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Stress and corticosteroid modulation of seizures and synaptic inhibition in the hippocampus

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ABSTRACT

The role of stress hormones in the initiation of epileptic seizures has been studied extensively in the past decade, with conflicting observations, from suppression to exacerbation of spontaneous seizures. We have now studied the effects of an acute stress on reactivity of juvenile rats to kainic acid (KA), which produces epileptic seizures. With a short (30 s) stress-KA delay, stress exacerbated epilepsy via activation of mineralocorticosterone receptors (MR). With a long (60 min) stress-KA delay, seizures were suppressed through activation of a glucocorticosterone receptor (GR). In a parallel study with CA1 pyramidal neurons in acute hippocampal slices, activation of MRs reduced the frequency of mIPSCs, whereas activation of GRs produced a slow onset, 2.5 fold increase in amplitudes of mIPSCs. GR effects were not mediated by protein synthesis, but did require activation of some protein kinases. These experiments suggest that stress can either facilitate or suppress seizures, in a time and receptor dependent manner.

Introduction

In epileptic patients, acute stress has been reported to exacerbate or even trigger an epileptic discharge (Frucht et al., 2000). Specifically, patients report that the frequency of their seizures increase if they are exposed to stress (Arida et al., 2009; Haut et al., 2003) and accordingly, emotional stress has so far been regarded as the most frequent self-reported seizure precipitant (Nakken et al., 2005). Numerous studies in experimental animals have attempted to provide a better understanding of the complex stress–epilepsy relations; however, both facilitating and suppressive effects of prior stress on epilepsy onset/intensity and subsequent neuronal damage have been reported (Joels, 2009). The reason for this inconsistency could be related to the fact that stress has profound time and region-selective effects on synaptic plasticity and cognitive functions associated with the hippocampus and other limbic structures (Garcia, 2002; Kim et al., 2006). Furthermore, corticosterone (Cort), the main stress hormone in rodents, activates two receptor types: the high affinity mineralocorticosteroid receptor (MR) and the low affinity glucocorticosteroid receptor (GR). Both receptors act at different time scales: a fast one via membrane receptors or a slow one, ranging from hours to days, via nuclear receptors. Even though the effects of Cort on intrinsic and synaptic properties of hippocampal neurons, acting via both genomic and nongenomic routes have been studied extensively in recent years (Joels, 2008), the distinction between the preferential actions at the two locations is not always clear. Needless to say, the effects of stress are more complex than just the release of steroid hormones, and central monoamines and other neuromodulators may affect the stress-induced alteration in seizure onset.

We have now examined the time course and receptor types involved in the effect of acute stress on kainic acid (KA)-induced seizures in the juvenile rat. Stress was found to have a profound effect on seizure onset and development, depending on the delay between the acute stress and the injection of KA. Furthermore, these effects are correlated with the effects of GRs and MRs on inhibitory synaptic currents in the hippocampal slice, suggesting that stress effects on seizure onset may be mediated by steroid regulation of inhibitory synapses in the hippocampus.

Material and methods

Stress

Experiments were approved by the Weizmann Institutional Animal Care and Use Committee in accordance with EU directives. 28 days old, prepubertal male Wistar rats were used (Jacobson-Pick and Richter-Levin, 2010; Lephart and Ojeda, 1990; Ojeda et al., 1980). The forced swim stress (FSS) protocol consisted of exposing a naive rat to a forced swim in a circular water tank for 15 min (diameter, 0.5 m; height, 0.5 m) (Maggio and Segal, 2007, 2009a, 2009b). Water depth was 40 cm, and temperature was maintained at 23 ± 1 °C. Stress exposure was always initiated at 9:00 A.M., when the plasma Cort levels are low (Finn et al., 2003; Krugers et al., 2005). An alternative stressful treatment, involving the placement of the rat on an elevated platform for

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30 min (Maggio and Segal, 2011), was used in selected experiments to verify that FSS is not unique in its action.

Induction of seizures

Kainic acid (KA, 10 mg/kg) was injected ip either 30 s or 1 h after exposure to the FSS. Similar to others (Dudek et al., 2006), the Racine's scale (Racine, 1972) was adapted to describe the different classes of behavioral seizure activity: Class I—Facial automatisms, Class II—head nodding, grooming and "wet-dog shakes", Class III—forelimb clonus and lordotic posture, Class IV—continuous forelimb clonus even when the animal rears, Class V—generalized tonic–clonic activity with loss of postural tone. Seizures were halted as soon as animals reached stage 5 (or sooner, if they did not reach it within 65 min) by injection of Diazepam (ip, 10 mg/kg).

Drugs

For the *in vivo* experiments, the following drugs were used: the MR antagonist spironolactone (20 mg/kg), the antiprogestinic and GR antagonist RU38486 (mifepristone, 20 mg/kg), the MR agonist aldosterone (100 μg/kg) and the GR agonist dexamethazone (100 μg/kg). Drugs were prepared from frozen stocks and injected ip 15 min prior to FSS. For the in-vitro experiments, drugs were superfused into the recording medium at known concentrations.

Slice procedures

Naïve male Wistar rats (28 days old of age) were rapidly decapitated and 400 μm horizontal hippocampal slices were prepared on a vibroslicer. Slices were incubated for 1.5 h in a humidified, carbogenated (5% CO2/95% O2) gas atmosphere at room temperature and were perfused with ACSF [containing (in mM) 124 NaCl, 2 KCl, 26 NaHCO3, 1.24 KH2PO4, 2.5 CaCl2, 2 MgSO4, and 10 glucose, pH 7.4]. Slices were then transferred to a recording chamber, placed on the stage of an Olympus BX51WI upright microscope and perfused with ACSF at a rate that allowed a given drug concentration to fully equilibrate within 6 min after onset of its perfusion. Single hippocampal CA1 neurons were visualized using infrared Nomarski optics and mIPSCs were recorded by perfusing the slices with DNQX (20 μM) APV (50 μM) and TTX (1 μM) while clamping neurons at −60 mV using a CsCl-based intracellular solution [containing (in mM): 136 CsCl, 10 KCl, 5 NaCl, 10 HEPES, 0.1 EGTA, 0.3 Na-GTP, 1 Mg-ATP, and 5 phosphocreatine, pH 7.2] to produce large inward currents carried by chloride ions that reversed at 0 mV. Bicuculline (20 μM) reliably blocked these synaptic currents. Signals were amplified with Axopatch 200A, digitized at 5KHz and recorded with PClamp-9 (Axon Instruments). Routinely, 5 min epochs of recordings were stored so as to accumulate sufficient mIPSCs for analysis.

Analysis

The behavioral data were summarized and analyzed off-line using Origin 8.0 Pro while the electrophysiological recordings were filtered and analyzed off-line using MiniAnalysis software (Synaptosoft) with a detection threshold of 7 pA. Noise analysis was performed on unfiltered data, 1 s recording epochs, using the RMS measurements in the noise analysis tool of MiniAnalysis software. This algorithm measures the root-mean-square noise level, and it was performed on a recording window that did not include any visible mIPSCs. Comparisons among groups were made using one or two way ANOVA, followed by Tukey's comparisons, as the case may be. Data are shown as mean ± SEM.

Results

Effects of prior stress on reactivity to KA

Control rats injected with KA developed full blown class V seizures over a period of 5–30 min (Figs. 1A and B) with a smooth transition

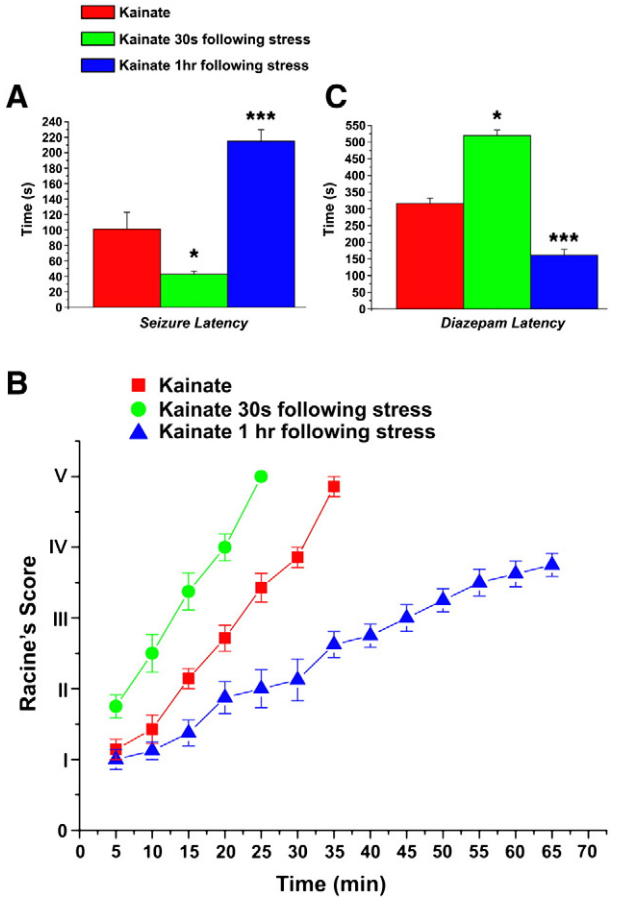

Fig. 1. Stress modulates seizures onset and severity in a time dependent manner. Injection of Kainic Acid (KA, 10 mg/kg) 30 s following FSS decreases seizure latency (A), worsens seizure progression (B) and reduces the efficacy of diazapam in halting seizures (C). Conversely, injection of KA 1 h following FSS increases seizure latency (A), reduces seizure progression (B) and increases the efficacy of Diazepam in arresting seizures. (n = 8 rats per group, see text for details), *p<0.05, ***p<0.001.

from class I (threshold of 101.2 s) to class V, reaching it within about 35 min at which point diazepam injection fully blocked the seizures within 5 min (Fig. 1C). The reactivity to KA was considerably enhanced when the drug was injected immediately following the swim stress: seizures threshold was reached within 42.3 s, while class V was seen in less than 25 min (Fig. 1B). In these rats, diazepam had a longer delay, reaching threshold at 500 s after the injection (Fig. 1C). Strikingly, a delay of 1 h between the stress and KA injection caused a significant slowing down of the appearance of the seizures, with a threshold time of 214.9 ± 15 s, and class V was not reached within the 65 min of observation time. The difference was statistically significant among groups ($p<0.001$, $F = 51.52$. *post hoc* Tukey's test revealed a significant difference between KA and 30 s following stress ($p<0.05$), between KA and 1 h following stress ($p<0.001$) and between 30 s following stress and 1 h following stress ($p<0.0001$)). On the other hand, diazepam was faster than controls to have an effect. The difference in diazepam action was statistically significant among groups ($p<0.001$, $F = 121.35$. *post hoc* Tukey's test revealed a significant difference between KA and 30 s following stress ($p<0.001$) and between KA and 1 h following stress ($p<0.001$, n = 8 rats in each group)).

Interestingly, the remarkable effects of stress on reactivity to KA are not specific to the type of stressor employed; when an elevated platform stress was used in another set of animals (N = 7 rats in each group, Fig. 2), the results were similar, ie. a short delay between the stressor and the injection of KA sped up the response to the drug, whereas a one-hour delay between the stress stimulus and KA prolonged considerably the onset of seizures.

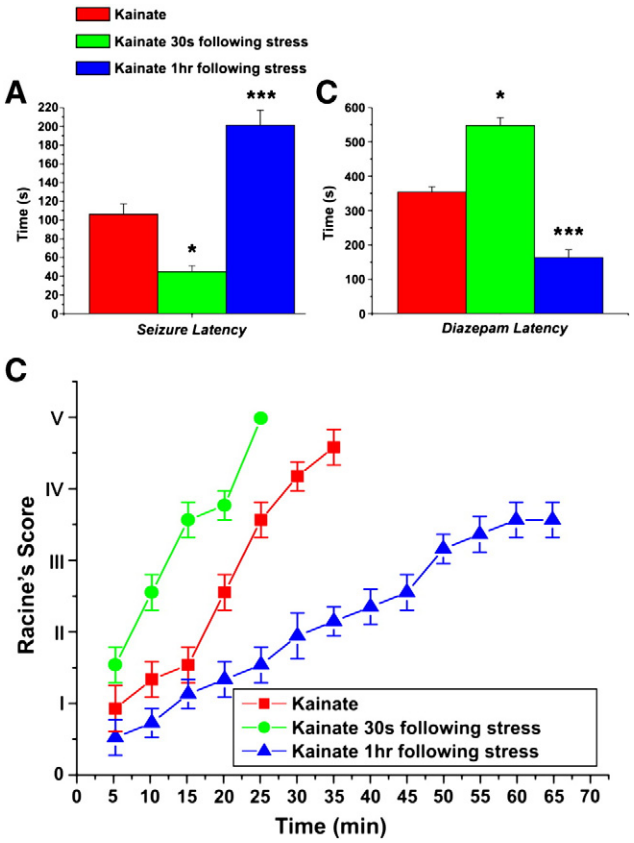

Fig. 2. Stress differently modulates seizures onset and severity independently of the type of protocol used for stress induction. Stress induced by an elevated platform protocol (30 min of exposure) shares similar effect on seizure modulation compared to stress induced by FSS. (n = 7 animals for each group), *p<0.05, ***p<0.001.

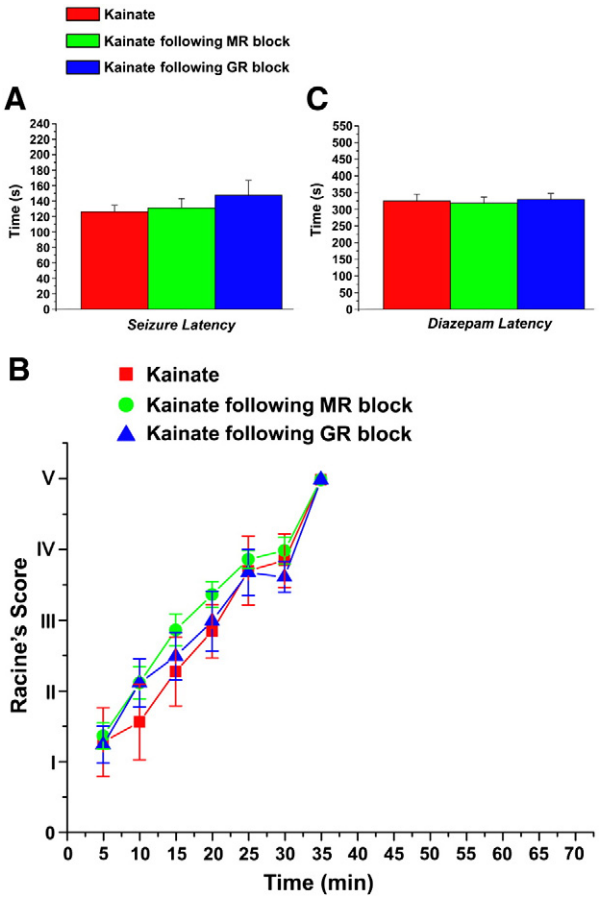

Fig. 3. Ambient corticosterone does not affect seizures. *In vivo* injection of either MRs or GRs antagonists in absence of FSS exposure does not cause a significant modulation in seizure latency (A), seizure progression (B) and diazepam latency (C). (n = 8 rats per group).

Differential modulation of stress effects by activation of MRs and GRs

To examine which of the two types of steroid hormone receptors, GRs and MRs, underlies the effects of stress on reactivity to KA, the respective antagonists were injected 15 min prior to the stress episode. Groups of 8 rats were used in this experiment. When injected alone, without being exposed to the FSS, there was no effects of either antagonist on latency to response to KA or to the effects of diazepam to block seizures (Fig. 3, Mean Values: KA 125.12 ± 8.50; spironolactone (MR blocker)—131.2 ± 12.1, mifepristone—(GR blocker)—147.6 ± 19.29. ANOVA, difference was not statistically significant among groups, F = 0.64248. At 20 min: KA 2.8 ± 0.37, spironolactone 3.37 ± 0.18, mifepristone 3 ± 0.42. ANOVA was not statistically significant among groups p = 0.275, F = 1.38. With respect to the effects of diazepam, KA 324.7 ± 20.18, spironolactone 318 ± 18.40, mifepristone 329.28 ± 17.84. ANOVA was not statistically significant among groups F = 0.105.)

In sharp contrast, blockade of either MRs or GRs by their respective antagonists had a profound effect on the FSS modulation of the response to KA (Fig. 4). MRs blockade prolonged the latency of response to KA, and slowed down significantly the progress of the response, to the extent that none of the animals reached Class V of the Racine's score within the 65 minute observation time. This was evident for both the short and the long delay between the FSS and the KA injection. Surprisingly, GRs blockade had no effect in the short FSS-KA delay, but it reversed the prolongation of latency and duration of response to KA applied 1 h after FSS (Fig. 4B, KA 30 s following MR block + FSS 597.9 ± 54.9, KA 30 s following GR block + FSS 51.125 ± 4.94, KA 30 s following FSS 52.5 ± 4.84, KA 1 h following MR block + FSS 1311.77 ± 60.73, KA 1 h following GR block + FSS 80.9 ± 7.21, KA 1 h following stress 232.9 ± 14.91. Two way ANOVA with *post hoc* Tukey's test was used for comparison. The overall ANOVA detected a significant difference (p<0.0001; F = 121.69) and a significant interaction (P<0.0001; F = 55.23). *post hoc* Tukey's test revealed a significant difference between the different times KA was administered to the animals (p<0.001) and when looking at the different groups of animals a significant difference was detected between groups MR block and KA, p<0.05).

In a similar albeit more complex way, diazepam affected the recovery from KA seizures: MRs blockade reduced the latency of action of diazepam with both the short and the long FSS-KA delays. GRs blockade, on the other hand, prolonged the latency to the effect of diazepam, with both the short and the long FSS-KA delays. (Fig. 4C, KA 30 s following MR block + FSS 336.9 ± 27.22, KA 30 s following GR block + FSS 859.6 ± 23.88, KA 30 s following FSS 544.2 ± 23.30, KA 1 h following MR block + FSS 166.4 ± 8.20, KA 1 h following GR block + FSS 584.1 ± 21.29, KA 1 h following FSS 163.75 ± 20.50. Here a two way ANOVA with *post hoc* Tukey's test was used for comparison. The factors for comparisons were A—the time KA was administered to the animal (either 30s or 1 h following the stress) and B—the different animal groups. The overall ANOVA detected a significant interaction between Factor A and Factor B (F = 11.83 p<0.001). *post hoc* Tukey's test revealed a significant difference between the different times KA was administered to the animals, p<0.0001, and when looking at the different groups of animals a significant difference was detected between groups MR block and KA, p<0.01, and GR block and KA, p<0.001).

Agonist effects

In a complementary series of experiments, the Cort agonists, aldosterone (for MRs) and dexamethasone (for GRs) were injected to

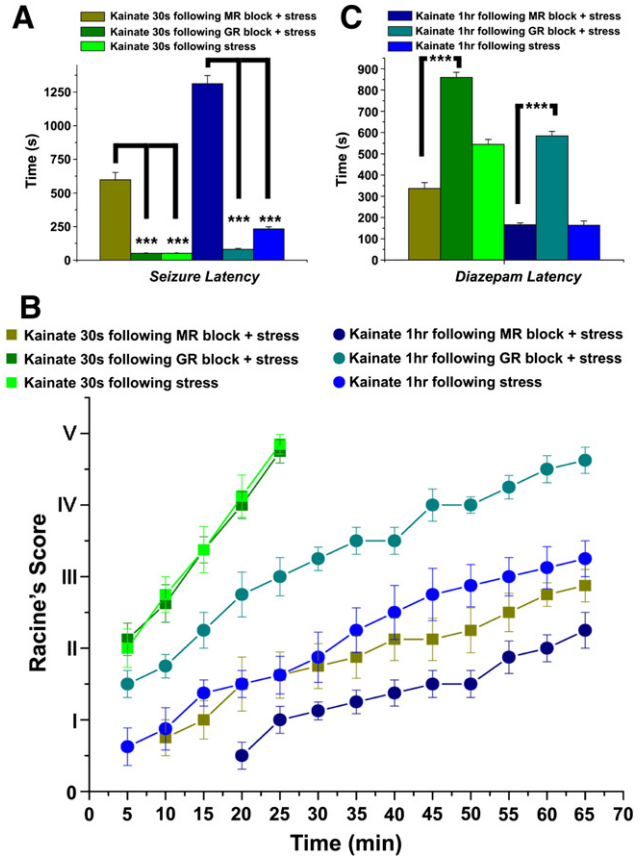

Fig. 4. Blockade of MRs or GRs prior to stress exposure differently modulate seizures. MR or GR antagonists were injected 15 min prior to FSS. Blockade of MRs increases seizure latency (A), reduces seizure progression (B) and enhances the efficacy of diazepam (C) when KA is injected either at 30s or at 1 h following FSS. GRs blockade prior to stress has a lower latency (A), higher progression (B) and responds less effectively to diazepam either 30 s or 1 h after stress (C). (n = 8 rats per group), ***p<0.001.

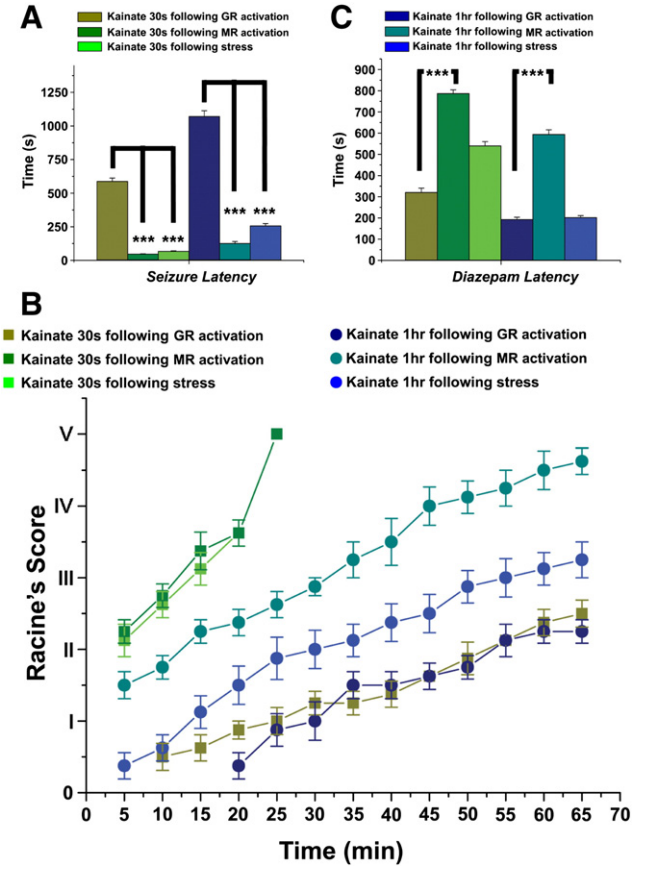

Fig. 5. MRs and GRs activation have different effects on seizures. Activation of GRs or MRs pharmacologically influences seizures in a different manner: Activation of GRs increases seizures latency (A), reduces seizure progression (B) and enhances the effects of diazepam (C). On the other hand, MRs activation lowers seizures onset, worsen seizure progression and lesser the effects of diazepam. (n = 8 animals for each group), ***p<0.001.

groups of 8 rats each, either 30 s or 60 min before KA injection, without exposing the rats to FSS (Fig. 5). Strikingly, GRs activation caused a delay in the response to KA, and a slowing down of the progression along the Racine scale, to the extent that these rats did not reach Class V within the 65 min observation time. In contrast, MRs activation by aldosterone sped up the response to KA, when applied at the short, 30 s interval before KA. Interestingly, MRs activation alone, when applied 60 min before KA, caused a delay in the development of the seizure, indicating that aldosterone by itself may have a dual action on the progression of epilepsy (Fig. 5B, Mean values at 20 min: KA 30 s following GR activation 0.87 ± 0.125, KA 30 s following MR activation 3.62 ± 0.18, KA 30 s following stress 3.62 ± 0.18; KA 1 h following GR activation 0.37 ± 0.21, KA 1 h following MR activation 2.37 ± 0.18, KA 1 h following stress 1.5 ± 0.26. 2-Way ANOVA using for comparisons factor A—the time KA was administered to the animal (either 30 s or 1 h following the stress) and factor B—the different animal groups, detected a significant effect for Factor A ($p<0.0001$, $F=67.94$) and for Factor B ($p<0.0001$, $F=86.75$) and a significant interaction ($P<0.001$; $F=8.97$). *post hoc* Tukey's test revealed a significant difference between the different times KA was administered to the animals, $p<0.0001$, and when looking at the different groups of animals a significant difference was detected between MR activation and KA, $p<0.05$). As seen before, the suppressive effect of diazepam on the KA-induced seizure is markedly affected by the priming stimulation, either stress or an injection of MRs/GRs agonists. Thus, activation of MRs delayed the action of diazepam, whereas activation of GRs sped up the action of diazepam. This was true for both the short delay between the agonist and KA, and the long delay (60 min) where the addition of dexamethasone acted like the exposure to FSS, which shortened the time to recovery from KA (Fig. 5C; Mean values: KA 30 s following GR activation 320.5 ± 20.03, KA 30 s following MR activation 786.37 ± 17.70, KA 30 s following stress 540.0 ± 20.03; KA 1 h following GR activation 192.75 ± 10.61, KA 1 h following MR activation 593.6 ± 22.24, KA 1 h following stress 201.6 ± 9.59. Here a two way ANOVA with *post hoc* Tukey's test was used for comparison. Once again, the factors for comparisons were A—the time KA was administered to the animal (either 30 s or 1 h following the stress) and B—the different animal groups. The overall ANOVA detected a significant interaction between Factor A and Factor B ($P<0.001$; $F=23.32$). *post hoc* Tukey's test revealed a significant difference between the different times KA was administered to the animals $p<0.0001$, and a significant difference was found between MR activation and KA, $p<0.05$).

To conclude this part of the study, stress, acting primarily via a fast MR, exacerbates the induction of epileptic seizures by KA, whereas its action through a slow GR, suppresses the induction of epileptic seizures. The relative contributions of the two receptors type is time dependent, in that MRs act rather fast, while GRs are slow to act, as predicted elsewhere (Maggio and Segal, 2009a, 2009b).

Corticosterone effects on inhibitory synaptic currents

Pyramidal cells of CA1 region of the hippocampus were patch-clamped at −60 mV, and spontaneous miniature IPSCs (mIPSCs) were recorded before and during perfusion of the slice with Cort (100 nM) or other agonists/antagonists (n = 14 cells). Two simultaneous effects were observed; a rapid (within 5–10 min) reduction in the frequency of mIPSC, which went down from 31.2 ± 1.16

events/2 min to 24.35 ± 0.65 at 25 min after onset of perfusion, to 24.07 ± 0.75 at 45 min (Fig. 6, ANOVA showed a significant difference among different time points, F = 1689.4, p<0.001. *Post hoc* Tukey's test for pair wise comparisons: a significant difference between pre 5 and post 5 (p<0.01), post 15, post 25, post 35, post 45, post 55 (p<0.001)). Simultaneously, there was a slow onset, and long duration of increase in mIPSCs amplitudes, which went up from 10.71 ± 1.1 pA in control condition, to 19.12 ± 2.05 pA 25 min after onset of Cort perfusion, to 28.9 ± 1.48 pA at 45 min after onset of perfusion, when in fact Cort was already perfused out of the slice. (Fig. 6A, one way repeated measure ANOVA was used for the analysis using different time points as a factor. The overall ANOVA detected a significant difference among different time points, F = 843.0, p<0.001. *Post hoc* Tukey's test was used for pairwise comparisons: a significant difference was detected between pre 5 and post 35, post 45, post 55 (p<0.001). There were no differences in the rise time and decay of the mIPSCs following exposure to Cort (data not shown).

The possibility that the two effects of Cort, i.e. the increase in mIPSCs amplitudes and the reduction in mIPSCs frequency (in the different time courses) are mediated by different steroid hormone receptors was then examined. To test for the involvement of GRs, the slices were either perfused with the GR agonist dexamethasone (100 nM), or with Cort (100 nM), in the presence of the MR antagonist spironolactone (500 nM). On the other hand, the MRs involvement was tested using either the MR agonist aldosterone (10 nM), or following perfusion with Cort (100 nM), in presence of the GR antagonist mifepristone (500 nM). Strikingly, activation of GRs mimicked the effects of Cort to increase the size of averaged mIPSCs (Fig. 7A, Control 11.8 ± 0.23pA, n = 14, MR + 13.0 ± 0.20, Cort (−GR) 12.97 ± 0.19, GR + 35.91 ± 0.63, Cort (−MR) 36.9 ± 0.59.

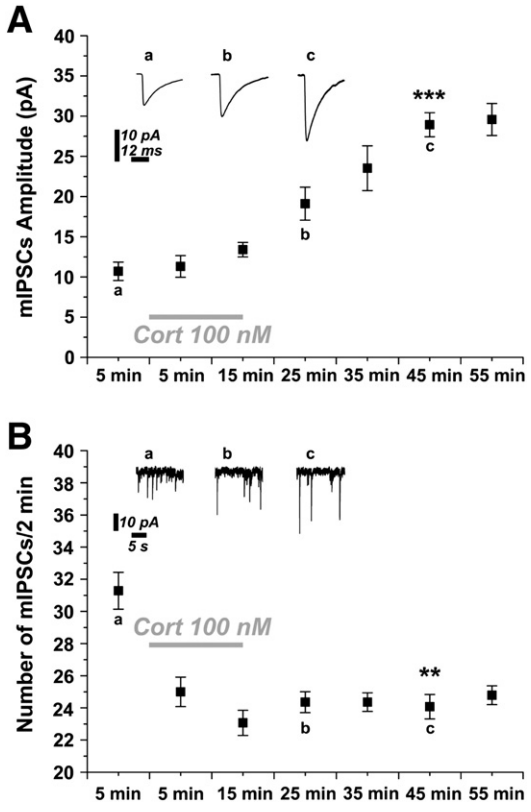

Fig. 6. Corticosterone increases mIPSCs amplitude while decreases mIPSCs frequency. Clamping neurons for >1 h showed that a short application (10 min) of Cort (Cort, 100 nM) (A) slowly changes mIPSCs amplitude while (B), simultaneously affecting rather fast mIPSCs frequency (5 min before Cort application, 31.2 ± 1.16 pA; 35 min after 10 min Cort application, 24.07 ± 0.75 pA; $n = 14$ cells, $p<0.001$). Representative traces (a, b, c) at indicated times are shown on top, **p<0.01; ***p<0.001.

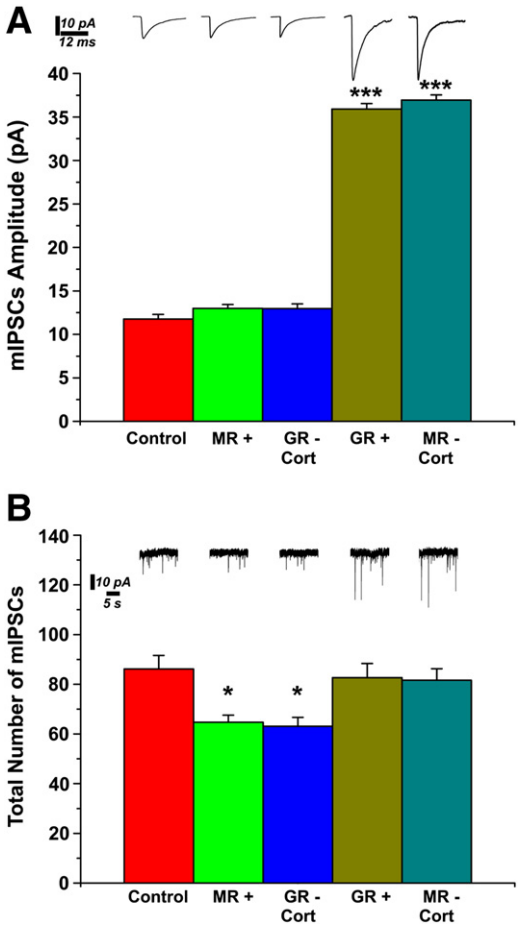

Fig. 7. GRs increase mIPSCs amplitude, and MRs decrease mIPSCs frequency. A: Activation of GRs by Dexa (100 nM) or Cort (100 nM) plus Spiro (500 nM), to block MR, mimicked the increase in mIPSCs amplitude mediated by Cort. Activation of MRs by Aldo (10 nM) or Cort (100 nM) plus RU (500 nM, to block GR) did not affect the amplitude of mIPSCs. Representative traces are shown on top, n = 14 cells per each group. B: Activation of MRs by Aldo (10 nM) or RU (500 nM) plus Cort (100 nM) significantly suppressed mIPSCs frequency while activation of GRs by Dexa (100 nM) or Cort (100 nM) plus Spiro (500 nM) did not have any effect on mIPSCs frequency. Representative traces are shown on top, n = 14 cells per each group, *p<0.05, ***p<0.001.

One way repeated measurements ANOVA detected a significant difference among treatments, F = 34.25, p<0.0001. *Post hoc* Tukey's test was used for pair wise comparisons: a significant difference was detected between Control, MR + and Cort (−GR) against GR + and Cort(−MR) (p<0.0001)), while the activation of MR had no such effect. Conversely, activation of MRs caused a reduction in frequency of mIPSCs, with no similar effects of activation of GRs (Fig. 7B, Control 86.14 ± 5.43, MR + 64.71 ± 2.86, Cort (−GR) 63.07 ± 3.59, GR + 82.64 ± 5.73, Cort (−MR) 81.64 ± 4.62. One way repeated measure ANOVA detected a significant difference among different treatments, F = 2.34, p<0.05. *Post hoc* Tukey's test for pair comparisons: a significant difference was detected between MR + and GR-Cort against Control, GR + and Cort (−MR) (p<0.05)).

These *in-vitro* experiments complement our behavioral experiments to suggest that Cort acts via activation of fast MRs to suppress GABAergic inhibition and increase excitability of the hippocampus, hence facilitate the epileptic action of KA, whereas the slow activation of GRs enhances GABAergic neurotransmission, and suppresses the ability of KA to cause seizures.

Molecular mechanisms of GRs

Having established a potent action of GRs on mIPSCs amplitudes in CA1 neurons of the hippocampus, we then explored possible

mechanisms mediating this effect. First, we established the slow time course of activation (Fig. 8A) also with the selective GR agonist dexamethasone (Dexa, 30 nM). As in the case with Cort, Dexa generated a slow rise in magnitude of mIPSCs that reached more than 2 fold increase over basal values some 30 min after application of the drug.

Unlike Cort, this effect was not accompanied by a change in frequency of mIPSCs (Fig. 8B). We then focused on Dexa as a selective agonist of the GRs.

To examine the possible involvement of nuclear receptors and subsequently, protein synthesis in the slow action of Dexa, slices were exposed to cycloheximide (Cyclo), prior to and simultaneously with Dexa. There was no apparent effect of blockade of protein synthesis on the action of Dexa to increase magnitudes of mIPSCs, in 14 cells tested (Fig. 9). On the other hand, blockade of protein kinase C, either with the selective antagonist GFx, or the less-selective antagonist H7, completely eliminated the effects of Dexa, while not affecting the basal magnitudes or frequencies of mIPSCs in the cells tested (14 cells in each group). These experiments indicate that Dexa exerts its action by interacting with protein kinase C, presumably downstream to the binding to membrane GRs.

Finally, in a previous study we noticed that low concentrations of GABA (5 μM) can increase membrane noise, and that Dexa counteracts this effect of GABA to reduce noise level back to control (Maggio and Segal, 2009a, 2009b). We have now expanded these observations (Fig. 10) to further explore the possibility that the agents that blocked the effects of Dexa on mIPSCs, also affect the changes in membrane noise produced by GABA. To this end, we exposed the slice to GABA, causing a rise in membrane noise from 4.95 ± 0.27 pA (RMS, Fig. 10A) to 7.97 ± 0.29 pA ($p<0.001$). In the same cells, mIPSC amplitudes went up from 12.57 ± 0.99 pA to 30.28 ± 1.15 pA ($p<0.001$). In the presence of Dexa, GABA-induced membrane noise went back down significantly to near control values of 5.96 ± 0.32 pA ($p<0.001$). Likewise,

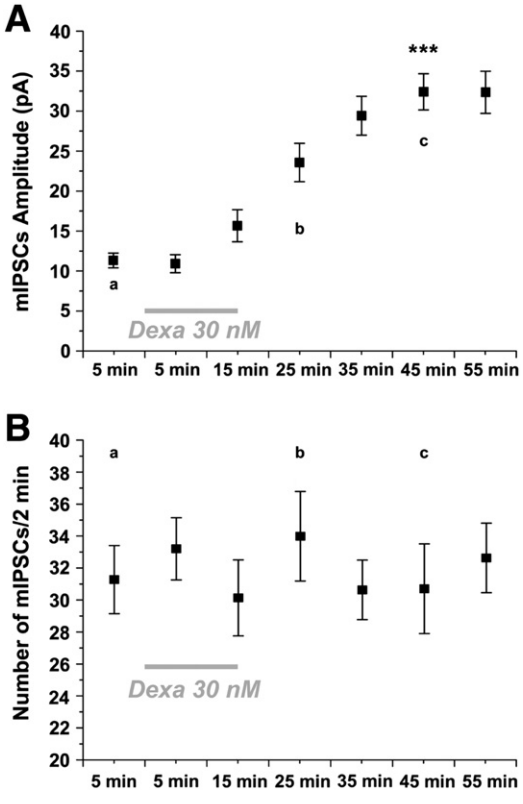

Fig. 8. Dexamethasone effects on mIPSCs. A low dose, short (10 min) application of Dexa produces a slow rising increase in mIPSC amplitudes, reaching a peak at least 20 min after washout of the drug (A), without a systematic effect on mIPSC frequencies (B) (n = 14 cells recorded for > 1 h each, ***$p<0.001$).

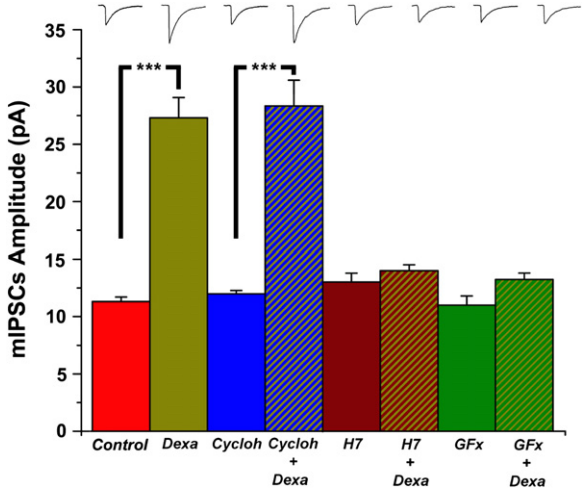

Fig. 9. The effects of Dexa on mIPSCs is not dependent on protein synthesis but on activation of protein kinase C. The presence of PKC antagonists H7 and GFx but not the protein synthesis inhibitor Cycloheximide blocked the Dexa-induced enhancement of mIPSC amplitudes. Representative traces shown on each bar, correspond to a single mIPSC recorded under this condition. N = 14 cells for each group, *** $p<0.001$.

mIPSCs amplitude, which by itself was enhanced by the presence of GABA, went up to a record high of 75.71 ± 2.11 pA (Fig. 10B). Interestingly, in the presence of cycloheximide, Dexa was as effective as in control conditions. In contrast, both H7 and GFx blocked the effects of Dexa on membrane noise, as well as on mIPSCs amplitudes, as seen above. Finally, the effects of Dexa on membrane noise are likely to be mediated by an interaction with the GABA receptor: in the presence of bicuculline, the averaged membrane noise went down from 4.95 ± 0.27 pA to 3.77 ± 0.23 pA (significant difference, $p<0.001$), but Dexa was not able to reduce membrane noise even further. In the same cells, the presence of bicuculline blocked all mIPSCs.

These experiments demonstrate that Dexa has a dual action, to increase the size of mIPSCs, probably by adding GABA receptors to the postsynaptic membrane of the inhibitory synapse, and to decrease GABA-associated membrane noise, probably by reducing the number of non-synaptic GABA receptors. Whether these two actions are two aspects of the same effect is subject to further experimentations.

Discussion

The present results demonstrate that an acute stress can have a complex effect on the onset and progression of epileptic seizure caused by an injection of KA. Typically, KA triggers the rapid development of seizures, which can result in death of the affected animal, if the seizure is not halted by enhancing GABAergic inhibition with diazepam. Exposure of the rat to a stressful stimulation, e.g. an inescapable swim in a water bucket, can have either a facilitatory action towards epilepsy, if KA is applied immediately after the stressful stimulation, or a suppressive action, if the drug is applied 1 h after the stressful stimulation. Further pharmacological experiments using selective agonists and antagonists indicate that the initial facilitatory action is likely to be mediated by activation of MRs, whereas the later suppressive action of stress is mediated by activation of GRs. It was hypothesized that these effects are likely to be mediated by steroid modulation of GABAergic neurotransmission, and indeed, the in-vitro experiments confirmed both the opposite effects of GRs and MRs on mIPSCs, as well as the difference in their time course of action, such that the MR effect is immediate, whereas the GR effect takes at least 30 min to develop. Furthermore, GRs activation affected mIPSCs size but not frequency, indicating a postsynaptic locus of action, and MRs activation affects frequency but not size of mIPSCs, indicating a presynaptic locus. Thus, it is likely that the two steroid

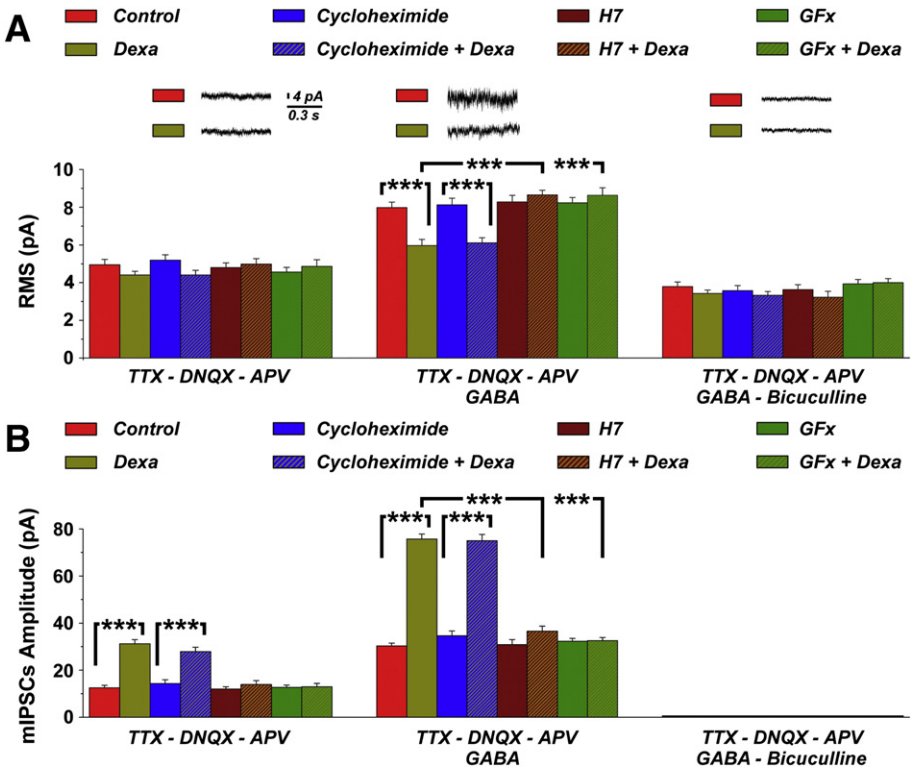

Fig. 10. Dual effect of Dexa on GABA-associated membrane noise and mIPSCs is mediated by the same mechanism. 5 μM GABA induced an increase in both membrane noise (*A*) and in miniature synaptic currents (*B*). Treatment with Dexa prevented the GABA-induced increase in noise while it amplified even further the GABA-mediated increase in synaptic currents. However Dexa in presence of Protein kinase C inhibitors failed both in suppressing GABA-induced increase in membrane noise and in increasing mIPSCs amplitude, $n = 14$ cells per group, $^{***}p < 0.001$.

receptor types are linked to different molecular mechanisms that eventually lead to changes in neuronal excitability.

Inconsistent effects of stress and Cort on reactivity of the hippocampus to neurotoxic insults, including KA and the GABAA antagonist picrotoxin, have been reported in the past decade. The measured variables include seizures threshold as well as subsequent cell death in the hippocampus. The steroid hormone was elevated by swim stress, or by manipulating the levels and the receptors for the steroid hormones. In some experiments the exposure to steroids was long lasting, and in only a handful of experiments were the effects of an acute stress on sensitivity to KA measured. In one such study, a 15 minute delay between the swim stress and the injection of the convulsant drug was used (Pericic et al., 2000) to confirm that swim does have an anticonvulsant effect, even though it is not clear if Cort is involved in this protective action. On the other hand, a 3 day elevation of Cort potentiated hippocampal damage following infusion of KA, in a strain-dependent manner (Scaccianoce et al., 1997). More recently, a protective effect of chronic Cort application on KA toxicity was reported (Benkovic et al., 2009). Once again, a 3 day overexposure to Cort potentiated the responses to KA, and the effect was mediated by activation of GRs (Talmi et al., 1995). In yet another study, stress or Cort exacerbated the neuronal cell loss caused by KA (Stein-Behrens et al., 1994). Another recent study examined the effects of an elevated platform stress on recurrence of seizures, to find that stress inhibited seizures in a time dependent manner, being effective with a 30–120 minute delay, but not with a longer, 4 hour delay (Zhou et al., 2010). These and similar studies were extensively reviewed in a recent article by Joels (2009).

Altogether, these studies do not provide a coherent picture on the stress–seizure relations, as they differ in the type of stressor, the duration of exposure to the stressor, the stress/neurotoxic delay, and the parameters studied (severity of epilepsy, neuronal damage). Our study attempted to clarify these relations by varying the stress/toxic delay and using specific activation of MRs and GRs. Based on our results, it is quite clear that MRs exacerbate epilepsy triggered by KA, whereas GRs suppress them, and that the two actions are sequential, MRs being fast, and GRs being slow. This fits with the general scheme of the effects of Cort, released during a stressful experience, on excitability of the hippocampus.

Less clear is what might be the molecular mechanisms activated by Cort to produce this dual action. Two general mechanisms should be considered, involving manipulation of excitatory glutamatergic or inhibitory GABAergic neurotransmission. Evidence for an effect of Cort on excitatory neurotransmission has been accumulated recently (Groc et al., 2008; Karst and Joels, 2005; Wang and Wang, 2009). However, previous studies (Orchinik et al., 2001), our recent results (Maggio and Segal, 2009a, 2009b), and the current one indicate that the GABAergic system is a more likely candidate for the modulation by steroid hormones of neuronal excitability in the hippocampus. In addition to the direct effects of steroid hormones on GABAergic synaptic currents, the striking effects of diazepam to stop the seizure, and do it in a differential manner following the two time delays after the stress indicates that the GABAergic system, which is affected by diazepam, is having a critical role in regulation of stress induced alteration in seizure progression.

While the differential effects of GRs and MRs activation, as well as their different kinetics are still being explored, it is quite clear that the MRs effects are membrane-associated, whereas the GRs effects are traditionally assumed to be mediated by nuclear receptors. Still, both GRs and MRs have membrane and nuclear receptors, and the possibility that they exert different effects in different parts of the brain has also been proposed recently (Segal et al., 2010). In the present study, we demonstrate that even the slow action of GRs is not mediated by protein synthesis, (i.e. by nuclear receptors) but is likely to involve activation of protein kinase C, known to be associated with membrane receptors, of the GABA-A type (Chou et al., 2010). This being the case, it is not entirely

clear why the effects of Dexa take so much time to develop (20–30 min). One of the most exciting possibility was alluded to in the last series of experiments, where membrane noise was analyzed. In these studies, the effects of Dexa on GABA associated increase in membrane noise paralleled its effect on size of mIPSCs, indicating that it is possible that non-synaptic GABA receptors, known to be rather mobile, move into synaptic locations, thus reducing GABA-associated membrane noise, while enhancing spontaneous GABAergic synaptic activity. It is still unclear which of the several subtypes of GABA receptors are diffusing into the synaptic location, as they are assumed to constitute different entities (Belelli et al., 2009), and further experiments are needed to explore these possibilities.

The effects of GRs activation in slowing seizures are indeed impressive. It has been previously hypothesized that steroids activation could perhaps influence seizures' threshold by either reducing vasogenic edema (Soffietti et al., 2002; Wen and Marks, 2002) or by stabilizing the Blood Brain Barrier (Kim et al., 2008), thus preventing the contact of epileptogenic substances with the brain tissue (Shlosberg et al., 2010). Here, however, we show that GRs enhance the amplitude of GABAA currents. This finding might bear important therapeutical implications. It has been proposed that a reduced surface expression of GABAA receptors (GABAARs) contributes to the pathogenesis of status epilepticus (SE) (Goodkin et al., 2007, 2008; Terunuma et al., 2008). As well, SE has been shown to induce altered expression of individual GABAARs subunits and to modify assembly of GABAARs (Sperk, 2007). Those changes have been assumed to result in altered GABAergic transmission leading to increased seizure susceptibility and reduced sensitivity of drugs acting on GABAergic transmission (Sperk, 2007). Based on previous evidence (Maggio and Segal, 2009a, 2009b), GRs might amplify the amplitude of GABAARs currents by increasing the number of receptors at the synapse, probably through the recruitment of extrasynaptic GABAARs. In this instance, therefore, GRs could both reduce the severity of seizures and increase the efficacy of GABAARs agonists (such as diazepam in our experiments) in being more effective to halt seizures. Even though the mechanisms by which GRs regulate GABAARs trafficking at the synapse are needed to be further explored, it is tempting to propose a therapeutic role of GRs in SE: administering GRs agonists during SE might enhance the beneficial effects of GABAARs agonists thus facilitating the arrest of prolonged seizures.

Conflict of interest

The authors declare no financial conflict of interest.

Acknowledgments

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