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Short communication

The effect of intraperitoneally administered dimethyl sulfoxide on absence-like epileptic activity of freely moving WAG/Rij rats

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ABSTRACT

Dimethyl sulfoxide (DMSO) is a widely used solvent for water-insoluble molecules and it has antioxidant, neuroprotective and cryopreservative effects. While DMSO is a regularly used solvent in research and a therapeutic agent, several cases of DMSO evoked seizures were reported in the literature. Therefore, we investigated the effect of different doses of DMSO on the absence-like epileptic activity of Wistar Albino Glaxo/Rijswijk (WAG/Rij) rats. We revealed that low doses of DMSO decreased whereas high doses of DMSO increased the absence-like epileptic activity of WAG/Rij rats.

1. Introduction

Several of the antiepileptic drug candidates are difficult to dissolve, thus various solvents are used, such as DMSO, for their delivery. DMSO is one of the most efficient solvent for water-insoluble drugs hence it is regularly used in biological studies and also a vehicle for drug therapy. DMSO also can be used as a therapeutic agent because of its antioxidant, neuroprotective and cryopreservative properties (Santos et al., 2003; Jacob and de la Torre, 2009). It also has several known side effects because of its influence on cell membranes and receptor affinity (Santos et al., 2003; Jacob and de la Torre, 2009).

Recently several cases of DMSO evoked seizures were reported (e.g., Bauwens et al., 2005; Marcacci et al., 2009). Furthermore, in some of the patients experiencing DMSO-associated encephalopathy altered neuroimaging patterns were found in the thalamus (Marcacci et al., 2009). Therefore in this study, the effect of different doses of DMSO was tested in genetically absence epileptic WAG/Rij rats generating seizures of cortico-thalamic origin (Snead, 1995; Coenen and Van Luijtelaar, 2003). We revealed that low doses of DMSO (1.65 mg/kg in either 1.5 ml/kg or 2.0 ml/kg) decreased whereas high doses of DMSO (825.3 mg/kg and 1650.6 mg/kg) increased the number of spike-wave discharges (SWDs) in the WAG/Rij rat.

2. Materials and methods

2.1. Animals

Treatments and surgery procedures of all animals were carried out according to the local ethical rules which are in conformity with the guidelines of the European Communities Council Directive 24 November 1986 (86/609/EEC). Eight months old male WAG/Rij rats (breeding colony of WAG/Rij rats at University of West Hungary, Savaria Campus, Szombathely, Hungary) weighting 280–320 g were used ($n = 35$). Animals were housed under standard laboratory conditions (light was on from 8.00 AM to 8.00 PM: 12:12 h light–dark cycle; Kovács et al., 2006), with free access to water and food. Rats were maintained in groups 3–4 and they were separated after surgery.

2.2. Implantation of animals for EEG recording

For EEG recording, all WAG/Rij rats were implanted under halothane–air mixture (1%) anesthesia. Stainless steel screw electrodes (0.8 mm o.d.) were implanted into the bone above the primary motor cortex and somatosensory cortex (A 0.8, L 1.8 and A 0.2, L 6.2) (Paxinos and Watson, 1997). We placed the ground electrode above the cerebellar cortex. Stainless steel reference

Abbreviations: ACSF, artificial cerebrospinal fluid; DMSO, dimethyl sulfoxide; i.p., intraperitoneal; SWD, spike-wave discharge; WAG/Rij, Wistar Albino Glaxo/Rijswijk.

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electrode (a plate of 3 mm × 4 mm with one side insulated) was implanted under the skin over the masseter muscle. Electrodes were soldered to a ten-pin socket that was fixed to the skull with dentacrylate cement.

2.3. Recording of SWDs and experimental paradigm

EEG were recorded by a differential preamplifier (SUPERTECH Bioamp 4, Hungary) attached to a CED 1401 μII data capture and analysis device (Cambridge, UK) using SPIKE 2 software (bandwidth of the EEG recording: 0.53–75 Hz; sampling rate: 500 Hz).

Rats were allowed to recover for at least 2 weeks. WAG/Rij rats were injected with 1.5 ml/kg non-pirogen artificial cerebrospinal fluid (ACSF; Szent Rókus Hospital and Institutions, Budapest, Hungary) intraperitoneally (i.p.) on 3 consecutive days (three-day control period) to establish averaged control SWD numbers and durations. On the fourth day, animals received a single i.p. injection of DMSO of a given concentration ($n = 5$–5). The pure DMSO liquid was diluted in ACSF and injected in a volume of 1.5 ml/kg. Injected DMSO solutions were 0.1, 1, 10, 30, 50 or 100% DMSO in ACSF in a volume of 1.5 ml/kg, which contained 1.65, 16.51, 165.06, 495.18, 825.3 and 1650.6 mg/kg DMSO, respectively.

The SWD numbers and durations were measured between 30 and 270 min after-injection (from 3.00 PM to 7.00 PM), since we revealed, in previous studies, that the i.p. injection could influence the number of SWDs in the 30 min period immediately after injection (Kovács et al., 2006).

To reveal whether the same amount of DMSO in slightly different volume injected has any different effect on SWDs, 1.65 mg/kg DMSO was also administered in 2 ml/kg ACSF (instead of 1.5 ml/kg; $n = 5$). In these experiments the EEG was recorded longer, from 1.00 PM to 7.00 PM.

On the fifth day, an ACSF control experiment (post-treatment control day; ACSF i.p.) was performed to disclose putative long lasting effects of DMSO on SWDs.

The recording periods were split into 30 min sections and these were evaluated separately. The SWDs were selected and cut off from the raw data files and were checked by FFT analysis as it was described earlier (Kovács et al., 2006). The changes of the SWDs (SWD number, total time of SWDs and average duration of SWDs) were expressed in percent of the corresponding average control values obtained from the ACSF injected control recordings (three-day control period), because there are individual variations in SWD parameters (Kovács et al., 2006, 2007). The changes were evaluated by Student's unpaired *t*-tests.

3. Results

3.1. Different doses of DMSO differentially changed the SWD number

The lowest amount of DMSO (1.65 mg/kg) injected in either 1.5 ml/kg or 2 ml/kg solution significantly decreased the SWD number equally between 30 and 90 min after i.p. injection (Fig. 1A and C). Injection of 16.51, 165.06 or 495.18 mg/kg DMSO had no effect on the absence-like epileptic activity of WAG/Rij rats during the whole recording period (Fig. 1A and B). Note however, that there is a non-significant decrease in the SWD number in all cases (16.51 mg/kg, 1%, between 60 and 90 min; 165.06 mg/kg, 10%, between 90 and 180 min; 495.18 mg/kg, 30%, between 150 and 210 min), which could be due to the normal metabolic decrease of the DMSO's blood concentration. Injection of 825.3 mg/kg DMSO significantly increased the SWD number ($p < 0.05$) for 90 min (between 60 and 150 min; Fig. 1A). Injection of pure DMSO (1650.6 mg/kg) significantly increased the SWD number ($p < 0.05$, $p < 0.005$) for 210 min (between 30 and 240 min; Fig. 1A and B). In each case, on the post-treatment control day, the SWD number returned to the baseline level (data not shown).

3.2. Effect of DMSO on the total SWD time

The total SWD time changed in parallel with the SWD number (Fig. 1D) as the average time of SWDs did not change (data not shown). Namely, the total SWD time decreased after 1.65 mg/kg DMSO injection (between 30 and 90 min in 1.5 ml/kg solution) while increased after 825.3 mg/kg DMSO (between 60 and 150 min) and 1650.6 mg/kg DMSO (between 30 and 240 min) injection.

4. Discussion

According to our knowledge, this is the first study that examines the effect of different doses of DMSO on the absence-like epileptic activity of WAG/Rij rats. We revealed that 1.65 mg/kg DMSO (in either 1.5 ml/kg or 2 ml/kg volume) decreased whereas 825.3 mg/kg and 1650.6 mg/kg DMSO increased the number of SWDs in freely moving WAG/Rij rats.

The physiological and pathophysiological effects of DMSO or the mechanisms of its side effects are not clearly understood, although it is widely used as a cryopreservative agent in stem cell transplantation (Bauwens et al., 2005; Marcacci et al., 2009). Additionally in neurosciences, DMSO is extensively used as an organic solvent to dissolve various pharmacons in both *in vitro* and *in vivo* studies (Santos et al., 2003).

It was demonstrated that DMSO has an antipsychotic effect and it is beneficial in the treatment of traumatic brain edema, memory dysfunction, ischemia and stroke (Santos et al., 2003; Jacob and de la Torre, 2009). Nevertheless, DMSO has several side effects such as intravascular hemolysis, hypernatremia, nausea, vomiting, anaphylactic reactions, etc. (Santos et al., 2003; Jacob and de la Torre, 2009). Furthermore, in a recent study it was revealed that DMSO may induce apoptosis in the developing central nervous system (Hanslick et al., 2009). Some case reports and experimental results demonstrated that DMSO may induce neurological toxicity evoked seizures in adult humans and rats (Bauwens et al., 2005). However, the exact role of DMSO in the neurological side effects and the pathomechanism of its neurological toxicity are undetermined.

The WAG/Rij rat is one of the models of human absence epilepsy spontaneously generating absence-like seizures evoked by a hyper-synchronous activity of the corticothalamic and thalamocortical loops (Coenen and Van Luijtelaar, 2003). DMSO has wide influence on ion currents, blocks the activation of Na^+ channels, decreases N-methyl-D-aspartate (NMDA) receptor-, α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptor- and γ-aminobutyric acid (GABA) receptor-induced ion currents, attenuates potassium currents and may change properties of T- and L-type calcium channels (Larsen et al., 1996; Santos et al., 2003; Jacob and de la Torre, 2009). As all of these receptors and channels are involved in the genesis of absence seizures (Snead, 1995), DMSO, by shifting the excitation/inhibition balance, can change absence epileptic activity.

Previously it was demonstrated that the effect of DMSO depended on the concentration it was applied (Larsen et al., 1996; Gurtovenko and Anwar, 2007). DMSO decreased the firing rate of neurons (Maclennan et al., 1996) and dose-dependently blocked the propagation of action potentials (Larsen et al., 1996). In high concentration, DMSO induced transient water pores into the membrane and degraded its structure and significantly perturbated the secondary protein structures of membrane proteins (Larsen et al., 1996; Gurtovenko and Anwar, 2007).

DMSO is frequently used to dissolve antiepileptic drug candidates in epilepsy research (e.g., Gebhardt et al., 2001). However, it was demonstrated that DMSO may enhance the proconvulsant

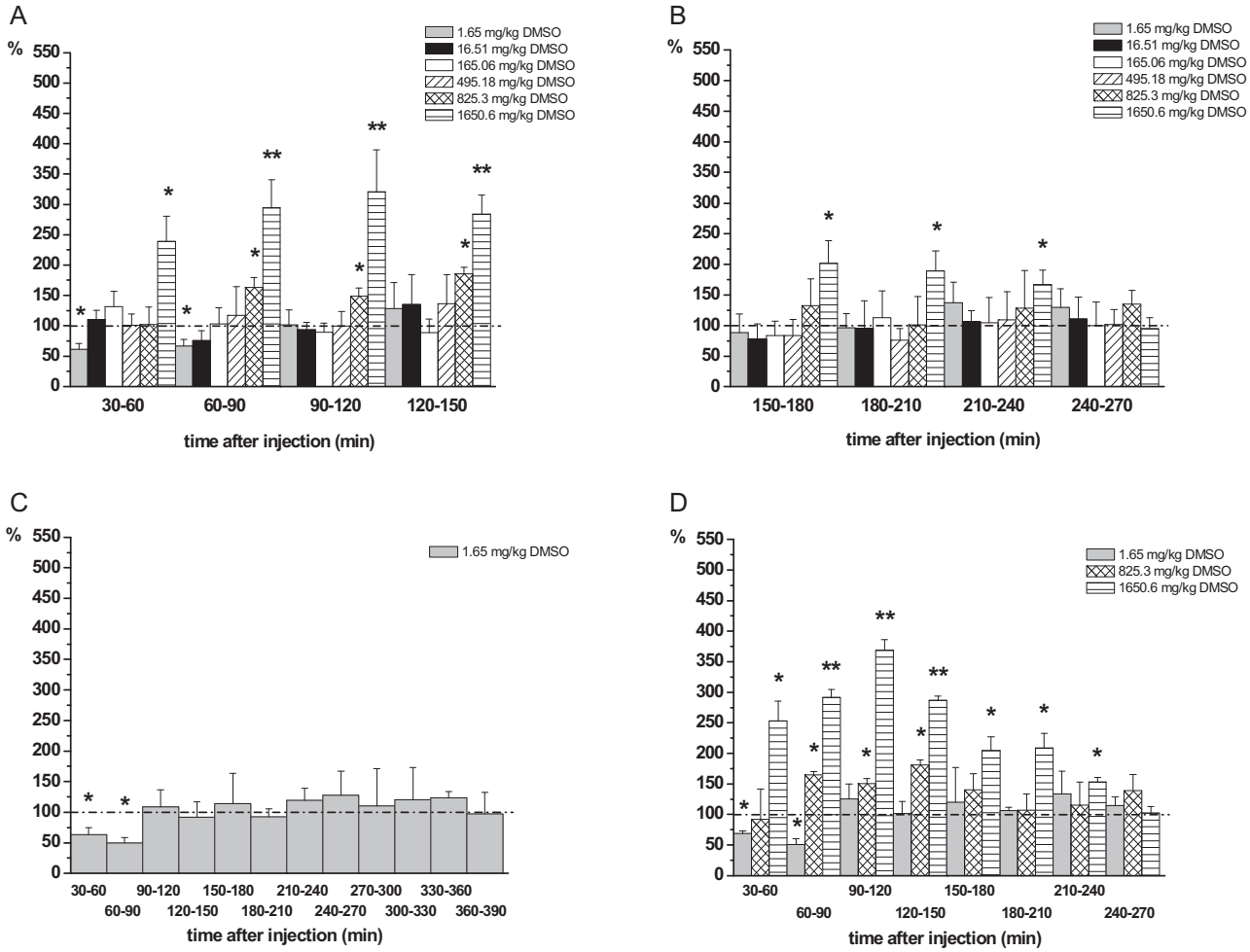

Fig. 1. Effect of different DMSO solutions (0.1, 1, 10, 30, 50 or 100% DMSO in ACSF in a volume of 1.5 ml/kg, which contained 1.65, 16.51, 165.06, 495.18, 825.3 and 1650.6 mg/kg DMSO) on SWD number in freely moving WAG/Rij rats (Part A: from 30 to 150 min; Part B: from 150 to 270 min). The effect of the lowest dose of DMSO (1.65 mg/kg) when administered in 2 ml/kg ACSF (instead of 1.5 ml/kg) on SWD number (Part C). Changes of the total SWD time in cases of the three effective DMSO doses (1.65, 825.3, 1650.6 mg/kg in 1.5 ml/kg; Part D). $^{*}p < 0.05$ and $^{**}p < 0.005$ level of significance.

activity of the dissolved drugs because DMSO may lower seizure threshold (Wong et al., 1988). Similarly, the antiepileptic effect of i.p. administered 2-methyl-4-oxo-3H-quinazoline-3-acetyl piperidine (Q5) in WAG/Rij rats (Kovács et al., 2007) was abolished if Q5 was diluted in 2 ml/kg 100% DMSO (2200.8 mg/kg; Kovács et al., unpublished data). Furthermore, i.c.v. injection of 3 μl 20% DMSO in GAERS rats (Genetic Absence Epilepsy Rats from Strasbourg) increased absence-like seizure incidence by about 60% (Landweer et al., unpublished data) suggesting that the way of application could be an important factor too.

In conclusion, i.p. administered DMSO changed the absence-like epileptic seizure activity of freely moving WAG/Rij rats. Small doses decreased while high doses increased the SWD activity. More studies are needed to clarify the exact mechanisms of these effects. However, as DMSO can modify the effects of the different antiepileptic drugs, particular care should be taken when evaluating the actions of these drugs administered in DMSO.

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