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Changing effect of GABA B receptor antagonist CGP46381 after status epilepticus in immature rats

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ABSTRACT

Possible proconvulsant action of $GABA_B$ receptor antagonist CGP46381 was studied 3 and 13 days after status epilepticus elicited in 12-day-old rats. GABAA-dependent activity was tested by pentylenetetrazol administration and found different in 15-day-old rats after status epiolepticus but not in the older group. The interaction of the two GABAergic systems should be studied in detail.

1. Introduction

Convulsive status epilepticus (SE) leads often to spontaneous seizures, i.e.to epilepsy. Changes underlying the transition from SE to spontaneous seizures (epileptogenesis) probably start very early after the arrest of SE, i.e.during a silent period (Curia et al., 2014). These changes might be studied in experimental animals – silent period to the first spontaneous seizures lasts 2–3 weeks in adult rats (Rattka et al., 2011), in immature rats even longer (Nehlig et al., 2002; Kubová and Mareš, 2013). Among changes in neurotransmitter systems inhibitory $GABA_A$ as well as $GABA_B$ systems were demonstrated to be compromised during this period in adult animals (Mangan and Lothman, 1996; Mangan and Bertram, 1997; André et al., 2001). There are only sparse data on changes of neurotransmitter systems in immature brain and they are usually focused on the late phase of silent period (e.g. glutamatergic - Doriat et al., 1999a – and adenosinergic - Doriat et al., 1999b).

Long silent period in developing rats offers an opportunity to study changes in neurotransmitter systems in detail. After description of only moderate changes in glutamatergic excitatory system focused on NMDA receptors with NR2B subunit (Abbasova et al., 2018) we started to study inhibitory systems. As the main inhibitory system – GABAergic – is concerned we decided to use a simple method of possible potentiation of pentylenetetrazol effects by an antagonist of $GABA_B$ receptors CGP46381. Immature rats after lithium-pilocarpine SE elicited on postnatal day 12 were studied in two different groups at postnatal days 15 and/or 25.

2. Methods

2.1. Animals

Experiments were performed on male Wistar rat pups. Rats were housed in a controlled environment (temperature 22 ± 1 °C, humidity 50–60%, lights on 6 a.m. – 6 pm) with free access to food and water. All procedures involving animals and their care were conducted according to the ARRIVE guidelines https://www.nc3rs.org.uk/arrive-guidelines in compliance with national (Act No 246/1992 Coll.) and international laws and policies (EU Directive 2010/63/EU for animal experiments) and the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). The experimental protocol was approved by the Ethical Committee of the Czech Academy of Sciences (Approval No. 128/2013). Institute of Physiology possesses an NIH Statement of Compliance with Standards for Humane Care and Use of Laboratory Animals (# A5820-01 valid until 1/31/2019).

2.2. Status epilepticus

Pilocarpine model of SE in developing rats was repeatedly described (e.g. Cavalheiro et al., 1987; Hirsch et al., 1992; Curia et al., 2008) and its lithium-pilocarpine modification is routinely used in our laboratory (Kubová et al., 2000; Suchomelová et al., 2002). Nine litters with 10 male pups were divided in status (N = 5–6) and control (N = 4–5) animals. All rats were pretreated with lithium chloride (127 mg/kg i.p.) at postnatal day 11 and 24 h later SE was elicited by pilocarpine (40 mg/kg i.p.) in 42 rats (SE groups). First signs of pilocarpine action

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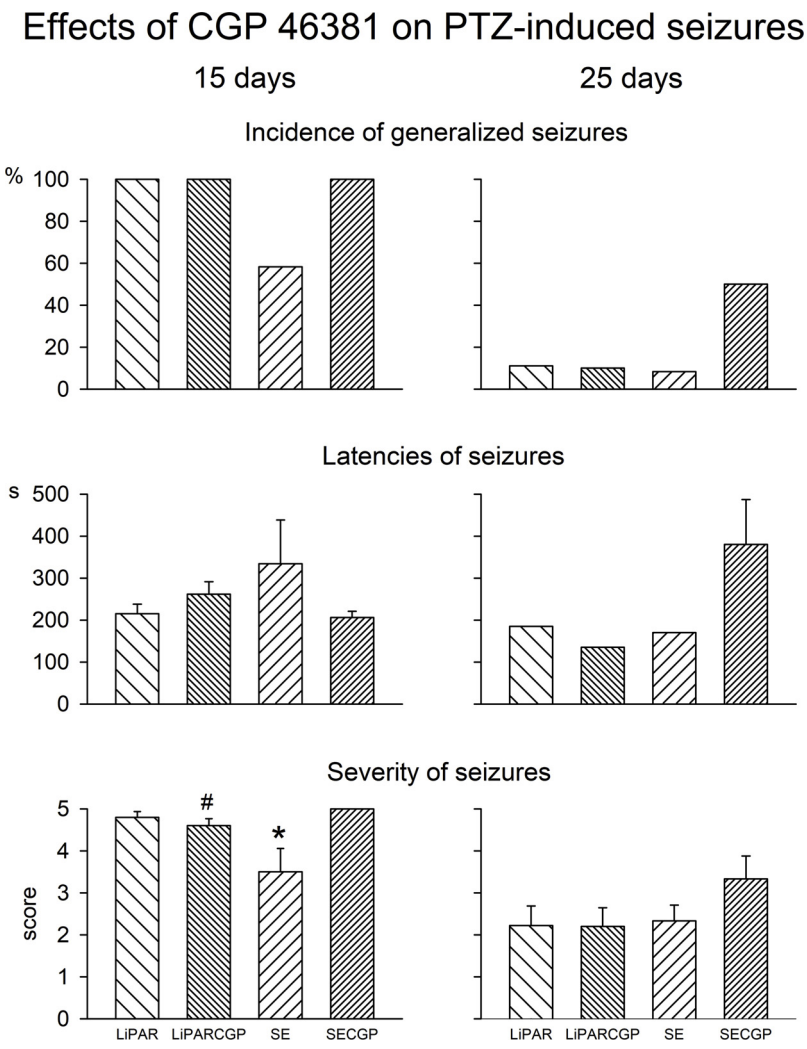

Fig. 1. Effects of CGP46381 on PTZ-induced generalized seizures. Left column: 15-day-old rats; right column: 25-day-old rats. From top to bottom: Incidence of generalized seizures; latencies of generalized seizures; seizure severity. X-axis in individual graphs from left to right: LiPAR group with PTZ only; LiPAR group pretreated with CGP46381; SE group with PTZ only; SE group pretreated with CGP46381. Y-axis in graphs from top to bottom: percent of animals exhibiting generalized seizures; latencies of generalized seizures; score expressing severity of seizures. Asterisk denotes significant difference between SE and SECGP groups, double cross between LiPARCGP and SECGP groups.

were epileptic automatisms (mostly scratching movements without contact with skin) appearing a few minutes after pilocarpine injection. Clonic seizures (stage 3 according to Racine 1972) started between 12 and 15 min after pilocarpine and then quickly progressed into sustained seizure activity characterized by Racine stages 3 and 4. Status was interrupted after 90 min of continuous convulsions by an injection of paraldehyde (0.07 ml/kg i.p.), then the animals were given 0.5 ml of physiological saline subcutaneously and at the moment they reacted to a light touch (usually 30–40 min) they were returned to their mother. Control animals (total N = 43; LiPAR groups) differed from SE rats only by replacement of pilocarpine by saline injection. They stayed out of the nest for the same time as SE pups. During the whole period rat pups were placed in plexiglass cages on the plate heated electrically to 34 °C.

Individual age and treatment groups were formed by 9–12 animals from at least two litters, each animal was used only once.

2.3. Pentylenetetrazol testing

Two age groups of rats were tested in this study – 15- and 25-day-old ones (i.e. 3 and 13 days after SE). Low dose of pentylenetetrazol (Sigma Aldrich, 60 mg/kg s.c.) was combined with a pretreatment with an antagonist of $GABA_B$ receptors CGP46381 (Tocris, 10 mg/kg i.p.). Interval between the two injections was 30 min. Rats were pretreated with CGP48361 after SE (SECGP) or lithium control administration (LiPARCGP). Animals were put individually into plexiglass boxes (again on a plate heated to 34 °C) and observed for 30 min after PTZ injection. Incidence and duration of minimal clonic and generalized tonic-clonic seizures as well as abnormal behavior were registered. Severity of seizures was expressed as a score using a 5-point scale (Pohl and Mareš, 1987).

2.4. Statistics

Statistics was calculated with SigmaStat program (SYSTAT). It started with a test of distribution of data and according to the result parametric (t-test, ANOVA) or nonparametric (Kruskall-Wallis, ANOVA on Ranks) tests were recommended. Holm-Sidak test was used as a posthoc test. Level of significance was put on 5%.

3. Results

3.1. Pentylenetetrazol action

Efficacy of the 60-mg/kg dose of PTZ was higher in 15- than in 25-day-old rats in both SE and LiPAR groups (Fig. 1). Animals receiving only PTZ exhibited generalized tonic clonic seizures in all 15-day-old LiPAR animals and in 7 out of 12 SE rats. The 25-day-old rats were more resistant to PTZ-induced generalized seizures, they were observed in only one animal in either group. Minimal clonic seizures (i.e.classical minimal metrazol seizures without a loss of righting ability) appeared only exceptionally in either group of 15-day-old rats and in 3 LiPAR and 6 SE rats 25 days old.

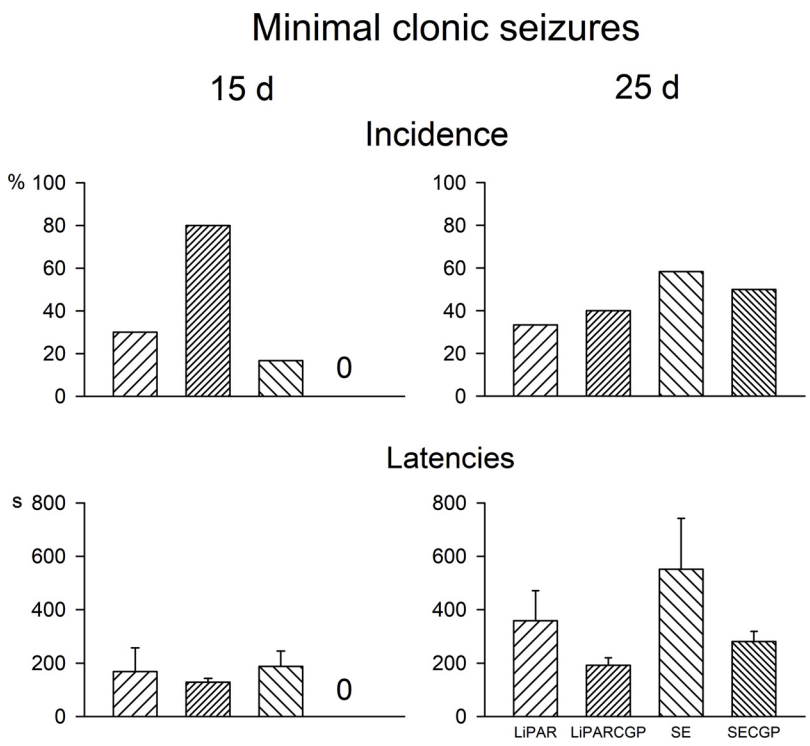

Fig. 2. Effects of CGP46381 on minimal clonic seizures.
Left column: 15-day-old rats; right column: 25-day-old rats. Top row: Incidence of minimal seizures; bottom row: latencies of minimal seizures. 0 means that this type of seizures was not present. There were no significant differences in this graph.

3.2. Effects of CGP46381

CGP46381 pretreatment resulted in generalized seizures in all 10 SE as well as LiPAR control 15-day-old rats but the tendency in the SE animals did not reach the level of statistical significance. Latency of generalized seizures tended to be shorter after CGP46381 pretreatment in SE animals but again the level of significance was not reached. Severity of seizures significantly increased in SE animals due to pretreatment with CGP46381 from 3.5 ± 0.56 to 5.0, this difference was significant ($p = 0.024$). In the LiPARCGP group two animals did not exhibit tonic phase of generalized seizures therefore severity of seizures in this group was significantly lower than in SECGP group ($p = 0.025$). Incidence of minimal clonic seizures was not significantly changed by CGP46381, the tendency to an increase in the LiPARCGP group (8 out of 10 rats) did not reach the level of statistical significance (Fig. 2). Latencies of seizures in the four groups of 15-day-old rats did not differ; when the seizures started, they lasted till the end of observation period (30 min).

Generalized seizures were observed in only one rat in SE and/or LiPAR groups in the 25-day-old animals. CGP46381 pretreatment increased the incidence of generalized seizures to 50% of animals after SE, no change was found in LiPAR control rats (Fig.1). Latency of generalized seizures could not be statistically evaluated, only the SECGP group exhibited these seizures in more than one animal. Severity of seizures was not significantly changed in SE or LiPAR animals, even an outlined difference between SE and SECGP groups did not reach the level of significance ($p = 0.143$). Incidence of minimal clonic seizures was not changed by CGP46381, their latencies tended to be shorter in animals pretreated with CGP46381 but due to a high variability in nonCGP groups the differences stayed below the level of statistical significance (Fig.2).

4. Discussion

There are two factors to be taken into account in the discussion. At first age-dependent change of proconvulsant action of CGP46381 which is more expressed in younger than in 25-day-old animals (Mareš, 2013) and at second the interval after SE (3 vs. 13 days - Mareš and Kubová, 2016). The second factor plays also an important role as demonstrated by Mangan and Bertram (1998) but it is impossible to separate these two factors in developing rats.

There was a tendency to a difference between SE and LiPAR animals after CGP treatment in 15-day-old animals – incidence of generalized seizures increased in SE rats. Opposite effect (again nonsignificant) was seen in minimal clonic seizures bringing another proof for different generators of these two types of seizures (Browning and Nelson, 1986). The only significant change was an increased seizure severity. No significant changes were found in 25-day-old rats. There were only moderate tendencies to increase in the incidence of generalized seizures and in the severity of seizures. Higher sensitivity of younger rats to $GABA_B$ receptor antagonist CGP46381 is in agreement with our previous data demonstrating a decrease of proconvulsant efficacy of CGP4638 with age in a model of cortical epileptic afterdischarges (Mareš, 2013).

Negative findings in 25-day-old rats in our experiments might be put together with data on adult rats 2–3 months after lithium-pilocarpine SE induced at postnatal day 10 (Nehlig et al., 2002) and pentylenetetrazol-induced SE at postnatal day 20 (Kouis et al., 2014). Both papers report no changes in sensitivity to pentylenetetrazol. It might demonstrate that changes in the sensitivity to pentylenetetrazol appear early after SE but not 10 days later. There might be a subsequent loss of this sensitivity which should be analyzed in future. This direction of further research is indicated by data from Virginia group – hippocampal pyramidal cells loose $GABA_A$ inhibition early after SE whereas $GABA_B$ system undergoes similar change approximately two weeks after SE (Mangan and Bertram, 1998).

Declaration of interests

None.

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