter release.
Slightly modified from Perea and Araque (2007, 2010).

found to be caused by stimulation of the Na^+/H^+ exchanger NHE1 (by lithium) or of the Na^+/HCO_3^- co-transporter NBCe1 (by carbamazepine and valproic acid) (Song et al., 2008; Peng et al., in press). Inhibition of *myo*-inositol uptake may explain a decrease of noradrenaline-stimulated increase in $[Ca^{2+}]_i$ following chronic treatment of astrocyte cultures with 1 mM lithium for 7–14 days (Chen and Hertz, 1996). The effect of a potential concomitant reduction in noradrenaline-mediated 'gliotransmitter' release is not known, but could be important in bipolar patients during both depressive and manic phases. In this context it should also be mentioned that Gs/Gi switching in response to β-adrenergic stimulation is important in cultured astrocytes (Du et al., 2010).

Chronic administration of tricyclic antidepressants, as well as electroconvulsive treatment, induces a down-regulation of β-adrenergic receptor binding and function in animal brains (Vetulani et al., 1976). This response is mimicked in astrocyte cultures, where treatment with the tricyclic antidepressant amitriptyline (1 μM) or the monoamine oxidase inhibitor tranylcypromine (5 μM) for at least two weeks reduces the cAMP accumulation induced by isoproterenol by ~30% (Hertz and Richardson, 1983). On account of the cAMP-mediated inhibition of I_{sAHP} (see above), this reduction of β-adrenergic activity might have a similar anti-depressant effect as fluoxetine-mediated inhibition of metabotropic activity of GluK2. Fluoxetine does not cause any down-regulation of the adenylate cyclase system in vivo (Mishra et al., 1979).

Potent up-regulation of $cPLA_2$ expression after treatment of cultured astrocytes with any of the 5 conventional SSRIs (Zhang et al., 2010) is evoked by stimulation of 5-HT_{2B} receptors and requires $ERK_{1/2}$ phosphorylation (Li et al., 2009). It replicates a similar up-regulation in rat brain (Rao et al., 2006). $cPLA_2$ is the enzyme releasing arachidonic acid, which stimulates glucose metabolism in cultured astrocytes (Yu et al., 1993). A similar up-regulation in astrocytes in vivo might contribute to the repeatedly reported ability of SSRIs to normalize regional decreases in energy metabolism, which occur in brain metabolism during major depression (Buchsbaum et al., 1997; Mayberg et al., 2000; Kennedy et al., 2001; New et al., 2004). It is in support of this idea that plasma concentrations of arachidonic acid in depressed patients are linearly correlated with regional brain glucose metabolism (Sublette et al., 2009). Moreover, treatment with 10 μM fluoxetine for 24 h, which might have sufficed to induce an increase in $cPLA_2$, leads to an increase in rate of glycolysis (2-deoxyglucose phosphorylation) in cultured astrocytes (Allaman et al., 2011). Acute exposure of astrocyte cultures to the same concentration of fluoxetine has no corresponding effect (L. Peng and L. Hertz, unpublished experiments).

4.3.3. Summary

Postmortem morphology and expression of astrocyte-specific proteins indicate astrocytic reduction in brains from patients having suffered from major depression. Among the few common effects of chronic treatment with lithium, carbamazepine and valproic acid is also development of intracellular alkalinization, which in turn may modify uptake of *myo*-inositol and thus influence PLC-mediated signaling. Chronic treatment with fluoxetine increases glycolysis in astrocytes, which may contribute to normalization of regional deficits in energy metabolism during successful treatment of depressed patients with an SSRI.

5. Concluding remarks

Astrocytes account for ~20% of the volume in brain cortex and a comparable or perhaps slightly larger part of its energy metabolism (Hertz, 2008). It has long been known that they play key roles in normal brain functions, such as active uptake of increased extracellular glutamate (Danbolt, 2001) and K^+ (Walz, 2000, 2004; Somjen et al., 2008), and that they are essential for glutamate synthesis, amidation to glutamine, oxidative degradation and uptake (see above). More recently, astrocytic expression of a plethora of receptors for neurotransmitters has been shown (Hansson and Rönnbäck, 2004; Domingues et al., 2010) together with the ability of many neurotransmitters to induce increase in astrocytic $[Ca^{2+}]_i$ and thereby promote Ca^{2+}-dependent release of 'gliotransmitters', such as glutamate and ATP (Araque et al., 1998; Parpura et al., 2010). 'Gliotransmitter'-mediated glutamatergic signaling is suspected to play a role in learning (Pereira and Furlan, 2010) and in epilepsy (Sierra-Paredes and Sierra-Marcuño, 2007; Beed et al., 2009) and seems also to do so in chronic pain (Ren,

2010). The present observations suggest an astrocyte-dependent glutamatergic mechanism of action for medication ameliorating depressed mood, and they tentatively identify stabilization of neuronal afterhyperpolarization as a potential aim for the development of new, faster-acting antidepressant therapy. Similar drug-induced alteration in glutamatergic activity seemed to explain the anti-depressant effect, regardless whether the depressive state was evoked by a depressive phase in bipolar disorder or by major depression. In the first case the GluK2 receptor was downregulated, and in the second it was upregulated and further edited.

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