

Anticonvulsant effect of celecoxib on pentylenetetrazole-induced convulsion: modulation by NO pathway

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This study aimed to examine whether celecoxib influences clonic seizure thresholds through modulation of nitric oxidergic (NO) pathway. The effect of celecoxib (1-5 mg/kg, p.o.) was investigated on clonic seizures induced by pentylenetetrazole (PTZ, 50 and 80 mg/kg, i.p.) in male Swiss mice. The interaction of celecoxib-induced effects with NO pathway was examined using a NO synthase (NOS) inhibitor, *N*(G)-omega-nitro-L-arginine methyl ester (L-NAME, 20 and 50 mg/kg, i.p.) and a NOS substrate, L-arginine (100 and 200 mg/kg, i.p.). The criteria for the development of seizure activity were the possibility for appearance of generalized clonus and prolongation of latency to the onset of convulsions following administration of 50 and 80 mg/kg of PTZ, respectively. Pretreatment with celecoxib (2.5 and 5 mg/kg) or L-NAME (50 mg/kg) induced anticonvulsant effect on the PTZ-induced clonic seizures. L-arginine at the dose of 200 mg/kg had proconvulsant effect. A sub-effective dose of celecoxib (1 mg/kg) induced an additive anticonvulsant effect when co-administered with L-NAME (20 mg/kg). Although L-arginine (100 mg/kg) *per se* did not influence PTZ-induced convulsion, it could attenuate the anticonvulsant effect of celecoxib (5 mg/kg). Our results indicate that celecoxib induces an anticonvulsant effect on clonic seizure threshold that may involve NO pathway.

Key words: seizure, celecoxib, L-NAME, L-arginine, pentylenetetrazole, nitric oxide, Cyclooxygenase type 2, mice.

INTRODUCTION

Cyclooxygenases (COXs) catalyze the initial step in the conversion of arachidonic acid to prostaglandins, which are key components of inflammatory response (Simmons et al. 2004, Vane et al. 1998). Two principal forms of the COX enzyme have been found; COX-1 is widely distributed in various cell types, while COX-2 is constitutively expressed in the central nervous system (CNS) and also induced after injury in inflammation sites (Choi et al. 2009). In the CNS, COX-2 is mainly expressed in glutamatergic neurons particularly within the hippocampus and cerebral cortex, the areas that demonstrate prominent role in the onset of seizures (Choi et al. 2009). It was found that COX-2 regulates

cell membrane excitability and long term synaptic plasticity in the hippocampus (Chen et al. 2002), suggesting that COX-2 may play a critical role in convulsive states. However, results of previous studies about the role of COX-2 in the genesis and maintenance of convulsion are controversial; for instance both proconvulsant and anticonvulsant role for COX-2 has been reported in kainic acid-induced seizure (Kelley et al. 1999, Kim et al. 2008). The effects of COX-2 inhibitors on the pentylenetetrazole (PTZ)-induced seizures are also controversial; Dhir and coauthors reported the anticonvulsant effect of COX-2 inhibitors (Dhir et al. 2006), while Akasura and coauthors showed that COX-2 inhibitors have neither anticonvulsant nor proconvulsant effects on PTZ-induced seizures (Akarsu et al. 2006).

Nitric oxide (NO), a gaseous messenger molecule is regarded as a *bona fide* neuronal transmitter (Zhou and Zhu 2009). It is synthesized from L-arginine by different subtypes of nitric oxide synthases (NOSs),

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including inducible, neuronal and endothelial NOS (Zhou and Zhu 2009). Inducible NOS like COX-2 is not expressed constitutively in cells and rather is induced following proinflammatory stimuli (Bredt 1999). NO has been demonstrated to be either proconvulsant or anticonvulsant depending on the seizure model. It attenuates electric currents- or kainic acid-induced seizures (Nidhi et al. 1999, Noh et al. 2006), whereas potentiates PTZ-induced seizures (Nidhi et al. 1999, Uzum et al. 2005, Riazi et al. 2006).

The simultaneous biosynthesis and release of both NO and prostaglandins in many tissues and their role in the pathophysiological mechanisms underlying the inflammatory responses implies possible interaction between NO and prostaglandins biosynthetic pathways (Salvemini 1997, Mollace et al. 2005). Further, it has been reported that NO might regulate COX activity (Salvemini 1997, Kim et al. 2005).

In spite of evidences for the effects of COX-2 and NOS inhibitors on seizures, the possible interaction of prostaglandins and NO biosynthetic pathways in seizures remains obscure. Here, we tried to examine the effects of celecoxib (a COX-2 inhibitor) on PTZ-induced seizure in mice. We also investigated the role of NO pathway in the effect of celecoxib on seizure threshold by using a NOS inhibitor, *N*(G)-omega-nitro-L-arginine methyl ester (L-NAME) and a NOS substrate, L-arginine.

MATERIALS AND METHODS

Animals

Adult male Swiss mice (Institute Pasteur of Iran, Tehran, Iran), aged 6-8 weeks and weighing 20-25 g were used. The animals were housed in a temperature-controlled (around 24°C) colony room. They were maintained in a 12-h on and 12-h off light/dark schedule with free access to food and water, except during experimental procedures. Groups consisted of 10-12 or 19-21 animals depending on the experiment and each animal was used only once. All animal procedures were conducted in accordance with the guidelines for the care and use of laboratory animals published by national institutes of health and with approval of Ethics Committee on Animal Experiments of Tehran University of Medical Sciences.

Drugs

All drugs were purchased from Sigma (St. Louis, MO, USA), except celecoxib which was obtained from Pfizer (New York, NY, USA). Celecoxib was suspended in 0.5% carboxymethyl cellulose and administrated *per os* (p.o.). PTZ, L-NAME and L-arginine were dissolved in sterile normal saline solution and injected intraperitoneally (i.p.) in a volume of 10 ml/kg. Dosage of PTZ used in the present study was based upon previous studies indicating that

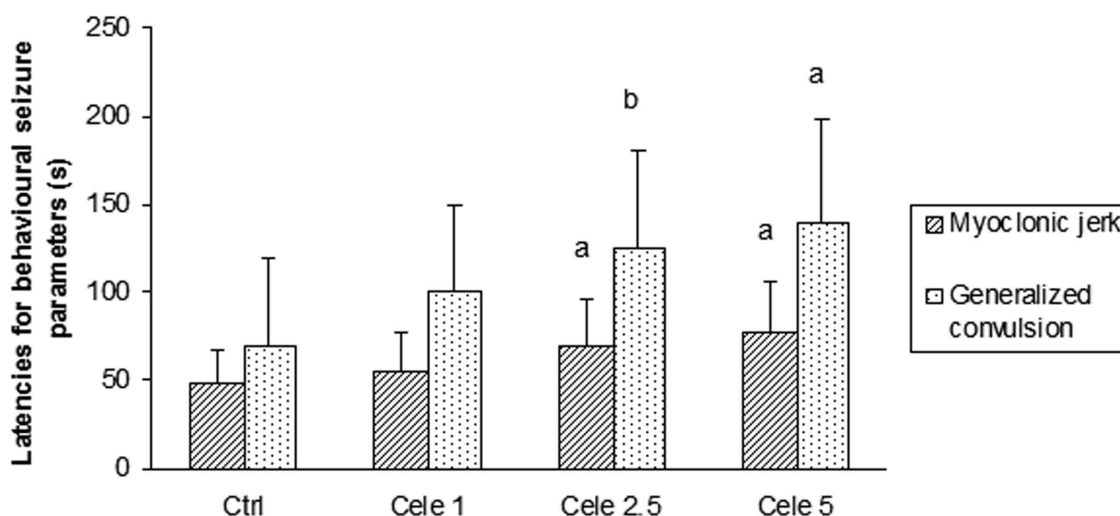


Fig. 1. Effect of celecoxib (1, 2.5 and 5 mg/kg p.o.) on the latency for myoclonic jerk and generalized convulsion induced by PTZ (80 mg/kg i.p.). Data represents median $\pm$ interquartile range of 10 to 12 mice. ^a $p < 0.01$ compared to control group; ^b $p < 0.05$ compared to control group. Ctrl, control; Cele, celecoxib.

80 and 50 mg/kg of PTZ can elicit clonic seizure in all and near to 50% of animals, respectively (Homayoun et al. 2002, Tutka et al. 2004, Wesolowska et al. 2006). In addition, mice were administered either celecoxib or 0.5% carboxymethyl cellulose vehicle. In experiments investigating the possible role of NO pathway in the celecoxib effects, mice were administered L-NAME, L-arginine or saline vehicle. The dosages and timing of administrations of celecoxib, L-NAME and L-arginine in the present study were based upon prior reports (Dhir et al. 2006, Gholipour et al. 2008, Oliveira et al. 2008).

Behavioral seizure evaluation

PTZ was administrated at the dose of either 50 or 80 mg/kg. Immediately after injection of PTZ, animals were transferred to a round open field (50 cm in diameter) and monitored for the appearance of convulsion for 20 min. Following the administration of 50 mg/kg of PTZ presence or absence of generalized clonus was determined. In contrast, when convulsion was elicited by 80 mg/kg of PTZ, time latencies for first myoclonic jerk and generalized clonus were measured. Latencies were calculated as a time between PTZ infusions to the onset of these stages. Generalized clonus was described by the involvement of all four limbs and tail, rearing, wild running and jumping, sudden loss of upright posture and autonomic signs such as hypersalivation and defecation.

Experiments

The current study was conducted in two different parts. In the first part, convulsion was stimulated by 80

mg/kg of PTZ and time latencies for first myoclonic jerk and generalized clonus were assessed. First, the effect of systemic administration of COX-2 inhibitors on seizure induced by PTZ was evaluated by treating animals with celecoxib (1, 2.5 or 5 mg/kg, p.o.) or its vehicle 60 min prior to the determination of the threshold of clonic seizures. Secondly, the possible involvement of nitric oxidergic pathway in seizure was evaluated. In this step, animals received pretreatment with L-NAME (20 or 50 mg/kg, i.p.) 30 min or L-arginine (100 or 200 mg/kg, i.p.) 45 min before PTZ administration.

Thereafter, possible involvement of the nitric oxidergic system in effects of celecoxib on clonic seizures was investigated. For this reason, time latencies for the development of first myoclonic jerk and generalized clonus after 80 mg/kg injection of PTZ as well as the possibility for the appearance of generalized clonus following administration of 50 mg/kg of PTZ were determined. Animals were treated with sub-effective dose of celecoxib (1 mg/kg) 60 min and sub-effective dose of L-NAME (20 mg/kg) 30 min before determination of seizure threshold or they received pretreatment with effective dose of celecoxib (5 mg/kg) and sub-effective dose of L-arginine (100 mg/kg), 60 and 45 min before PTZ administration, respectively.

Statistical analysis

Groups were compared using the Fisher's exact test for categorical variables and Kruskal-Wallis nonparametric one-way analysis of variance for continuous variables. Post hoc analyses were carried out by the Dunn's multiple comparison test. $p < 0.05$ was considered significant.

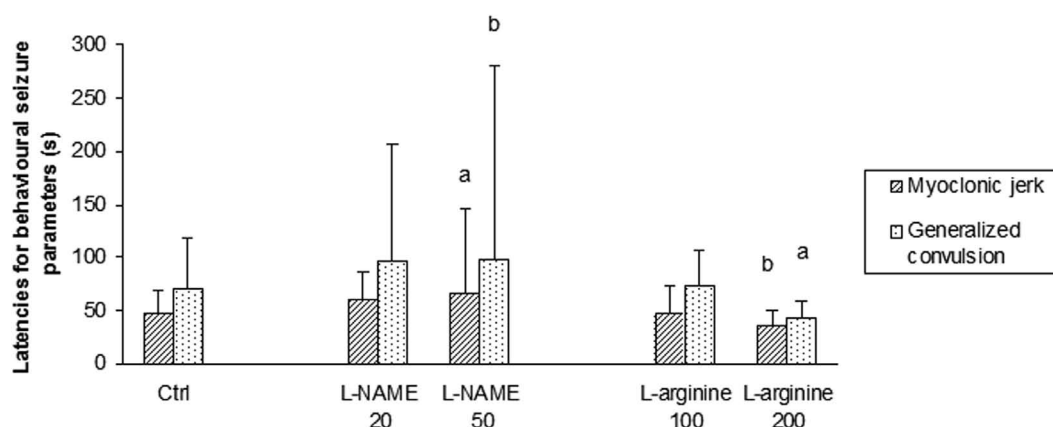


Fig. 2. Effects of L-NAME (20 and 50 mg/kg i.p.) and L-arginine (100 and 200 mg/kg i.p.) on the latency for myoclonic jerk and generalized convulsion induced by PTZ (80 mg/kg i.p.). Data represents median $\pm$ interquartile range of 10 to 12 mice. ^a $p < 0.05$ compared to control group; ^b $p < 0.01$ compared to control group. Ctrl, control.

Table I

Effects of celecoxib alone and in combination with L-NAME or L-arginine on the development of generalized convulsion following administration of 50 mg/kg of PTZ

	Ctrl	L-NAME 20	Cele 1	L-NAME 20 + Cele 1
Convulsions	10/19	9/19	10/21	4/21*
	Ctrl	L-arginine 100	Cele 5	L-arginine 100 + Cele 5
Convulsions	10/19	11/20	6/20	10/21

Data are expressed as a number of convulsing animals in relation to the total number of animals tested in the single experiment. * $p < 0.05$ compared to control and celecoxib 1 mg/kg group. Ctrl, control; Cele, celecoxib.

RESULTS

Kruskal-Wallis revealed a significant effect of celecoxib ($H_3=21.6$, $p < 0.01$ for myoclonic jerk and $H_3=19.3$, $p < 0.01$ for generalized convulsion). Post hoc analysis demonstrated that pretreatment with celecoxib at the doses of 2.5 and 5 mg/kg significantly increased time latencies for the first myoclonic jerk and generalized convulsion ($p < 0.05$, Fig. 1). Since 1 mg/kg of celecoxib could not attenuate PTZ-induced convulsion ($p > 0.05$), it was considered as a subeffective dose of celecoxib.

Figure 2 shows the effects of different doses of L-NAME (20 and 50 mg/kg) and L-arginine (100 and 200 mg/kg) on myoclonic jerk and generalized convulsion induced by PTZ. L-NAME at the dose of 50 mg/kg increased time latencies for the onset of myoclonic jerk and generalized convulsion ($p < 0.05$). On the contrary, 200 mg/kg of L-arginine reduced time latencies for first myoclonic jerk ($p < 0.05$) and generalized convulsion ($p < 0.01$). Lower doses of L-NAME (20 mg/kg) and L-arginine (100 mg/kg) were regarded as subeffective doses ($p > 0.05$).

Fig. 3 shows that concurrent administration of sub-effective doses of celecoxib (1 mg/kg) and L-NAME (20 mg/kg) had significant inhibitory effects on the appearance of myoclonic jerk and generalized convulsion ($p < 0.05$) elicited by PTZ. In addition, L-NAME (20 mg/kg) significantly enhanced the protective activity of celecoxib (1 mg/kg) against the development of generalized convulsion ($p < 0.05$, Fig. 3 and Table I). L-arginine at the dose of 100 mg/kg blunted the inhibitory effect of celecoxib on the appearance of generalized convulsion and myoclonic jerk ($p < 0.05$ compared to the animals treated with 5

mg/kg of celecoxib and $p > 0.05$ compared to the control group, Fig. 4). L-arginine (100 mg/kg) had a tendency to increase the incidence of generalized convulsion in animals received pretreatment with celecoxib (5 mg/kg), although the difference did not reach significant levels (Table I).

DISCUSSION

The results of the current study indicate that the selective COX-2 inhibitor, celecoxib at the doses of 2.5 and 5 mg/kg attenuates seizure induced by PTZ, an agent which blocks chloride channels in the γ -aminobutyric acid receptor (GABA) complex (Loscher and Schmidt 1988). Co-administration of sub-effective doses of L-NAME (20 mg/kg) and celecoxib (1 mg/kg) protected the animals against PTZ. In addition, L-NAME improved the anticonvulsant activity of celecoxib significantly. L-arginine at the dose which was not able to influence PTZ-induced convulsion, blunted the anticonvulsant effect of celecoxib.

COX-2 is responsible for the pathologic production of prostaglandins in response to a variety of stimuli from proinflammatory factors (e.g. cytokines and endotoxins), seizure activity, brain injury to the activation of N-methyl-D-aspartate (NMDA) receptors (i.e. main receptors for the excitatory neurotransmitter, L-glutamate) (Yang and Chen 2008). As mentioned earlier, COX-2 is also constitutively expressed in the CNS neurons, but enriched in the hippocampus and cortex (Choi et al. 2009). This study report that celecoxib can decrease or have no effect on PTZ-induced seizures, depending on the dose of drug administered. It appears that seizure activity incrementally causes an indiscrimi-

nate and widespread induction of long-term potentiation (LTP) (Reid and Stewart 1997). In addition, it has been shown that COX-2 expression is up-regulated by high frequency stimulation (HFS) associated with the induction of LTP (Bliss and Collingridge 1993, Yamagata et al. 1993). Likewise, another experiment showed that selective COX-2 inhibitors reduce HFS-induced LTP at hippocampal synapses (Chen et al. 2002). Moreover, exogenous application of prostaglandin E2 which is main product of COX-2 increases frequency of firing, excitatory postsynaptic potentials amplitude and temporal summation in hippocampal slices treated with a selective COX-2 inhibitor (Chen and Bazan 2005). In accordance with aforementioned findings, Yang and Chen has suggested that COX-2 along with prostaglandin E2 may dynamically regulate membrane excitability (Yang and Chen 2008). On the other hand, Both basal expression and seizure-induced over-expression of COX-2 are reduced by blocking NMDA receptors which are involved in the initiation of seizure (Gholipour et al. 2008, Toscano et al. 2008, Wojtal et al. 2003, Yamagata et al. 1993). Further, selective COX-2 inhibitors block NMDA neurotoxicity (Hewett et al. 2000, Strauss and Marini 2002). Therefore, it is possible that above mentioned mechanisms, may underlie the currently reported anticonvulsant effect of celecoxib.

In the CNS, NO behaves as a multifunctional messenger and neurotransmitter, influences various physiological and pathological functions (Hoffman 1991,

Moncada et al. 1991, Feil and Kleppisch 2008). The main intracellular action of NO is activation of the soluble guanylate cyclase which leads to the formation of cycline guanine monophosphate (cGMP) in the CNS (Garthwaite et al. 1988, Feil and Kleppisch 2008). An increase in cGMP follows stimulation of L-glutamate receptors mainly of the NMDA type (Garthwaite et al. 1988, Bredt and Snyder 1989, Feil and Kleppisch 2008). It has been shown that NO as a retrograde messenger that is synthesized postsynaptically and acts on presynaptic terminals, plays an important role in hippocampal LTP (Bon and Garthwaite 2003, Feil and Kleppisch 2008). Further, there is direct evidence from hippocampal cultures that NO can potentiate synaptic transmission (Arancio et al. 2001). In addition, Itoh and others has reported that PTZ kindling in mice is associated with an increase in the amount of neuronal NOS (Itoh et al. 2004). In concordance with aforementioned findings, NO is considered to be involved in the pathophysiology of epilepsy, although the results of experiments carried out by several authors are often conflicting (Wojtal et al. 2003). Different findings may arise as a consequence of discrepancies in the kinds of drugs, the model of seizures and the species of animals used in experiments. In this study we showed that L-NAME, a drug that inhibits all subtypes of NOS non-specifically, attenuates PTZ-induced convulsion dose-dependently which is consistent with previous studies (Hodayoun et al. 2002, Riazi et al. 2006, Gholipour et al. 2008).

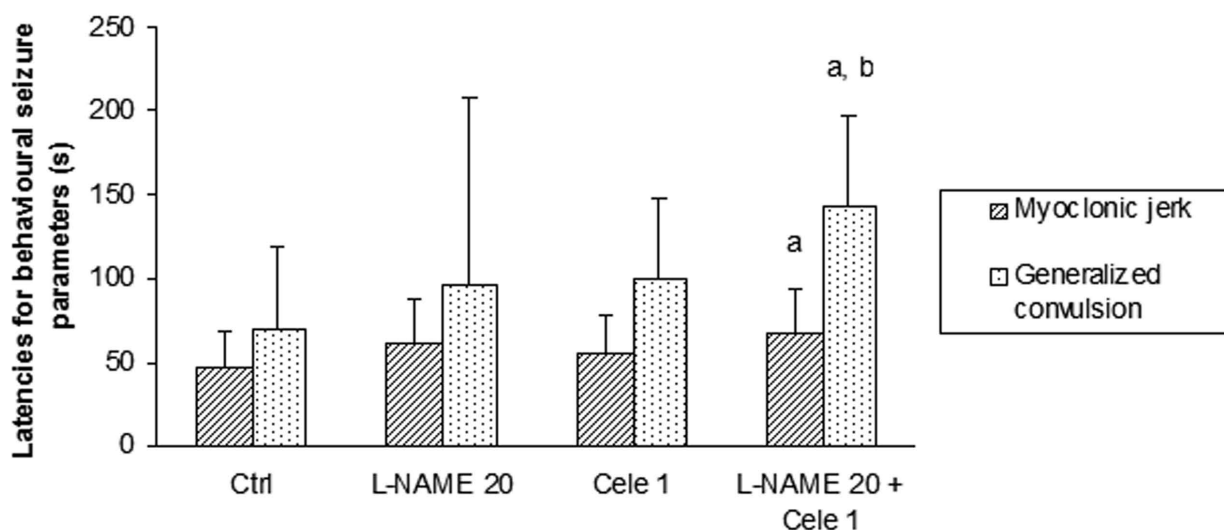


Fig. 3. Effect of concurrent administration of sub-effective doses of celecoxib (1 mg/kg) and L-NAME (20 mg/kg) on the latency for myoclonic jerk and generalized convulsion induced by PTZ (80 mg/kg i.p.). Data represents median $\pm$ interquartile range of 10 to 12 mice. ^a $p < 0.05$ compared to control group. ^b $p < 0.05$ compared to Cele 1 group. Ctrl, control; Cele, celecoxib.

There are evidences implying the possible interaction between NO and COX pathways in some pathophysiological states including osteoarthritis, angiogenesis, renal perfusion and endotoxin-induced cardiomyopathy (Abramson et al. 2001, Beierwaltes 2002, Davel et al. 2002, Mebazaa and coworkers 2001). Salvemini et al. for the first time discovered that COX activity is regulated by NO. They found that NO directly increases COX-1 activity which leads to 7-fold increase in prostaglandin E2 production (Salvemini et al. 1993). Similarly, it has been reported that inhibition of NOS inhibits not only NO but also prostaglandin production (Lazarewicz et al. 2000, Salvemini et al. 1995), suggesting that COX enzymes are targets for pathophysiological roles of NO (Salvemini 1997). Kim and coauthors reported that apart from activation of COX-2 by free NO molecules, inducible NOS binds to COX-2 and activates it (Kim et al. 2005). There are evidences regarding that the interaction between NO and prostaglandin production pathways occurs at multilevels. For example, COX-2 expression is also modulated, and up-regulated by NO (Hughes et al. 1999). In addition, it has been shown that inducible NOS inhibitors ameliorate the antihyperalgesic effect of COX-2 inhibitors (Dudhgaonkar et al. 2007). Most of these findings indicate the interaction between NOS and COX-2 in inflammatory responses. However, to our knowledge it has not been studied whether there is possible “cross talk” between

NO and prostaglandin biosynthetic pathways in a seizure model.

The major limitation of this study is the possible pharmacokinetic interaction between agents affecting NO synthesis and celecoxib. Therefore, when interpreting data this possibility needs to be considered and it may in part contribute to the observed effects.

In this study we showed that co-administration of sub-effective doses of celecoxib and L-NAME attenuates PTZ-induced convulsion. Moreover, we found that L-arginine blunts the protective effect of celecoxib against generalized convulsion. These findings for the first time extend the observations for the interaction between NO and prostaglandins biosynthetic pathways to a seizure model in mice. The molecular mechanisms involved in this interaction require further investigation. Our findings may have therapeutic relevance. Drugs that modulate either of these two pathways apart from influencing the other pathway indirectly, can also affect susceptibility of epileptic patients to seizure. Since, these two pathways influence the anticonvulsant action of different antiepileptic drugs (Dhir and Kulkarni 2006, Luszczki et al. 2007, Tayal et al. 2008), further investigations in this field can help clinicians to regulate the therapeutic dose of antiepileptic drugs in those patients who simultaneously use anti-inflammatory agents. However, because NOS and COX-2 inhibitors can either promote or attenuate seizures, depending on

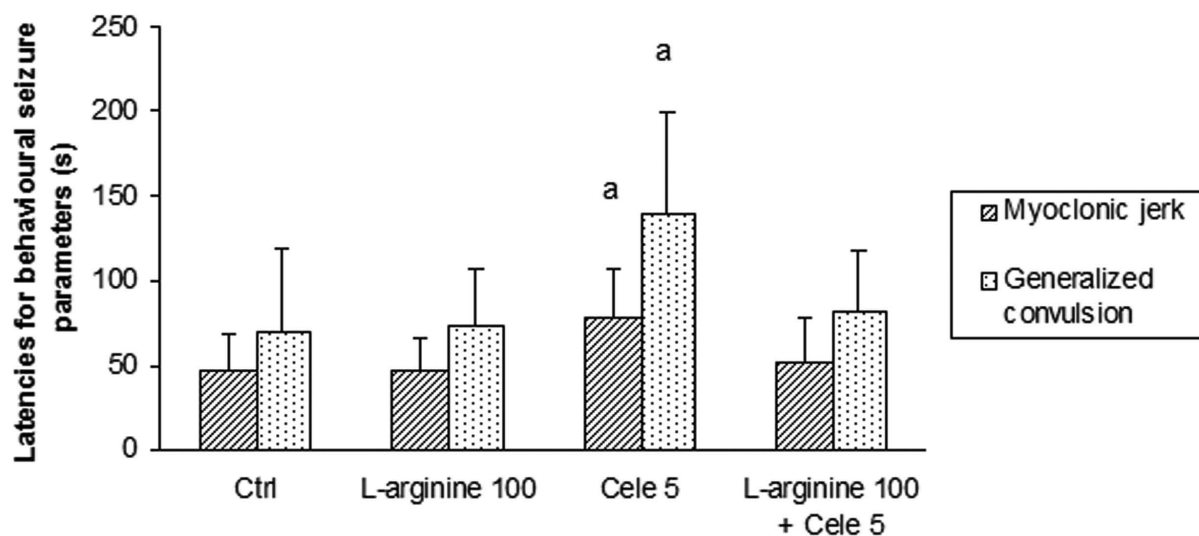


Fig. 4. Effect of concurrent administration of effective dose of celecoxib (5 mg/kg) and subeffective dose of L-arginine (100 mg/kg) on the latency for myoclonic jerk and generalized convulsion induced by PTZ (80 mg/kg i.p.). Data represents median $\pm$ interquartile range of 10 to 12 mice. * $p < 0.05$ compared to other groups. Ctrl, control; Cele, celecoxib.

the experimental convulsive model used, further studies should be performed to elucidate which epileptic patients can get benefit from these kinds of interventions.

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