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Effect of acute caffeine administration on PTZ-induced seizure threshold in mice: Involvement of adenosine receptors and NO-cGMP signaling pathway

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ABSTRACT

Purpose: Caffeine is a non-selective antagonist of A_1 and A_{2A} adenosine receptors (ARs). In this regard, nitric oxide (NO) is partly involved in the central effects of caffeine. In this study, we examined the effect of acute caffeine administration on pentylenetetrazole (PTZ)-induced seizure threshold by focusing on A_1Rs, A_{2A}Rs, and NO-cGMP signaling pathway.

Methods: NMRI male mice (25–30 g) received caffeine (5, 50, and 100 mg/kg) alone, whereas 8-CPT (1 and 5 mg/kg, a selective A_1Rs antagonist), SCH-442416 (5 and 10 mg/kg, a selective A_{2A}Rs antagonist) or sildenafil (5 and 10 mg/kg, a phosphodiesterase 5 inhibitor) were administrated alone or as pre-treatment before caffeine. Seizure threshold was assessed by intravenous infusion of PTZ. Nitric oxide metabolites (NOx) were measured with the Griess method.

Results: When administrated alone, caffeine (5 and 50 mg/kg) and 8-CPT (1 and 5 mg/kg) significantly decreased seizure threshold, while 100 mg/kg of caffeine, SCH-442416 or sildenafil did not change it. Only pre-treatment with SCH-442416 (5 and 10 mg/kg) or sildenafil (5 and 10 mg/kg) before 100 mg/kg of caffeine significantly decreased seizure threshold. Moreover, NOx levels significantly decreased following alone administration of caffeine (100 mg/kg) or 8-CPT (5 mg/kg).

Conclusion: The results of present study showed that 5 and 50 mg/kg of caffeine had a proconvulsant effect but caffeine at a dose of 100 mg/kg had no effect on seizure threshold. In addition, it seems that the effect caffeine on seizure threshold is partly mediated through ARs or modulation of the NO-cGMP signaling pathway.

1. Introduction

Caffeine (1,3,7-trimethylxanthine) belongs to the family of purine alkaloids and is the main active component of energy drinks and caffeine-containing beverages such as tea and coffee (Nieber, 2017). Caffeine is the non-selective antagonist of adenosine receptors (ARs). Adenosine as a potent inhibitory neuromodulator has four G protein-coupled receptors: A_1, A_{2A}, A_{2B}, and A_3 (El Yacoubi et al., 2001). A_1Rs and A_{2A}Rs are the preferential targets of caffeine. However, affinities of caffeine and other methylxanthines to A_1Rs and A_{2A}Rs (with Ki values of 29 and 48 μM, respectively) are nearly equal (Tchekalarova et al., 2009).

Some human reports (Bonilha and Li, 2004; Kaufman and Sachdeo, 2003) and animal studies (Cutrufo et al., 1992; Loscher, 2009) show that caffeine is a proconvulsant agent, but its potential pro-epileptic effect is still a matter of debate. Caffeine may induce seizures in rodents when consumed in high doses (over 400 mg/kg) (Chroscinska-Krawczyk et al., 2011). Caffeine has shown a mitigatory effect on seizure threshold in some animal models of epilepsy (Chu, 1981; De Sarro et al., 1997). Moreover, based on the results of a systematic review caffeine increases seizure susceptibility (van Koert et al., 2018). However, there are some reports that caffeine at doses far less than its convulsive dose has no significant effect on seizure threshold (Bankstahl et al., 2012; Jargiello-Baszak et al., 2016). Besides, there are additional reports regarding no effect of caffeine against seizures following chronic administration (Germe et al., 2015; El Yacoubi et al., 2008). These paradoxical effects of caffeine may be partly related to its effects on ARs. It is well established that activation of A_1Rs leads to anticonvulsant effect and proconvulsant effect of caffeine to somewhat is mediated by antagonizing of A_1Rs (Boison, 2011). On the other hand, A_{2A}R is a stimulatory receptor and its activation may aggravate seizures (El Yacoubi et al., 2008). Thus, it is possible that the paradoxical effect of caffeine on seizures to be partly due to its dose-dependent effect on A_1Rs or A_{2A}Rs.

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Nitric oxide (NO), a known neurotransmitter/neuromodulator in the brain, is involved in many physiological and pathological processes. NO is generated from L-arginine by various isoforms of nitric oxide synthase (NOS), namely neuronal NOS, inducible NOS, and endothelial NOS (Akula et al., 2008). Activation of soluble guanylate cyclase (sGC) by NO leads to elevation of intracellular second messenger cyclic guanosine monophosphate (cGMP) levels. Levels of cGMP are regulated by cyclic nucleotide phosphodiesterases (PDEs), which catalyze the hydrolysis of cyclic adenosine monophosphate (cAMP) and cGMP (Kaster et al., 2005). It has been suggested that NO exerts both proconvulsant (Royes et al., 2005; Riazi et al., 2006) or anticonvulsant effects (Yahyavi-Firouz-Abadi et al., 2006) depending on the route of administration and model of seizure. In addition, the results of several studies demonstrate the modulatory role of the NO-cGMP pathway on the anticonvulsant effect of adenosine (Akula et al., 2008; Kaku et al., 2001). There is obvious evidence that caffeine can modulate NO production (Lopez-Munoz et al., 1996; Kayir and Uzbay, 2004). For example, NO production decreases as the result of A_1Rs activation, whereas activation of A_{2A}Rs leads to elevation of NO generation (Bruce et al., 2002). On the other hand, the non-selective NOS inhibitor, N^{ω}-Nitro-L-arginine methyl ester (L-NAME) potentiates the anticonvulsant effect of adenosine, while either L-arginine or sodium nitroprusside attenuate the anticonvulsant effect of adenosine (Akula et al., 2008). These results show that the NO-cGMP pathway is probably involved in the central effects of adenosine.

Overall, it seems that the mechanism of actions of caffeine is not solely by antagonizing of ARs and probably other signaling pathways such as the NO-cGMP pathway may be involved. Hence, the aim of the current study is to investigate the effects of acute administration of caffeine on PTZ-induced clonic seizure threshold in mice. Moreover, the involvement of A_1Rs and A_{2A}Rs is investigated using selective antagonists of these receptors. Finally, the involvement of the NO-cGMP pathway is evaluated using sildenafil as selective PDE5 inhibitor and measurement of NO metabolites.

2. Materials and methods

2.1. Animals

A total of 126 male mice of the NMRI strain, bred in the animal house of Physiology Research Center, Kashan University of Medical Sciences (age 5–6 weeks and weight 25–30 gr) were used. Animals were housed (seven animals per cage) in standard polypropylene cages, at a constant temperature of 22 ± 2 ℃ and humidity of 50–55%, with automatically controlled 12/12 h light/dark cycles. All animals were fed a regular mice chow and water ad libitum. All experiments were performed in accordance with the guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, revised 1985) and were approved by the Research and Ethics Committee of Kashan University of Medical Sciences (ethics code: I.R KAUMS.MEDNT.REC.1396.18), Kashan, Iran.

2.2. Drugs

Chemicals and drugs used in this study were: PTZ, caffeine, 8-CPT (a selective A_1Rs antagonist), SCH-442416 (a selective A_{2A}Rs antagonist), and sildenafil (a PDE5 inhibitor). PTZ was purchased from Sigma (USA) and all of the other drugs were supplied from Tocris (UK). PTZ (0.5% solution) was prepared in heparinized sterile saline 0.9% and administered as intravenous (i.v.) infusion. Caffeine was dissolved in normal saline solution at desired doses. Sildenafil, 8-CPT, and SCH-442416 were dissolved in dimethyl sulfoxide (DMSO) according to recommended protocol of the manufacturer. All the mentioned drugs were injected intraperitoneally (i.p.) in a volume of 5 ml/kg of body weight. Drug doses used in this study were based on the results of previous studies (Faingold et al., 2016; Germe et al., 2015; Riazi et al., 2006) and pilot experiments.

2.3. Experiments

The animals were randomly divided into 18 groups. Each experimental group consisted of 7 mice based on pilot experiments and previous study on this seizure model (Heydari and Davoudi, 2017).

In experiment 1(including 4 groups), animals received a i.p. injection of different doses of caffeine (5, 50, and 100 mg/kg) 30 min before the determination of PTZ- induced clonic seizure threshold. Control animals received the same volume of saline. Based on this experiment, a dose of 100 mg/kg of caffeine was used in subsequent experiments.

In experiment 2 (including 7 groups), mice were acutely administrated with DMSO as control or different doses of 8-CPT (1 and 5 mg/kg, i.p), SCH-442416 (5 and 10 mg/kg, i.p) or sildenafil (5 and 10 mg/kg, i.p) 30 min before determination of PTZ-induced clonic seizure threshold.

In experiments 3 (including 7 groups), we examined the effect of pre-treatment with the same doses of 8-CPT (1 and 5 mg/kg, i.p), SCH-442416 (5 and 10 mg/kg, i.p) or sildenafil (5 and 10 mg/kg, i.p) 30 min before the selected dose of caffeine. Control animals received DMSO before the selected dose of caffeine. In all pre-treatment groups, PTZ-induced clonic seizure was determined 30 min after caffeine administration.

2.4. Seizure induction

PTZ was dissolved in heparinized sterile saline (0.9%) to prepare a fresh solution with a dose of 5 mg/ml before i.v. infusion. The dose and infusion rate of administered PTZ was 5 mg/ml in saline and 0.5 ml/min, respectively. Before testing, each mouse was weighed and placed in a clear acrylic plastic restrainer, followed by immersing its tail in a warm water bath (40–45 ℃) for 1 min to dilate the tail veins. Lateral tail vein of the mouse was catheterized with a 30 dental carpule attached to a length of No.10 polyethylene (PE) tubing, which was secured to the tail by a narrow piece of adhesive tape. The PE tubing (approximately 50 cm in length) was attached to a 10 ml plastic syringe containing the PTZ solution (5 mg/ml PTZ in 0.9% heparinized saline) mounted into a syringe pump (Top, Japan). The PTZ solution was subsequently infused into the tail vein of freely moving mouse at a constant rate of 0.5 ml/min. Times (in seconds) from the start of the infusion to the appearance of general clonus (forelimb clonus followed by full clonus of the body) was recorded for each mouse. The recorded times were then converted to mg/kg PTZ for each mouse depend on PTZ dose administered and time-related (Heydari and Davoudi, 2017; Mesdaghinia et al., 2010).

2.5. Measurement of NOx

After induction of PTZ-induced seizure, the animals were sacrificed and their brains were removed to determine NOx. The Griess reaction was used to assay NOx as previously described (Heydari and Davoudi, 2017). First, standard curves for nitrite were prepared. Brain tissue was homogenized in Tris HCl 50 M containing 0.1 mM EDTA. Afterward, 100 µl of tissue suspensions were added to the Griess reagent including 100 µl of vanadium (III) chloride (VCl3), 50 µl of sulfanilamide, and 50 µl of N-(1-Naphthyl) ethylenediamine dihydrochloride (NEDD). VCl3 was used to reduce nitrate to nitrite. The proteins were subsequently precipitated by the addition of 50 µl of 10% trichloroacetic acid. The contents were incubated for 45 min and then centrifuged. Next, supernatants were transferred to a 96-well flat-bottomed microplate. Absorbance was read at 540 nm using microplate reader and final values were calculated from nitrite standard curves. NOx levels were measured in all groups except sildenafil receiving groups because sildenafil is a phosphodiesterase 5 inhibitor.

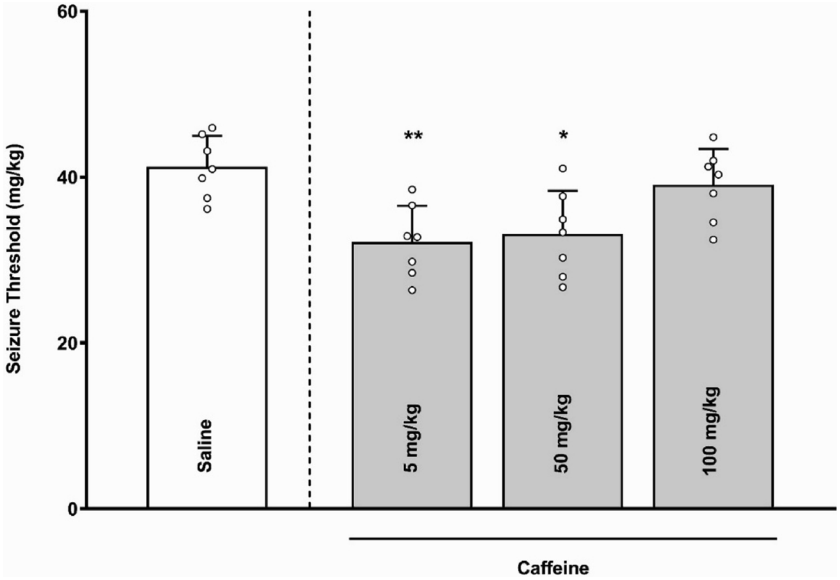

Fig. 1. Effect of different doses of caffeine (5, 50, and 100 mg/kg, i.p.) on the PTZ-induced clonic seizure threshold; *P < 0.05 and **P < 0.01 in comparison with saline-treated control group (mean ± SD, n = 7).

2.6. Statistical analysis

Experimental factors were caffeine (5, 50, and 100 mg/kg), 8-CPT (1 and 5 mg/kg), SCH-442416 (5 and 10 mg/kg) and sildenafil (5 and 10 mg/kg). All data were expressed as mean ± standard deviation (SD). Significance of differences in seizure threshold and NOx levels was done by one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons. Significance of difference between two groups with the same dose of 8-CPT, SCH-442416 or sildenafil in the absence or presence of caffeine was calculated by a two-tailed Student's t-test. Differences were considered statistically significant if P < 0.05. The SPSS version 23.0 software was used for all analyses.

3. Results

3.1. Effect of different doses of caffeine on PTZ-induced seizure threshold

Fig. 1 illustrates the effect of acute administration of caffeine at different doses (5, 50, and 100 mg/kg, i.p.) on PTZ-induced clonic seizure threshold. One-way ANOVA revealed a significant effect ($F_{3,24} = 6.98$, P = 0.002). *Post hoc* analysis showed that compared to saline-treated control group (41.25 ± 3.72 mg/kg), caffeine at doses of 5 and 50 mg/kg had a significant proconvulsant effect [32.19 ± 4.35 (p = 0.004) and 33.14 ± 5.2 mg/kg (p = 0.011) respectively). In comparison, caffeine at dose of 100 mg/kg did not significantly change the seizure threshold [39.06 ± 4.34 mg/kg, (p = 0.79)]. Proconvulsant effect of caffeine and its underlying mechanisms have been previously described in detail. Our results confirmed and extended the previous report (Chroscinska-Krawczyk et al., 2011) that caffeine at doses far below its convulsive potential (400 mg/kg) did not change the seizure threshold. Thus, a dose of 100 mg/kg of caffeine was selected for further experiments to investigate the possible role of A_1Rs and A_{2A}Rs and also NO-cGMP signaling pathway.

3.2. Effect of 8-CPT, a selective antagonist of A_1Rs, alone and as pre-treatment before 100 mg/kg of caffeine on PTZ-induced seizure threshold

Fig. 2 shows the effect of acute administration of 8-CPT (1 and 5 mg/kg, i.p.) alone or as pre-treatment before 100 mg/kg of caffeine on PTZ-induced clonic seizure threshold. When 8-CPT was administrated alone, one-way ANOVA revealed a significant effect ($F_{2,18} = 24.31$, P = 0.000). *Post hoc* analysis showed that compared to DMSO-treated control group (45.64 ± 5.54 mg/kg), 8-CPT at doses of 1 and 5 mg/kg significantly decreased PTZ-induced seizure threshold [35.63 ± 5.11 mg/kg (p = 0.002) and 28.77 ± 2.31 mg/kg (p = 0.000), respectively].

In pre-treatment groups, the same doses of 8-CPT (1 and 5 mg/kg, i.p.) were acutely administrated 30 min before 100 mg/kg of caffeine ($F_{2,18} = 2.71$, P = 0.094). Compared to DMSO + Caffeine (100 mg/kg) control group (44.85 ± 6.56 mg/kg), pre-treatment with 8-CPT at doses of 1 and 5 mg/kg did not significantly change PTZ-induced seizure threshold [43.9 ± 8.73 mg/kg (p = 0.971) and 52.58 ± 7.51 mg/kg (p = 0.170), respectively]. Pre-treatment of 5 mg/kg of 8-CPT before 100 mg/kg caffeine resulted in a significant (p = 0.000) increase in seizure threshold compared to 5 mg/kg of 8-CPT alone group. These results show that despite pre-treatment with 8-CPT and its antagonizing effect on A_1Rs, administration of caffeine at a dose of 100 mg/kg had no proconvulsant effect.

3.3. Effect of SCH-442416, a selective antagonist of A_{2A}Rs, alone and as pre-treatment before 100 mg/kg of caffeine on PTZ-induced seizure threshold

Fig. 3 shows the effect of acute administration of SCH-442416 (5 and 10 mg/kg) alone or as pre-treatment before 100 mg/kg of caffeine on PTZ-induced clonic seizure threshold. When SCH-442416 was administrated alone, one-way ANOVA did not reveal a significant effect ($F_{2,18} = 3.35$, P = 0.058). *Post hoc* analysis showed that compared to DMSO-treated control group (45.64 ± 5.54 mg/kg), SCH-442416 at doses of 5 and 10 mg/kg did not significantly change PTZ-induced seizure threshold [40.45 ± 5.88 mg/kg (p = 0.21) and 38.19 ± 5.11 mg/kg (p = 0.053), respectively].

When the same doses of SCH-442416 pre-treated before 100 mg/kg of caffeine, one-way ANOVA revealed a significant effect ($F_{2,18} = 40.55$, P = 0.000). Post hoc analysis showed that compared to DMSO + Caffeine (100 mg/kg) control group (44.85 ± 6.54 mg/kg), pre-treatment with SCH-442416 at doses of 5 and 10 mg/kg significantly decreased PTZ-induced seizure threshold [29.62 ± 3.02 mg/kg (p = 0.000) and 24.58 ± 2.39 mg/kg (p = 0.000), respectively].Meanwhile, pre-treatment of 5 and 10 mg/kg of SCH-442416 before 100 mg/kg caffeine resulted in a significant decrease in

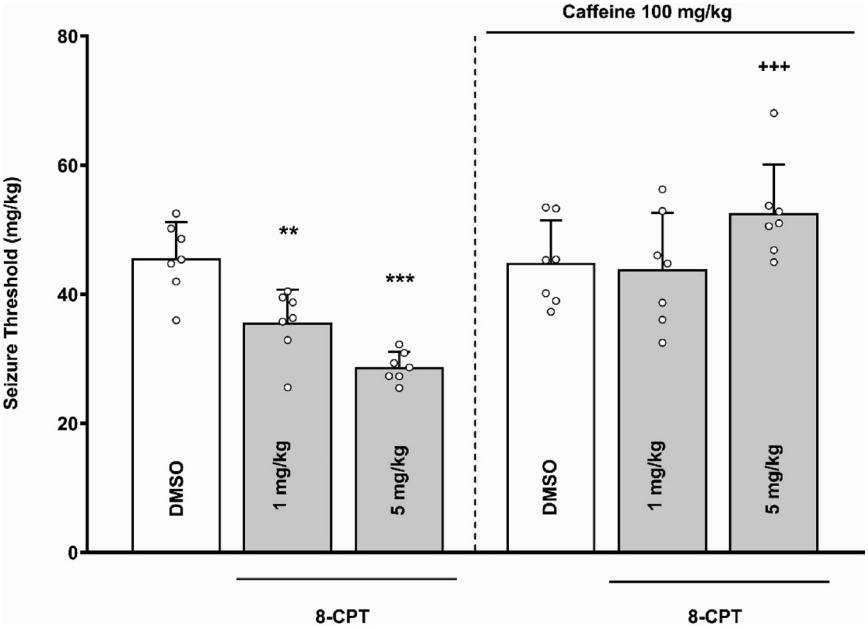

Fig. 2. Effect of different doses of 8-CPT (1 and 5 mg/kg, i.p.) alone and as pre-treatment before 100 mg/kg of caffeine on the PTZ-induced clonic seizure threshold; **P < 0.01 and ***P < 0.001 in comparison with DMSO-treated control group, + + +P < 0.001 in comparison with 5 mg/kg of 8-CPT alone group (mean ± SD, n = 7).

the seizure threshold compared to 5 and 10 mg/kg of SCH-442416 alone groups (p = 0.001 and p = 0.000 respectively).

3.4. Effect of pre-treatment with sildenafil, a selective inhibitor of PDE5, alone and as pre-treatment before 100 mg/kg of caffeine on PTZ-induced seizure threshold

Fig. 4 shows the effect of acute administration of sildenafil (5 and 10 mg/kg) alone or as pre-treatment before 100 mg/kg of caffeine on PTZ-induced clonic seizure threshold. When sildenafil was administrated alone, one-way ANOVA did not reveal a significant effect ($F_{2,18}$ = 2.25, P = 0.134). *Post hoc* analysis showed that compared to DMSO-treated control group (45.64 ± 5.54 mg/kg), sildenafil at doses of 5 and 10 mg/kg did not significantly change PTZ-induced seizure threshold [42.06 ± 4.98 mg/kg (p = 0.36) and 40.28 ± 3.73 mg/kg (p = 0.12), respectively].

When the same doses of sildenafil pre-treated before 100 mg/kg of caffeine, one-way ANOVA revealed a significant effect ($F_{2,18}$ = 34.21, P = 0.000). Post hoc analysis showed that compared to DMSO + Caffeine (100 mg/kg) control group (44.85 ± 6.54 mg/kg), pre-treatment with sildenafil at doses of 5 and 10 mg/kg significantly decreased PTZ-induced seizure threshold [32.86 ± 1.5 mg/kg (p = 0.000) and 27.32 ± 2.04 mg/kg (p = 0.000), respectively]. Also, pre-treatment of 5 and 10 mg/kg of sildenafil before 100 mg/kg caffeine resulted in a significant decrease in seizure threshold compared to 5 and 10 mg/kg of sildenafil alone groups (p = 0.001 and p = 0.000 respectively).

3.5. Effect of different doses of caffeine on NOx levels in the brain

Fig. 5 illustrates the effect of acute administration of caffeine at different doses (5, 50, and 100 mg/kg, i.p.) on NOx levels in the brain. One-way ANOVA revealed a significant effect ($F_{3,24}$ = 3.11, P = 0.045). *Post hoc* analysis showed that compared to saline-treated control group (69.85 ± 9.48 μM/g tissue), caffeine 100 mg/kg significantly decreased the NOx levels [58.73 ± 5.37 μM/g tissue

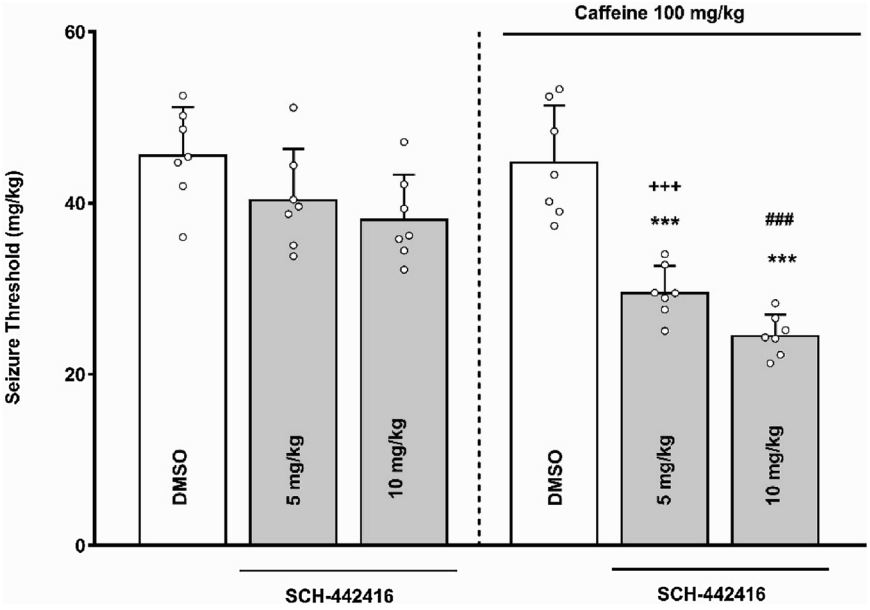

Fig. 3. Effect of different doses of SCH-442416 (5 and 10 mg/kg, i.p.) alone and as pre-treatment before 100 mg/kg of caffeine on the PTZ-induced clonic seizure threshold; ***P < 0.001 in comparison with DMSO + Caffeine group, + + +P < 0.001 in comparison with 5 mg/kg of SCH-442416 alone group, ###P < 0.001 in comparison with 10 mg/kg of SCH-442416 alone group (mean ± SD, n = 7).

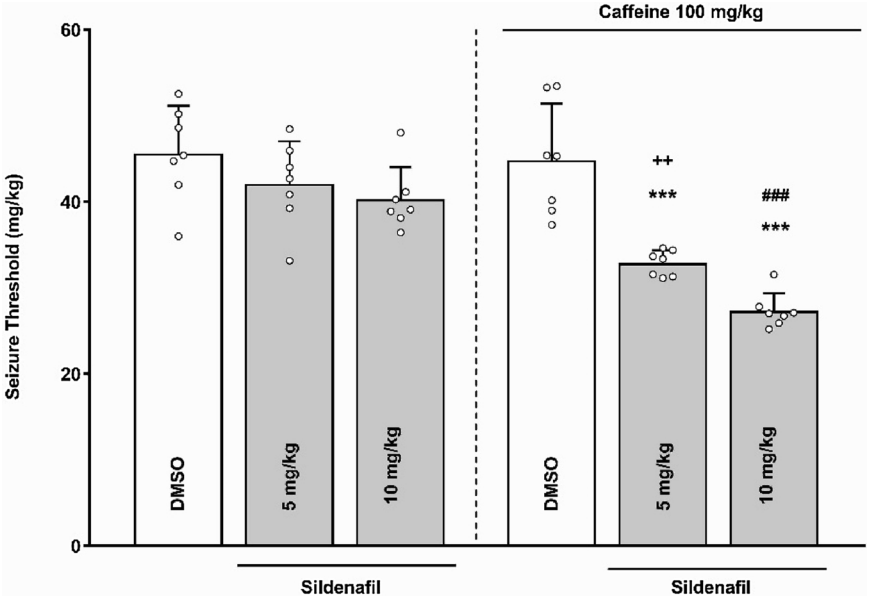

Fig. 4. Effect of different doses of sildenafil (5 and 10 mg/kg, i.p.) alone and as pre-treatment before 100 mg/kg of caffeine on the PTZ-induced clonic seizure threshold; ***P < 0.001 in comparison with DMSO + Caffeine group, ++P < 0.01 in comparison with 5 mg/kg of sildenafil alone group, ###P < 0.001 in comparison with 10 mg/kg of sildenafil alone group (mean ± SD, n = 7).

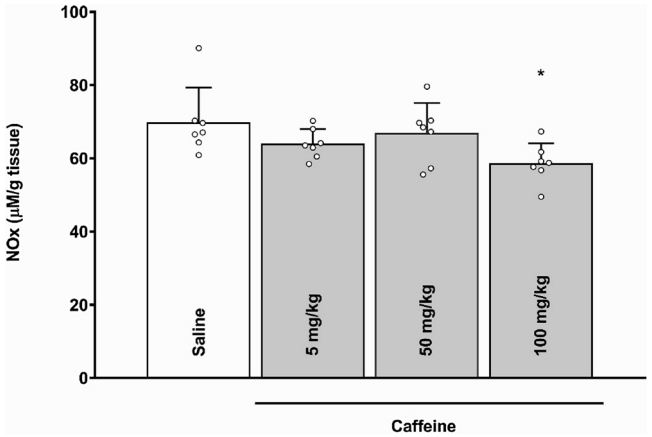

Fig. 5. Effect of different doses of caffeine (5, 50, and 100 mg/kg, i.p.) on NOx levels in the brain; *P < 0.05 compared with the saline-treated control group (mean ± SD, n = 7).

(p = 0.035)]. In comparison, caffeine at doses of 5 and 50 mg/kg did not significantly change NOx levels [63.99 ± 4.07 µM/g (p = 0.43) and 66.91 ± 8.2 µM/g tissue (p = 0.86) respectively].

3.6. Effect of 8-CPT, a selective antagonist of A_1Rs, alone and as pre-treatment before 100 mg/kg of caffeine on NOx levels in the brain

Fig. 6 shows the effect of acute administration of 8-CPT (1 and 5 mg/kg, i.p.) alone or as pre-treatment before 100 mg/kg of caffeine on NOx levels in the brain. When 8-CPT was administrated alone, one-way ANOVA did not reveal a significant effect ($F_{2,18}$ = 1.48, P = 0.254). Compared to DMSO-treated control group (55.85 ± 11.71 µM/g tissue), 8-CPT at doses of 1 and 5 mg/kg did not significantly change NOx levels [59.54 ± 8.23 µM/g tissue (p = 0.79) and 65.57 ± 11.7 µM/g tissue (p = 0.23), respectively].

When the same doses of 8-CPT pre-treated before 100 mg/kg of caffeine, one-way ANOVA revealed a significant effect ($F_{2,18}$ = 6.13, P = 0.009). *Post hoc* analysis showed that compared to DMSO + Caffeine (100 mg/kg) control group (58.69 ± 7.34 µM/g tissue), 8-CPT at a dose of 5 mg/kg significantly decreased NOx levels [48.06 ± 5.82 µM/g tissue (p = 0.007)]. Compared to DMSO + Caffeine (100 mg/kg) control group, 8-CPT at a dose of 1 mg/kg did not significantly change NOx levels [53.06 ± 3.02 µM/g tissue, (p = 0.181)]. Meanwhile, pre-treatment of 5 mg/kg of 8-CPT before 100 mg/kg caffeine resulted in a significant decrease in NOx levels compared to 5 mg/kg of 8-CPT alone group (p = 0.004).

3.7. Effect of SCH-442416, a selective antagonist of A_{2A}Rs, alone and as pre-treatment before 100 mg/kg of caffeine on NOx levels in the brain

Fig. 7 shows the effect of acute administration of SCH-442416 (5 and 10 mg/kg, i.p.) alone or as pre-treatment before 100 mg/kg of caffeine on NOx levels in the brain. When SCH-442416 was administrated alone, one-way ANOVA did not reveal a significant effect ($F_{2,18}$ = 0.465, P = 0.636). Compared to DMSO-treated control group (55.85 ± 11.71 µM/g tissue), SCH-442416 at doses of 5 and 10 mg/kg did not significantly change NOx levels [53.68 ± 9.98 µM/g tissue (P = 0.91) and 50.78 ± 7.41 µM/g tissue (P = 0.61), respectively].

When the same doses of SCH-442416 pre-treated before 100 mg/kg of caffeine, one-way ANOVA did not reveal a significant effect ($F_{2,18}$ = 1.33, P = 0.29). *Post hoc* analysis showed that compared to DMSO + Caffeine (100 mg/kg) control group (58.69 ± 7.34 µM/g tissue), SCH-442416 at doses of 5 and 10 mg/kg did not significantly change NOx levels [56.58 ± 10.66 µM/g tissue (p = 0.88) and 51.84 ± 5.23 µM/g tissue (p = 0.27), respectively].

4. Discussion

The results of the present experiment showed that acutely administrated caffeine at doses of 5 and 50 mg/kg had proconvulsant effect, while 100 mg/kg of caffeine did not change the seizure threshold. When administrated alone, 8-CPT (1 and 5 mg/kg) had proconvulsant effect, while SCH-442416 (5 and 10 mg/kg) or sildenafil (5 and 10 mg/kg) did not change seizure threshold. On the other hand, pre-treatment with the same doses of SCH-442416 or sildenafil before 100 mg/kg of caffeine significantly decreased seizure threshold, while proconvulsant effect of 8-CPT (1 and 5 mg/kg) disappeared in the presence of caffeine. Only 100 mg/kg of caffeine significantly decreased NOx levels. When administrated alone, 8-CPT (1 and 5 mg/kg) or SCH-442416 (5 and 10 mg/kg) did not significantly decrease NOx levels. In pre-treated groups, only pre-treatment of 8-CPT (5 mg/kg) before 100 mg/kg of caffeine significantly decreased NOx levels.

Caffeine is the non-selective antagonist of ARs and its primary targets are A_1Rs and A_{2A}Rs (Tchekalarova et al., 2010). Adenosine behaves

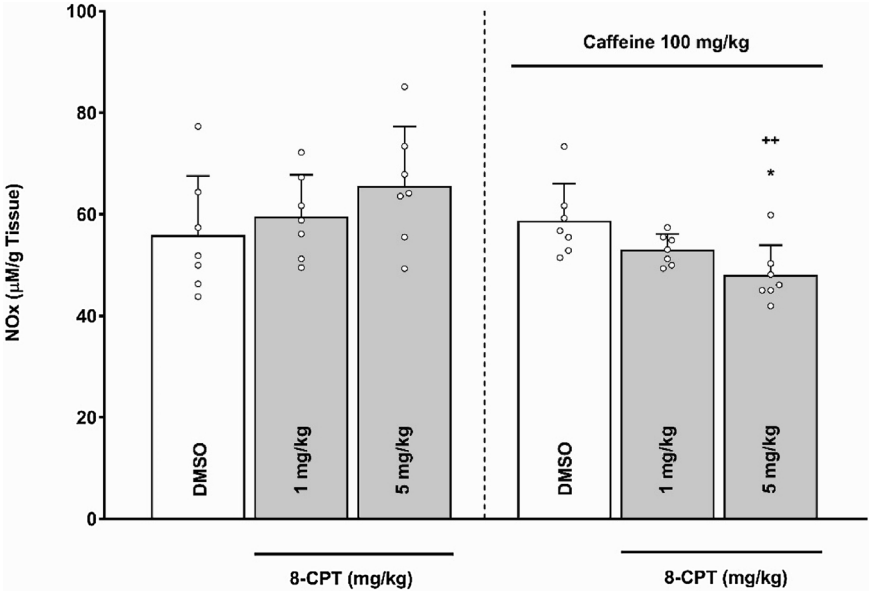

Fig. 6. Effect of different doses of 8-CPT (1 and 5 mg/kg, i.p.) alone and as pre-treatment before 100 mg/kg of caffeine on NOx levels in the brain; *P < 0.05 comparison with DMSO + Caffeine group, ++P < 0.01 in comparison with 5 mg/kg of the 8-CPT alone group (mean ± SD, n = 7).

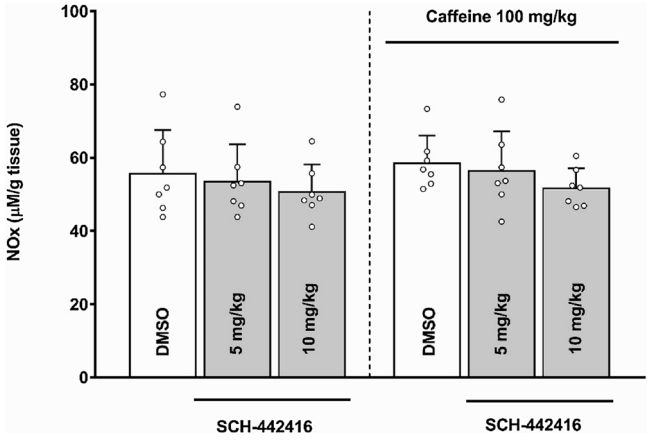

Fig. 7. Effect of different doses of SCH-442416 (5 and 10 mg/kg, i.p.) alone and as pre-treatment before 100 mg/kg of caffeine on NOx levels in the brain (mean ± SD, n = 7).

as an inhibitory neuromodulator in the brain and has anticonvulsant action through activation of A_1Rs (Dragunow, 1990). It seems that locally released adenosine has a crucial role in localizing epileptic focus through activation of A_1Rs (Fedele et al., 2006). In this context, A_1Rs knockout mice were more susceptible to seizures following experimental traumatic brain injury (Kochanek et al., 2006). Nevertheless, several pieces of evidence suggest caffeine as a proconvulsant agent (Chu, 1981; De Sarro et al., 1997).

To explore the effect of A_1Rs, we examined the effect of acute administration of 8-CPT, a selective A_1Rs antagonist, alone and as pre-treatment before 100 mg/kg of caffeine. Our results showed that low concentrations of 8-CPT (2 and 5 mg/kg, i.p.), when administered alone, significantly decreased seizure threshold. The declined seizure threshold by the 8-CPT suggests proconvulsant effect of A_1Rs antagonists. Thus, proconvulsant effect of 5 and 50 mg/kg of caffeine in our study was probably due to antagonizing effect on A_1Rs. Nevertheless, 8-CPT did not alter the seizure threshold when administered before 100 mg/kg of caffeine. Also, pre-treatment of 5 mg/kg of 8-CPT before 100 mg/kg caffeine resulted in a significant increase of seizure threshold compared to 5 mg/kg of 8-CPT alone group. Different effects of 8-CPT on seizure threshold in the absence and presence of caffeine suggest the involvement of other signaling pathways such as the effect of caffeine on A_{2A}Rs or involvement of NO-cGMP pathway in the effect of caffeine on a seizure.

Results obtained from the administration of 100 mg/kg of caffeine confirmed and extended previous results that some doses of caffeine had no effect against seizures. Our results are not the first report regarding no effect of caffeine against seizures. It has been previously reported that acute administration of 60 or 80 mg/kg of caffeine did not significantly change the PTZ-induced seizure threshold (Bankstahl et al., 2012). In another study, acute administration of 46.2 and 92.4 mg/kg of caffeine showed no proconvulsant effect in maximal electroshock in mice (Jargiello-Baszak et al., 2016). Moreover, the acute administration of caffeine (20 mg/kg, P.O) did not change the seizure threshold in juvenile and adult rats (Himmel, 2008). In contrast to our results, caffeine at a dose of 92.4 mg/kg significantly reduced the threshold for PTZ-induced clonic seizures in mice (Luszczki et al., 2006). In another study, caffeine at doses of 150–200 mg/kg produced the clonic seizures in mice (Marangos et al., 1981).

The role of A_{2A}Rs in the modulation of seizure susceptibility has been recently well established. Attenuation of PTZ-induced clonic seizure in A_{2A}Rs knockout mice drinking water only was mimicked in wild-type mice receiving caffeine in drinking water for 14 days (El Yacoubi et al., 2008). In addition, A_{2A}Rs knockout mice were partially resistant to limbic seizures (El Yacoubi et al., 2009). Considering these findings, it is suggested that the effect of acutely administrated 100 mg/kg of caffeine on seizure is not due to its antagonizing effect on A_{2A}Rs. Therefore, we used different doses of SCH-442416 (5 and 10 mg/kg) before 100 mg/kg of caffeine. When administered alone, SCH-442416 had no significant effect on PTZ-induced seizure threshold. Pre-treatment with both doses of SCH-442416 before caffeine decreased PTZ-induced seizure threshold. Since SCH-442416 is the selective antagonist of A_{2A}Rs, it seems that the proconvulsant effect of caffeine in the presence of SCH-442416 is partly due to its effect on another signaling pathway. Because SCH-442416 did not change seizure threshold per se in our investigation, we concluded that the proconvulsant effect of caffeine is probably through antagonizing effect on A_1Rs.

Although caffeine can modulate seizure susceptibility mainly through antagonizing effect on ARs, other mechanisms may also be involved in the central effects of caffeine. In an unpublished work, we have previously examined the involvement of the NO-cGMP pathway using L-NAME or L-arginine in the central effect of caffeine. Our results

showed that L-arginine attenuated the effect of 100 mg/kg of caffeine and had proconvulsant effect. Since A_{2A} receptor stimulation leads to increase of NO production (Bruce et al., 2002), inhibitory effect of caffeine on A_{2A}Rs may lead to decrease of NO production. To further investigate the role of the NO-cGMP pathway, we measured NOx levels following acute administration of different doses of caffeine. Based on our results, 100 mg/kg of caffeine significantly decreased NOx levels in the brain tissues. This dose of caffeine was the same dose that did not change the seizure threshold. Thus, it is probable that the effect of 100 mg/kg of caffeine is partly due to a decrease in NO and attenuation of the NO-cGMP pathway effect on seizure activity. In this context, proconvulsant effect of 5 mg/kg of 8-CPT disappeared and NOx levels decreased in the presence of 100 mg/kg of caffeine. This probably points to the involvement of the NO-cGMP pathway in the effect of caffeine on seizure activity. As previously mentioned, the activation of A_{2A}Rs results in an increase in the NO production (Bruce et al., 2002). Thus, antagonizing the effect of 100 mg/kg of caffeine on A_{2A}Rs is likely the main mechanism of the decrease in the NOx levels in the context of the selective antagonizing effect of 8-CPT. In comparison, 5 and 10 mg/kg of SCH-442416 showed proconvulsant effect in the presence of 100 mg/kg of caffeine without any significant change in the NOx levels. One possible mechanism is the effect of caffeine on A_1Rs without affecting A_{2A}Rs in the presence of SCH-442416.

We also examined the effect of pre-treatment with sildenafil before 100 mg/kg of caffeine on PTZ-induced seizure threshold. Pre-treatment with sildenafil resulted in proconvulsant effect in the presence of 100 mg/kg of caffeine. Sildenafil is a selective PDE5 inhibitor that potentiates the NO-mediated signaling pathway by preventing the cGMP degradation (Kaster et al., 2005). Thus, it is obvious that the increase in cGMP as a result of PDE5 inhibition is the main mechanism of proconvulsant effect of pre-treatment with sildenafil. Although caffeine per se is a weak and non-selective inhibitor of PDEs at high doses, the effect of 100 mg/kg of caffeine does not appear to be due to an elevation of cGMP levels. Besides, results obtained from different doses of sildenafil indicate that some effect of 100 mg/kg of caffeine may be due to inhibition of A_{2A}Rs and a subsequent decrease in NO production. One explanation is that the decrease in NO and the resultant decrease in cGMP are more important than the weak inhibitory effect of caffeine on PDE5. Moreover, it has been shown that several PDE inhibitors such as theophylline inhibit sGC activity and decrease the level of cGMP (Francis et al., 2011). However, there is no data regarding the inhibitory effect of caffeine on sGC activity.

There are some limitations to our study. The Griess assay has been utilized extensively as a reliable method to estimate the overall NO production (Heinzen and Pollack, 2002). However, this method indirectly measures total NOx, therefore non NO-related sources of NOx may affect the results. There are more precise methods for measuring NO content, such as electron paramagnetic resonance (EPR) spectroscopy (kozlov et al., 1995). On the other hand, use of ARs knockout mice instead of ARs antagonists could possibly exhibit more accurate results.

5. Conclusions

In conclusion, we showed that acute administration of caffeine at low doses (5 and 50 mg/kg) had a proconvulsant effect, while higher dose of caffeine (100 mg/kg) did not change the seizure threshold. The effect of 100 mg/kg of caffeine on seizure threshold confirmed and extended the previous results showing that some doses of caffeine do not change seizure threshold. Then, we demonstrated that the effect of caffeine is partly mediated through A_1Rs and A_{2A}Rs receptors. Our results suggest that higher dose of caffeine probably has more inhibitory effect on A_{2A}Rs. This may explain why higher dose of caffeine increases the seizure threshold with some doses of A_1Rs antagonist. Finally, we showed the involvement of the NO-cGMP pathway in the central effect of caffeine.

Conflict of interest

The authors declare that they have no conflict of interest.

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