European Journal of Pharmacology 791 (2016) 523–534

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Neuropharmacology and analgesia

Pharmacokinetic-pharmacodynamic influence of N-palmitoylethanolamine, arachidonyl-2′-chloroethylamide and WIN 55,212-2 on the anticonvulsant activity of antiepileptic drugs against audiogenic seizures in DBA/2 mice

Rita Citraro [a], Emilio Russo [a], Antonio Leo [a], Roberto Russo [b], Carmen Avagliano [b], Michele Navarra [c], Antonio Calignano [b], Giovambattista De Sarro [a,*]

[a] Science of Health Department, Clinical Pharmacology Unit, School of Medicine, University "Magna Graecia" of Catanzaro, Italy
[b] Department of Pharmacy, University of Naples "Federico II", Naples, Italy
[c] Department of Experimental Pharmacology, University of Messina, Messina, Italy

ARTICLE INFO

Article history:
Received 17 March 2016
Received in revised form
16 September 2016
Accepted 19 September 2016
Available online 20 September 2016

Keywords:
PEA
Cannabinoid compounds
PPAR-α receptors
Audiogenic seizure model
Antiepileptic drugs
Drug interaction

ABSTRACT

We evaluated the effects of ACEA (selective cannabinoid (CB_1) receptor agonist), WIN 55,212-2 mesylate (WIN; non-selective CB_1 and CB_2 receptor agonist) and N-palmitoylethanolamine (PEA; an endogenous fatty acid of ethanolamide) in DBA/2 mice, a genetic model of reflex audiogenic epilepsy. PEA, ACEA or WIN intraperitoneal (i.p.) administration decreased the severity of tonic-clonic seizures. We also studied the effects of PEA, WIN or ACEA after co-administration with NIDA-41020 (CB_1 receptor antagonist) or GW6471 (PPAR-α antagonist) and compared the effects of WIN, ACEA and PEA in order to clarify their mechanisms of action. PEA has anticonvulsant features in DBA/2 mice mainly through PPAR-α and likely indirectly on CB_1 receptors, whereas ACEA and WIN act through CB_1 receptors. The co-administration of ineffective doses of ACEA, PEA and WIN with some antiepileptic drugs (AEDs) was examined in order to identify potential pharmacological interactions in DBA/2 mice. We found that PEA, ACEA and WIN co-administration potentiated the efficacy of carbamazepine, diazepam, felbamate, gabapentin, phenobarbital, topiramate and valproate and PEA only also that of oxcarbazepine and lamotrigine whereas, their co-administration with levetiracetam and phenytoin did not have effects. PEA, ACEA or WIN administration did not significantly influence the total plasma and brain levels of AEDs; therefore, it can be concluded that the observed potentiation was only of pharmacodynamic nature. In conclusion, PEA, ACEA and WIN show anticonvulsant effects in DBA/2 mice and potentiate the effects several AEDs suggesting a possible therapeutic relevance of these drugs and their mechanisms of action.

1. Introduction

The endocannabinoid system has been demonstrated to play a protective role in many central nervous system disorders including epilepsy (Friedman and Devinsky, 2015; Goffin et al., 2011; Leo et al., 2016; Mattace Raso et al., 2014). It has been shown that both some natural cannabinoids (especially, Δ9-tetrahydrocannabinol and cannabidiol) and cannabinoid receptor agonists possess different anticonvulsant effects in several animal models of epilepsy also acting through non cannabinoid receptors (Devinsky et al., 2014; Hill et al., 2012; Jones et al., 2011). Different studies indicated that the endogenous cannabinoid ligands anandamide (AEA) and 2-arachidonylglycerol (2-AG) and the related non-cannabinoid ligand N-palmitoylethanolamine (PEA; an endogenous bioactive fatty acid amide) have anticonvulsant properties in numerous in vitro and in vivo models (Citraro et al., 2013a, 2013b; Lambert et al., 2001; Sheerin et al., 2004; Wallace et al., 2003, 2002); in agreement, (R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinyl-methyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1 naphthalenyl-methanone mesylate (WIN 55,212-2 mesylate, a non-selective CB_1/CB_2 receptor agonist) and arachidonyl-2-chloroethylamide (ACEA, a selective cannabinoid CB_1 receptor agonist) exerted anticonvulsant properties in several animal models of epilepsy (Citraro et al., 2013a; Kozan et al., 2009; Shafaroodi et al., 2013; Vilela et al., 2013; Wallace et al., 2003, 2002). PEA, an endogenous fatty acid amide analogue of the endocannabinoid AEA, produced on-demand within the lipid bilayer (Costa et al., 2008; Petrosino et al., 2010) plays a crucial role in the regulation of several pathophysiological processes, such as pain, inflammation,

* Corresponding author.
E-mail address: desarro@unicz.it (G. De Sarro).

http://dx.doi.org/10.1016/j.ejphar.2016.09.029
0014-2999/

neurotoxicity and seizures (Calignano et al., 2001; Citraro et al., 2013b; D'Agostino et al., 2012; Lambert et al., 2002; Mattace Raso et al., 2014; Re et al., 2007). In particular, PEA has protective effects both against maximal electroshock seizures (MES) and tonus in the pentylentetrazole (PTZ) model of seizures (Lambert et al., 2001). Moreover, it has also been observed that a pretreatment with PEA increased the latency to seizures' onset as well as decreased the duration of PTZ-induced seizures (Aghaei et al., 2015). PEA also increased the latency to clonus in the kindling amygdaloid model without, however, affecting duration of clonus and after discharge (Sheerin et al., 2004). Finally, the anticonvulsant effects of PEA have also been reported in a genetic absence epilepsy model (Citraro et al., 2013b). Several mechanisms have been proposed to explain the effects of PEA (Petrosino et al., 2010); to date it is widely recognized that PEA does not bind directly to CB_1/CB_2 receptors, whereas its pharmacological effects are mainly mediated by activation of peroxisome proliferator-activated receptor (PPAR-α) (Di Cesare Mannelli et al., 2013; Hansen, 2010; Lo Verme et al., 2005; O'Sullivan and Kendall, 2010; Raso et al., 2011; Sugiura et al., 2000; Howlett et al., 2004). It has been recognized that WIN potentiates the anticonvulsant activity of diazepam, carbamazepine, phenytoin, phenobarbital, lamotrigine, pregabalin, topiramate and valproate in the MES model (Luszczki et al., 2011b, 2013; Naderi et al., 2008); it also enhances the anticonvulsant activity of ethosuximide, phenobarbital and valproate in the mouse PTZ-induced seizures model (Luszczki et al., 2011a). ACEA increased the anticonvulsant effects of valproate and phenobarbital (Andres-Mach et al., 2012; Luszczki et al., 2006; Luszczki et al., 2010). It has been described that PEA might potentiate the effect of AEA on CBRs and/or vanilloid receptor 1 (TRPV1) (Costa et al., 2008; De Petrocellis et al., 2001; Ho et al., 2008). In certain situations (i.e. after repeated treatments), PEA reduces AEA hydrolysis employing fatty acid amide hydrolase (FAAH) (Ben-Shabat et al., 1998; Di Marzo et al., 2001; Lambert and Di Marzo, 1999). Nevertheless, in WAG/Rij rats (an animal model of absence epilepsy), we showed that PEA exerts anticonvulsant effects mainly acting on PPAR-α receptor and only indirectly acting on CBRs (Citraro et al., 2013b).

Based on this evidence, we have investigated the anticonvulsant mechanisms of WIN, ACEA and PEA and determined their interaction with some AEDs (i.e. carbamazepine, diazepam, felbamate, gabapentin, lamotrigine, levetiracetam, oxcarbazepine, phenytoin, phenobarbital, topiramate and valproate) against audiogenic seizures in the DBA/2 mouse, an experimental model of audiogenic seizures (Citraro et al., 2011; De Sarro et al., 2015; Donato Di Paola et al., 2007). Finally, we have also detected free plasma and total brain concentrations of some AEDs in order to ascertain any pharmacokinetic contribution to the observed interactions between tested drugs.

2. Materials and methods

2.1. Animals

Male DBA/2 mice weighing 8–12 g (22–26 days old) or 20–28 g (48–56 days old) were used. Mice were purchased from Harlan Italy srl (Correzzana, Milan, Italy), housed in groups and kept under controlled conditions of humidity (60 ± 5%) and temperature (21 ± 2 °C), with a reversed light/dark (12/12 h) cycle (light on at 19.00). Mice were allowed free access to standard laboratory chow and tap water until the time of experiments. Procedures involving animals and their care were performed in agreement with international and national law and policies (EU Directive 2010/63/EU for animal experiments, ARRIVE guidelines and the Basel declaration including the 3 R concept). All efforts were made to minimize animal suffering and to use only the number of animals necessary to have reliable scientific data.

2.2. Drugs

Felbamate (Schering Plough, Milano, Italy), carbamazepine and oxcarbazepine (Novartis, Basel, Switzerland) and diazepam (Hoffman La Roche, Basel, Switzerland) were dissolved in a 1% solution of Tween 80. Gabapentin (Sigma-Aldrich, Milan, Italy), levetiracetam (UCB Pharma, Braine-l'Alleud, Belgium), valproate (Mg^{2+} salt; Sigma Tau, Pomezia, Italy), phenobarbital (Na^+ salt; Sigma-Aldrich, Milan, Italy), lamotrigine (Glaxo-Wellcome, Verona, Italy), phenytoin (Na^+ salt; Sigma-Aldrich, Milan, Italy) and topiramate (personal gift from Dr. R.P. Shank; Johnson & Johnson Pharmaceutical Research & Development LCC, USA) were dissolved in 0.9% sterile saline. N-palmitoylethanolamine (PEA, Sigma-Aldrich, Milan, Italy), (R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)-pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl]-1 naphthalenyl-methanone mesylate (WIN 55,212-2, Sigma-Aldrich, Milan, Italy), arachidonyl-2-cyclopropylamide (ACEA, Tocris Bioscience, Bristol, UK) and GW6471, a PPAR-α antagonist (Sigma-Aldrich, Milan, Italy) were suspended in a 5% solution of Tween 80, 5% polyethylene glycol (PEG) and in 90% sterile saline before i.p. administration. 1-(2,4-Dichlorophenyl)-5-(4-methoxyphenyl)-4-methyl-N-(1-piperidinyl)-1H-pyrazole-3-carboxamide (NIDA-41020; Sigma-Aldrich, Milan, Italy) was dissolved in a minimum amount of dimethyl sulfoxide (DMSO); the final volume was made up with sterile saline before i.p. administration. All drugs were administered i.p. at a volume of 0.1 ml/10 g of body weight. Control animals received equivalent volumes of the vehicle at the respective times before the test as previously described (Russo et al., 2013).

2.3. Experimental design

DBA/2 mice were treated with: PEA (5–40 mg/kg, i.p.) 30, 60, 90 or 120 min before auditory stimulation; ACEA (0.5–30 mg/kg; i.p.) 30, 60 or 90 min before auditory stimulation; WIN (2.5–60 mg/kg; i.p.) 10, 20 or 30 min before auditory stimulation; NIDA-41020 (0.5–2 mg/kg; i.p.) 45 min before auditory stimulation and GW64721 intracerebroventricularly (i.c.v.) (0.5–4 μg/mouse) or vehicle 30 min before auditory stimulation. The different times and route of drugs administration were chosen according to previously published articles or personal pilot studies (Andres-Mach et al., 2012; Citraro et al., 2013a, 2013b; Payandemehr et al., 2015).

AEDs were administered according to previously published studies (see Results sections) (De Sarro et al., 2000; De Sarro et al., 1998). Mice, under fluothane anesthesia, were injected (i.c.v.) in the left or right lateral ventricle (coordinates 1 mm posterior and 1 mm lateral to the bregma; depth 2.4 mm) through a 5 μl Hamilton microsyringe fitted with a nylon cuff on the needle, as previously described (De Sarro et al., 2004). Sound stimulation (12–16 kHz; 109 dB) was applied for 1 min or until tonic extension occurred (Sarro et al., 2012).

2.4. Determination of plasma and brain levels of AEDs and effects on motor coordination

DBA/2 mice were administered i.p. with vehicle or one compound studied (PEA, ACEA, WIN, NIDA-41020 or GW6471) plus one AED before audiogenic test. Behavioral and pharmacokinetic studies were carried out under the same protocol (i.e. timing and dosing). Drug level determination was performed by HPLC or immunoenzymatic assay in older mice (see Section 2.1) in order to avoid difficulties in blood sampling, as previously described (Russo et al., 2013). No pharmacokinetic changes were described between 21–26 and 48–56 days old mice (De Sarro et al., 2002; Sarro et al., 2012). Rotarod test (U. Basile, Comerio, Varese, Italy) was carried out

in groups of 10 DBA/2 mice administering all drugs under investigation alone or in combination in order to determine their TD_{50} values (± 95% confidence limits) by the method of Litchfield and Wilcoxon (1949), as previously reported (Russo et al., 2013).

2.5. Statistical analysis

Groups of control and drug-treated mice were statistical analyzed by Fisher's exact probability test or analysis of variance (ANOVA) and Dunnett's test whereas ED_{50} or TD_{50} values were calculated and statistically analyzed using the method of Litchfield and Wilcoxon as previously reported (Russo et al., 2013). The percent occurrence of seizure phases of the administered compounds and the dose-response curves were close-fitting using linear regression method. The lines of best fit of AEDs plus vehicle or one cannabinoid were compared using a $\chi 2$-test, as previously described De Sarro et al. (2012). The plasma levels of the studied compounds are expressed as means ± S.E.M. of at least eight determinations and Student's *t*-test was performed for statistical comparison. $P \leq 0.05$ was considered statistically significant. The statistical software used was GraphPad Prisma 6.0 (La Jolla, CA, USA).

3. Results

3.1. Effects of PEA, ACEA and WIN against audiogenic seizures in DBA/2 mice

Administration of vehicles had no effects on all phases of audiogenic seizures (data not shown). The effects of PEA, ACEA and WIN were studied at different times after administration, on different parameters and various doses (Section 2.2). PEA (5–40 mg/kg), ACEA (0.5–30 mg/kg) and WIN (5–60 mg/kg) administered i.p. reduced the severity of tonic and clonic phases of audiogenic seizures in DBA/2 mice in a dose-dependent manner (Figs. 1–3); the obtained ED_{50} values (± 95% confidence limits) for each compound are reported in Table 1. ACEA and PEA were also effective against the wild running phase of the audiogenic seizures (Table 1, Figs. 1 and 2) whereas,

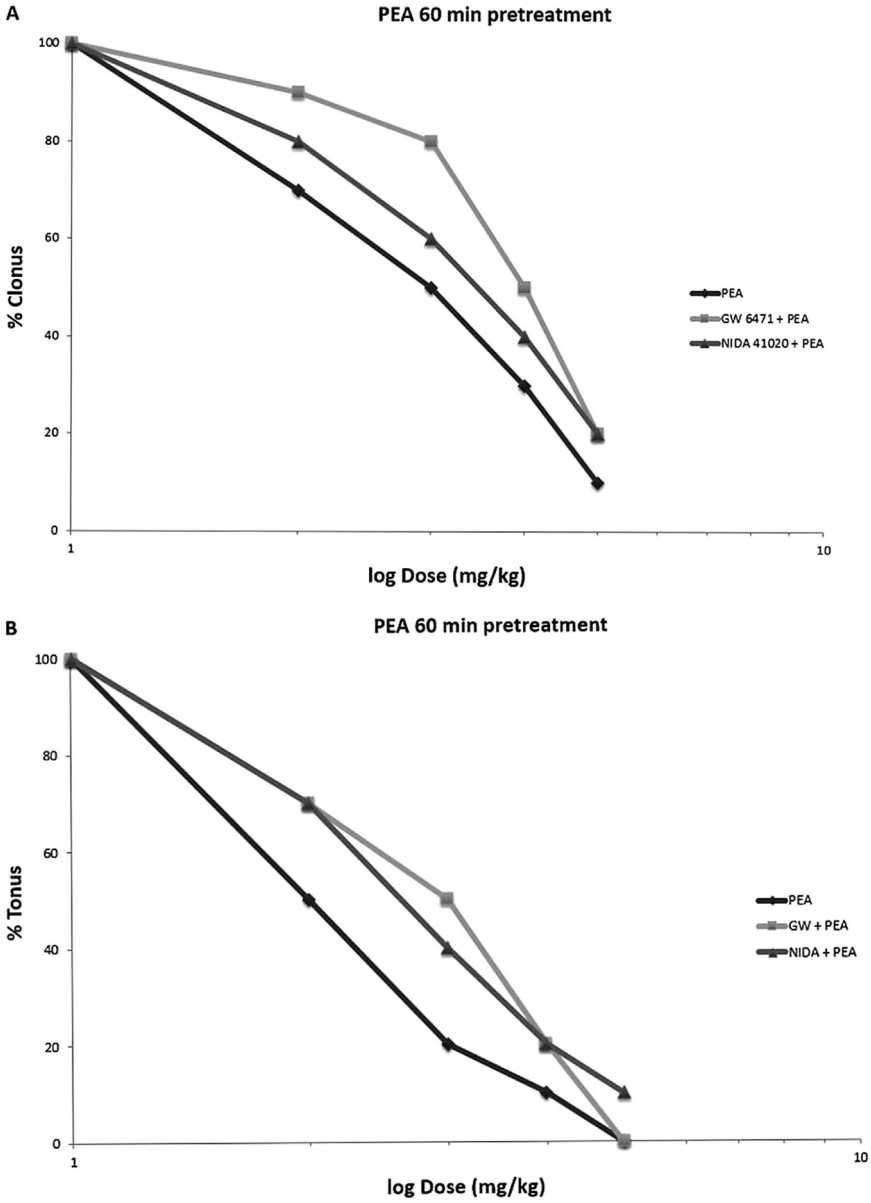

Fig. 1. Dose-response curves of the anticonvulsant effects of PEA (5–40 mg/kg) 60 min after i.p administration alone or in combination with GW6471 (2 μg/mouse, i.c.v.; 30 min after PEA) and NIDA-41020 (1 mg/kg, i.p.; 15 min after PEA). Abscissa shows the log doses, ordinate shows (A) percentage of clonic seizures, (B) percentage of tonic seizures.

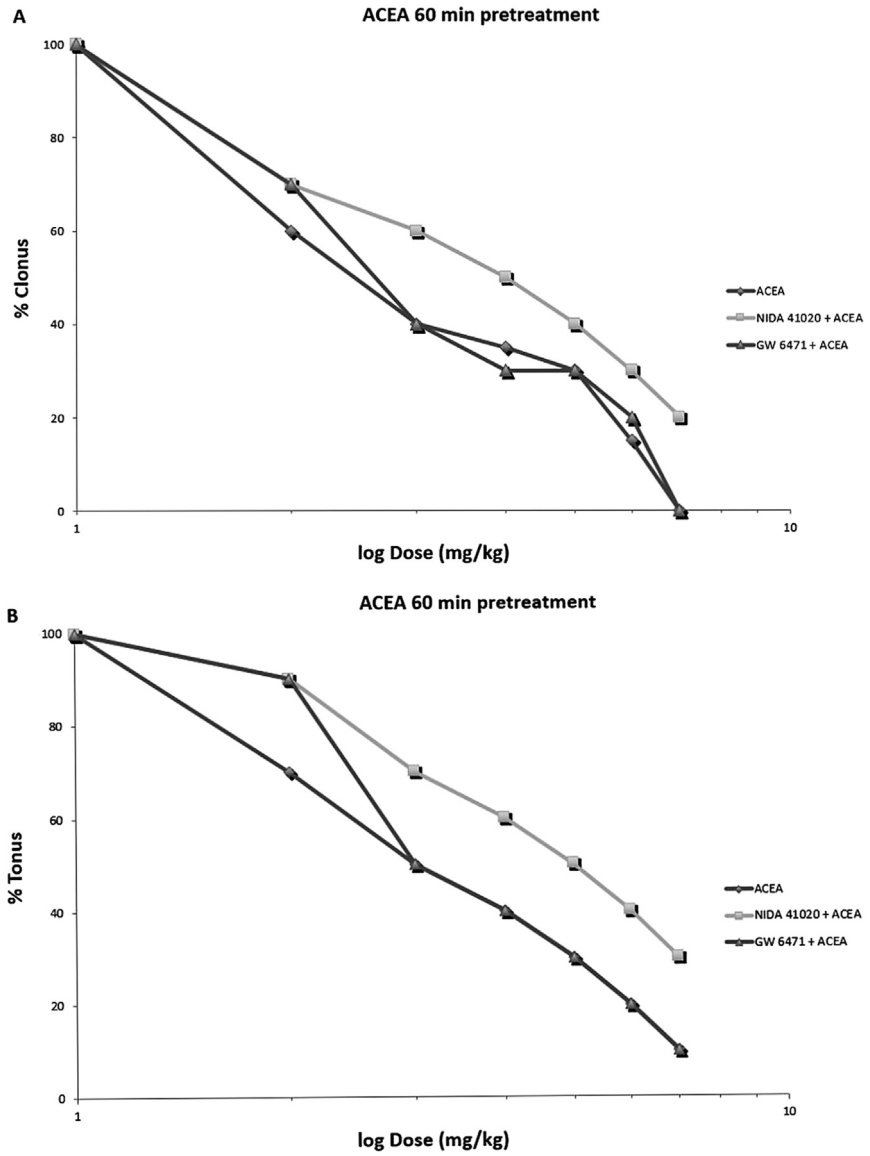

Fig. 2. Dose-response curves of the anticonvulsant effects of ACEA (0.5–30 mg/kg) 60 min after i.p administration alone or in combination with GW6471 (2 μg/mouse, i.c.v.; 30 min after ACEA) and NIDA-41020 (1 mg/kg, i.p.; 15 min after ACEA). Abscissa shows the log doses, ordinate shows (A) percentage of clonic seizures, (B) percentage of tonic seizures.

WIN was not effective on this latter phase of audiogenic seizures up to 60 mg/kg (Table 1 and Fig. 3). The rank order of potency measured by the lowest ED_{50} values (expressed in μmol/kg) for tonus and clonus is: ACEA tonus (18.77 μmol/kg) clonus (25.63 μmol/kg) > PEA tonus (34.46 μmol/kg) clonus (38.88 μmol/kg) > WIN tonus (66.73 μmol/kg) clonus (84.64 μmol/kg). The anticonvulsant properties of these drugs had a maximum effect 90 min after administration of PEA, 60 min after ACEA and 20 min after WIN (Table 1).

NIDA-41020 (0.5–2 mg/kg, i.p.) and GW6471 (0.5–4 μg/mouse, i.c.v.) had any anticonvulsant effects against the wild running, clonic or tonic phase of audiogenic seizures in DBA/2 mice when administered alone (data not shown).

Since PEA (5 mg/kg i.p.), ACEA (2 mg/kg) and WIN (5 mg/kg i.p.) did not significantly affect reflex seizures per se as it was also observed in previous studies (Citraro et al., 2013a; Luszczki et al., 2011b, 2013); these doses were selected for the following section of the present experimental protocol to be tested in co-administration with AEDs (see Section 2.3). The doses of these three drugs used did not induce any locomotor impairment as measured in the rotarod test as well as did not modify body temperature (data not shown; see Section 2.3).

3.2. Effects of GW6471 and NIDA-41020 on the anticonvulsant effects of PEA, WIN and ACEA in DBA/2 mice

GW6471 (2 μg/mouse, i.c.v.) and NIDA-41020 (1 mg/kg, i.p.) were able to significantly antagonize the anticonvulsant properties of PEA shifting to the right its dose-response curves, whereas NIDA-41020 only (1 mg/kg, i.p.), but not GW6471 (2 μg/mouse, i.c.v.), was able to significantly reduce the anticonvulsant properties of both ACEA and WIN (Figs. 1–3; Table 1).

3.3. Effects of PEA, WIN and ACEA upon the anticonvulsant activity of conventional AEDs versus audiogenic seizures in DBA/2 mice

All AEDs studied showed anticonvulsant effectiveness against audiogenic seizure of DBA/2 mice (Table 2), as previously reported (De Sarro et al., 2015). PEA's effects upon the activity of the AEDs on reflex audiogenic seizures were different depending on the AED tested.

PEA pretreatment (5 mg/kg, i.p.) produces a consistent shift to the left of the dose-response curves for some AEDs; a significant decrease of ED_{50} values against clonus and tonus was found for all drug combinations except levetiracetam and clonus for phenytoin (Table 2).

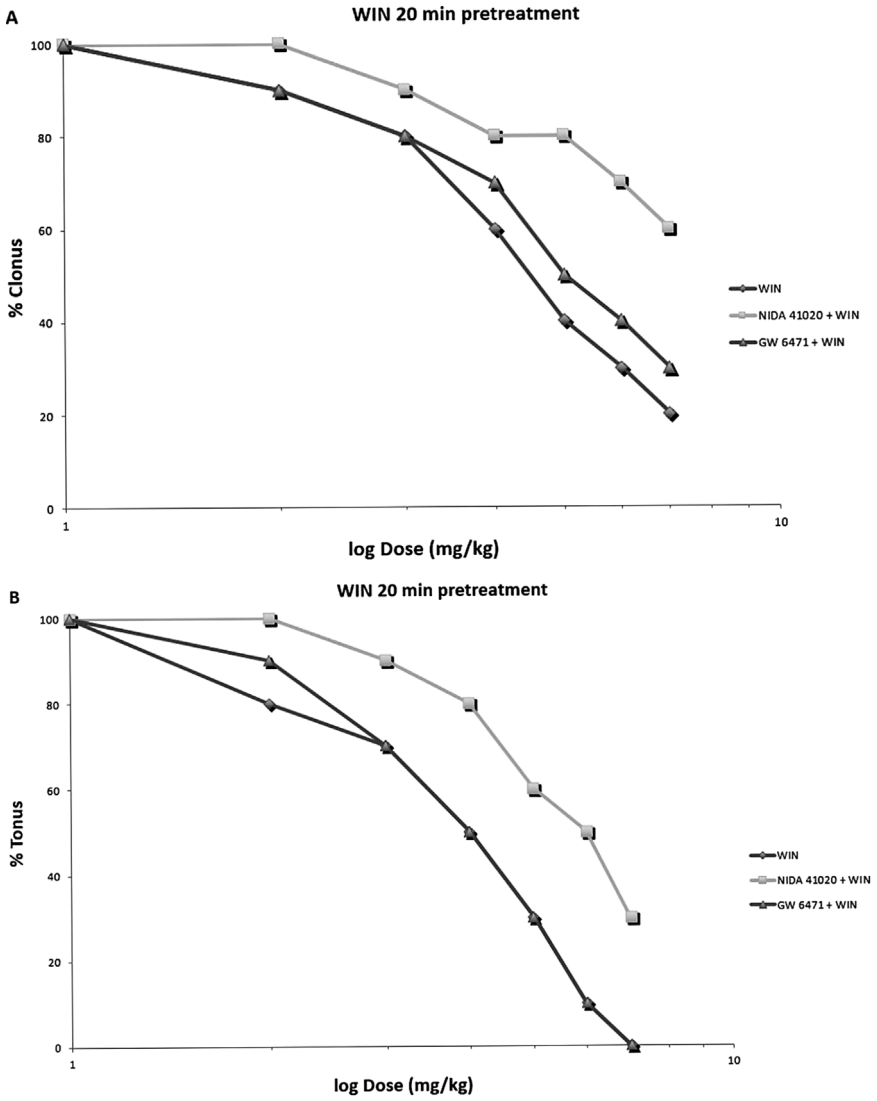

Fig. 3. Dose-response curves of the anticonvulsant effects of WIN (2.5–60 mg/kg) 20 min after i.p administration alone or in combination with GW6471 (2 μg/mouse, i.c.v.; 10 min before WIN) and NIDA-41020 (1 mg/kg, i.p.; 25 min before WIN). Abscissa shows the log doses, ordinate shows (A) percentage of clonic seizures, (B) percentage of tonic seizures.

ACEA (2 mg/kg, i.p.) or WIN (5 mg/kg, i.p.) treatment induced a consistent shift to the left of the dose-response curves and a significant reduction (P at least < 0.05) of ED_{50} values for clonus and/or tonus of some AEDs (Table 2). In particular, combined treatment of AEDs with ACEA (2 mg/kg, i.p.) significantly reduced all ED_{50} values for clonus except those of lamotrigine, levetiracetam, oxcarbazepine, phenytoin and topiramate and for tonus also for oxcarbazepine. Similarly, WIN (5 mg/kg, i.p.) significantly reduced ED_{50} values for both clonus and tonus of all AEDs except levetiracetam, oxcarbazepine, and phenytoin. Therefore, comparing WIN and ACEA the main difference was the ability of WIN to potentiate at this dose of also lamotrigine and topiramate for clonus only (Table 2).

3.4. *Effects of NIDA 41020 and GW6471 upon the anticonvulsant activity of AEDs versus audiogenic seizures in DBA/2 mice*

NIDA-41020 (1 mg/kg i.p.; 45 min before auditory stimulation) or GW6471 (2 μg/mouse i.c.v.; 30 min before auditory stimulation) were unable to significantly modify the dose-response curves for all AEDs studied (Table 2). All dose-response curves of wild running were parallel with the exception of diazepam plus NIDA-41020, levetiracetam plus GW6471, phenytoin plus WIN, topiramate plus GW6471 or valproate plus NIDA-41020 (data not shown). Indeed, all dose-response curves of clonus were parallel with the exception of carbamazepine plus GW6471, levetiracetam plus ACEA, valproate plus GW6471.

3.5. *Effects of PEA, WIN and ACEA upon the motor impairment induced by AEDs*

All AEDs, used at doses equal to their ED_{50} values against the clonic phase of the audiogenic seizures, did not modify motor performance of DBA/2 mice on the rotarod test (Sarro et al., 2012). Higher doses were required to induce motor deficit. TD_{50} values were significantly reduced by co-administration of PEA with all AEDs with the exception of carbamazepine and levetiracetam. The concomitant treatment of WIN or ACEA with AEDs resulted usually in an increase of motor impairment. TD_{50} values were significantly decreased by WIN and ACEA in combination with all AEDs with the exceptions of carbamazepine, levetiracetam, and phenytoin for WIN and carbamazepine, levetiracetam and oxcarbazepine for ACEA. Although TD_{50} was lowered, the therapeutic index (TI) of all AEDs co-administered with PEA, ACEA or WIN was generally comparable to AEDs alone if not higher (Table 3).

Table 1
ED50 values (± 95% confidence limits) for PEA, WIN, ACEA, alone or co-administered with NIDA 41020 or GW6471 on audiogenic seizures in DBA/2 mice at different time points after drug administration.

Treatment drug	Seizure phase		
(Time)	Wild running	Clonus	Tonus
PEA (30 min)	47.0 (34.58–63.88)	31.23 (26.23–37.19)	18.23 (13.38–24.83)
PEA (60 min)	27.21 (21.83–33.93)	19.41 (14.61–25.65)	11.53 (8.14–16.34)
PEA (90 min)	22.94 (17.23–30.54)	11.80 (7.63–18.26)	10.46 (6.38–17.16)
PEA (120 min)	29.04 (23.24–36.3)	21.35 (15.12–30.14)	12.04 (8.10–17.90)
NIDA 41020 (45 min)+PEA (60 min)	36.96 (26.88–50.82)	26.48 (21.36–32.84)	24.88 (17.87–34.65)^{aa}
NIDA 41020 (45 min)+PEA (90 min)	29.08 (20.33–41.58)	20.49 (15.05–27.89)^{a}	17.35 (10.75–27.99)^{a}
GW6471 (30 min)+PEA (60 min)	37.09 (32.49–42.35)^{a}	30.01 (25.73–30.17)^{a}	21.32 (15.17–29.97)^{a}
GW6471 (30 min)+PEA (90 min)	33.94 (26.13–44.07)	24.36 (17.71–33.52)^{aa}	18.77 (12.66–27.84)^{a}
WIN (10 min)	NM	56.84 (32.30–65.05)	47.32 (26.56–84.30)
WIN (20 min)	51.51 (40.48–65.54)	36.10 (22.71–57.39)	28.46 (19.18–42.23)
WIN (30 min)	58.64 (44.82–76.74)	47.32 (26.56–84.30)	33.96 (25.88–44.56)
NIDA 41020 (45 min)+WIN (20 min)	75.62 (55.78–102.53)^{a}	65.66 (49.08–80.42)^{a}	51.77 (33.79–79.34)^{a}
GW6471 (30 min)+WIN (20 min)	57.05 (40.48–80.42)	38.03 (31.01–46.63)	28.88 (19.61–40.76)
ACEA (30 min)	23.86 (14.6–39.0)	19.97 (14.29–27.91)	11.22 (9.03–13.94)
ACEA (60 min)	12.68 (9.71–16.56)	9.38 (7.48–11.77)	6.87 (4.71–10.03)
ACEA (90 min)	19.97 (14.29–27.91)	16.96 (12.15–23.67)	10.45 (8.15–13.41)
NIDA 41020 (45 min)+ACEA (60 min)	28.88 (17.87–34.65)^{aa}	18.33 (12.33–27.28)^{a}	12.08 (6.92–21.09)^{a}
GW6471 (30 min)+ACEA (60 min)	13.74 (8.56–22.03)	9.08 (6.39–12.90)	6.82 (4.47–10.40)

All data are expressed as mg/kg and were calculated according to the method of Litchfield and Wilcoxon (1949). NM = not measurable up to the dose of 60 mg/kg. Significant differences among concurrent groups are marked by $^{a}P < 0.05$ and $^{aa}P < 0.01$.

Table 2
ED_{50} values (± 95% confidence limits) for vehicle plus the antiepileptic drugs or in combination with PEA (5 mg/kg, i.p.), NIDA 41020 (1 mg/kg, i.p.), WIN (5 mg/kg, i.p.), ACEA (2 mg/kg, i.p.) or GW64716 (2 μg/mouse i.c.v.) against audiogenic seizures in DBA/2 mice.

	Drug	+Vehicle	+PEA	+NIDA 41020	+WIN	+ACEA	+GW6471
Wild running	Carbamazepine	10.6 (8.1–13.8)	7.8 (6.5–9.4)^{a}	10.2 (8.0 – 13.0)	8.1 (6.3–10.4)^{a}	7.8 (6.1–9.97)^{a}	10.4 (8.2–13.2)
	Diazepam	0.49 (0.34–0.71)	0.31 (0.21–0.46)^{a}	0.49 (0.32 – 0.75)	0.35 (0.27–0.45)^{a}	0.33 (0.22–0.44)^{a}	0.46 (0.32–0.66)
	Felbamate	114.6 (92–142.7)	82 (64–105.1)^{a}	119 (95 – 149.1)	85 (62–116)^{a}	87 (61–124)^{a}	108 (88–132.5)
	Gabapentin	38 (16–51)	22.4 (17.1–29.3)^{aa}	38.2 (19.3 – 75.6)	23.8 (18.2–31.1)^{aa}	23.2 (17.1–31.5)^{aa}	37.2 (24.1–57.42)
	Lamotrigine	6.1 (4.6–8.1)	3.6 (2.8–4.63)^{aa}	6.4 (4.9 – 8.36)	3.9 (3.2–4.7)^{a}	3.7 (3.1–4.4)^{a}	5.9 (4.3–8.1)
	Levetiracetam	15.4 (12.2–19.5)	13.2 (11.2–15.6)	15.8 (12.6 – 19.8)	14.8 (11.7 – 18.7)	14.4 (12.8 – 16.2)	15.6 (11.8–20.6)
	Oxcarbazepine	11.4 (9.7–13.3)	7.4 (6.2–8.83)^{a}	11.8 (9.8 – 14.2)	8.9 (7.5 – 10.6)	8.7 (7.4–10.2)	12.4 (9.6–16.0)
	Phenobarbital	7.1 (5.6–9.0)	4.19 (3.6–4.88)^{aa}	6.5 (5.4–7.8)	5.47 (4.5–6.6)	5.8 (4.6–7.3)	6.5 (4.8–8.8)
	Phenytoin	4.3 (3.1–6.0)	3.7 (2.4–5.7)	4.1 (3.0–5.8)	4.3 (2.9–6.4)	3.9 (2.6–5.8)	4.4 (3.1–6.25)
	Topiramate	22.9 (15.8–33.9)	12.1 (10.2–14.4)^{aa}	23.4 (18.4–29.7)	14.3 (11.8 – 17.3)^{aa}	14.6 (12.1–17.6)^{aa}	23.4 (16.2 – 33.8)
	Valproate	84 (63–114)	51 (37–70.3)^{aa}	79 (65–96.0)	52.5 (38–72.5)^{aa}	54 (38–76.7)^{a}	80.2 (59.1–108.8)
Clonus	Carbamazepine	4.4 (3.6–5.4)	2.7 (2.0–3.6)^{aa}	4.6 (3.7 – 5.7)	3.1 (1.9–5.1)^{a}	2.9 (2.1–4.0)^{aa}	3.7 (2.5–5.6)
	Diazepam	0.28 (0.2–0.39)	0.18 (0.13–0.25)^{aa}	0.29 (0.21–0.40)	0.19 (0.14–0.25)^{aa}	0.19 (0.14–0.26)^{aa}	0.26 (0.20–0.34)
	Felbamate	48.8 (35–67)	30.2 (24.8–36.8)^{aa}	49.8 (39.6–62.6)	32 (26–39.4)^{aa}	34.6 (26.9–44.5)^{a}	45 (30–69)
	Gabapentin	20.3 (13.7–30.2)	12.4 (9.6–16.02)^{aa}	20.9 (15.8 – 27.65)	13.3 (10.3–17.2)^{aa}	13.5 (10.7–17.0)^{aa}	21.1 (16.1–26.3)
	Lamotrigine	3.5 (2.4–5.1)	2.1 (1.6–2.7)^{aa}	3.8 (2.6 – 5.55)	2.2 (1.5–3.2)^{a}	2.9 (2.3–3.65)	3.9 (2.6–5.8)
	Levetiracetam	9.8 (7.2–13.2)	9.5 (7.4–12.2)	9.9 (7.8 – 12.6)	8.8 (6.8–11.4)	9.6 (7.3–12.6)	9.6 (7.1–13.5)
	Oxcarbazepine	4.2 (3.0–5.88)	2.7 (2.0–3.6)^{a}	4.4 (3.4–5.69)	3.7 (2.6–6.53)	3.9 (2.8–5.4)	4.1 (2.9–5.8)
	Phenobarbital	3.4 (2.3–5.0)	2.0 (1.5–2.67)^{aa}	3.3 (2.1–5.19)	2.1 (1.7–2.6)^{aa}	2.2 (1.8–2.69)^{a}	3.3 (2.4–4.54)
	Phenytoin	2.5 (1.8–3.5)	2.1 (1.7–2.6)	2.3 (1.9–2.8)	2.2 (1.8–2.7)	2.2 (1.8–2.7)	2.6 (1.9–3.6)
	Topiramate	12.1 (6.9–21.2)	6.5 (5.5–7.68)^{aa}	12.4 (7.7–19.9)	8.96 (6.34–13.57)^{a}	10.1 (8.0–12.8)	12.1 (8.0–18.3)
	Valproate	43 (33–56)	22.7 (18.4–28.0)^{aa}	41.7 (26.2–66.4)	25.9 (20.2–33.2)^{aa}	25.3 (19.4–33.0)^{aa}	44 (34–56.9)
Tonus	Carbamazepine	3.0 (2.6–3.8)	1.7 (1.3–2.22)^{aa}	3.1 (2.7–3.56)	2.1 (1.6–2.76)^{a}	1.9 (1.45–2.49)^{aa}	3.1 (2.7–3.56)
	Diazepam	0.24 (0.15–0.39)	0.15 (0.11–0.20)^{aa}	0.22 (0.18–0.27)	0.16 (0.12 – 0.21)^{aa}	0.17 (0.12–0.24)^{a}	0.24 (0.15 – 0.39)
	Felbamate	23.1 (18.15–9.4)	14.2 (9.8–20.6)^{aa}	23.8 (18.3–30.9)	17.8 (14.2–22.4)^{a}	16.1 (12.2 – 21.2)^{aa}	24.2 (18.6–31.5)
	Gabapentin	13.9 (8.7–22.3)	6.5 (4.9–8.6)^{aa}	13.3 (10.9–16.2)	7.2 (5.46 – 9.26)^{aa}	6.8 (5.6 – 18.26)^{aa}	14.1 (12.49 – 15.92)
	Lamotrigine	1.1 (0.7–1.8)	0.6 (0.4–0.9)^{aa}	1.21 (0.9–1.63)	0.8 (0.5–1.3)^{a}	0.9 (0.7–1.1)	1.2 (0.9–1.6)
	Levetiracetam	7.9 (5.9–10.6)	7.3 (5.8–9.2)	7.8 (6.1–9.97)	7.5 (6.3–8.9)	7.6 (6.3–9.2)	8.1 (6.5–10.4)
	Oxcarbazepine	3.2 (2.7–3.79)	2.0 (1.4–2.86)^{aa}	3.0 (2.4–3.75)	2.5 (1.8–3.5)	2.2 (1.6–3.0)*	3.2 (2.3–4.5)
	Phenobarbital	2.4 (1.7–3.4)	1.3 (0.9–1.88)^{aa}	2.2 (1.8–2.7)	1.5 (1.2–1.88)^{aa}	1.5 (1.1–2.05)^{aa}	2.2 (1.8–2.7)
	Phenytoin	2.0 (1.6–2.5)	1.4 (1.1–1.8)^{a}	1.9 (1.5–2.41)	1.6 (1.2–2.13)	1.7 (1.4–2.1)	2.1 (1.6–2.76)
	Topiramate	6.12 (4.8–9.18)	3.25 (2.58–4.09)^{aa}	6.4 (5.2–7.88)	3.94 (2.42–6.41)^{aa}	3.84 (2.78–5.3)^{aa}	6.1 (4.6–8.1)
	Valproate	31 (22–43)	16.4 (12.5–21.5)^{aa}	30.2 (20.4–44.7)	18.2 (14.7–22.5)^{aa}	17.6 (14.2–21.8)^{aa}	31 (21–46)

All data are expressed as mg/kg and were calculated according to the method of Litchfield and Wilcoxon (1949). Vehicle, PEA 90 min , WIN 20 min , ACEA 60 min or GW6471 30 min before rotarod test, respectively. AEDs: lamotrigine, oxcarbazepine and gabapentin 45 min , carbamazepine, diazepam, levetiracetam and felbamate 60 min , phenobarbital and valproate 60 min , phenytoin 120 min , topiramate 90 min before auditory stimulation. Significant differences among groups are marked by $^{a}P < 0.05$ and $^{aa}P < 0.01$.

Table 3
TD_{50} values (± 95% confidence limits) for various antiepileptic drugs plus vehicle or in combination with PEA (5 mg/kg, i.p.), NIDA 41020 (1 mg/kg, i.p.), WIN (5 mg/kg, i.p.), ACEA (2 mg/kg, i.p.) or GW64716 (2 μg/mouse i.c.v.) in the rotarod test.

Treatment	TD_{50}	TI	Treatment	TD_{50}	TI
Carbamazepine + Vehicle	46.5 (37.9–57)	10.5	Lamotrigine + Vehicle	81 (55–118)	23.1
Carbamazepine + PEA	39.8 (28.7–55.2)	14.7	Lamotrigine + PEA	44.7 (34.9–57.2)[aa]	21.3
Carbamazepine + NIDA 41020	47.4 (36.3–61.2)	10.3	Lamotrigine + NIDA 41020	79 (58–108)	20.8
Carbamazepine + WIN	38.0 (30.2–47.2)	12.3	Lamotrigine + WIN	44.8 (35–57.3)[aa]	20.4
Carbamazepine + ACEA	41.2 (34.1–49.8)	14.2	Lamotrigine + ACEA	43.4 (33–57.1)[aa]	15
Carbamazepine + GW64716	48.1 (36.4–64)	13	Lamotrigine + GW64716	82.7 (63–109)	21.2
Diazepam + Vehicle	3.8 (3.0–4.8)	13.5	Phenobarbital + Vehicle	139 (115–168)	40.9
Diazepam + PEA	2.4 (1.6–3.6)[a]	13.3	Phenobarbital + PEA	97.7 (81–118)[a]	48.8
Diazepam + NIDA 41020	3.8 (2.8–5.2)	13.1	Phenobarbital + NIDA 41020	135 (114–160)	40.9
Diazepam + WIN	2.2 (1.4–3.46)[a]	11.5	Phenobarbital + WIN	88.7 (72–109.3)[a]	42.2
Diazepam + ACEA	2.6 (1.8–4.8)[a]	13.7	Phenobarbital + ACEA	98 (78–123.1)[a]	44.5
Diazepam + GW64716	3.7 (2.8–4.9)	14.2	Phenobarbital + GW64716	131.4 (103–167.6)	39.7
Felbamate + Vehicle	816 (590–1024)	16.7	Topiramate + Vehicle	162 (110–238.6)	13.4
Felbamate + PEA	582 (523–648)[a]	19.3	Topiramate + PEA	102.9 (89.3–118.6)[aa]	15.8
Felbamate + NIDA 41020	783 (581–1055)	15.8	Topiramate + NIDA 41020	154.4 (116–205)	13
Felbamate + WIN	532 (424–667)[a]	16.6	Topiramate + WIN	103.7 (82.5–130.4)[aa]	11.6
Felbamate + ACEA	526 (431–642)[a]	15.2	Topiramate + ACEA	116.5 (95.9–141.5)[a]	11.5
Felbamate + GW64716	776 (547–1101)	17.2	Topiramate + GW64716	158 (119–210)	13.1
Gabapentin + Vehicle	290.3 (218.3–386)	14.3	Valproate + Vehicle	290 (240–251)	7.3
Gabapentin + PEA	194 (138–273)[a]	15.6	Valproate + PEA	201.7 (182–223.6)[a]	8.9
Gabapentin + NIDA 41020	261 (179–380.6)	12.5	Valproate + NIDA 41020	280.2 (229–343)	7.1
Gabapentin + WIN	192 (148–249)[a]	14.4	Valproate + WIN	161.5 (137–190.3)[aa]	6.2
Gabapentin + ACEA	171 (119–246)[a]	12.7	Valproate + ACEA	209.5 (177–247.9)[a]	8.3
Gabapentin + GW64716	276 (187–407)	13.1	Valproate + GW64716	287(223–369.4)	6.5
Oxcarbazepine + Vehicle	60.9 (52.1–71.2)	19	Levetiracetam + Vehicle	1601 (1334.2–1921.1)	163.4
Oxcarbazepine + PEA	41.7 (33.5–51.8)[a]	20.8	Levetiracetam + PEA	1327 (1105.8–1592.4)	139.7
Oxcarbazepine + NIDA 41020	56.8 (49.2–65.6)	18.9	Levetiracetam + NIDA 41020	1578 (1254–1985)	159.4
Oxcarbazepine + WIN	41.5 (34.3–50.2)[a]	16.6	Levetiracetam + WIN	1254 (1019.5–1542.4)	142.5
Oxcarbazepine + ACEA	40.3 (32.8–49.5)	14.9	Levetiracetam + ACEA	1512 (1239.3–1768)	157.5
Oxcarbazepine + GW64716	57.2 (48.6–67.3)	17.9	Levetiracetam + GW64716	1586 (1306–1926)	165.2
Phenytoin + Vehicle	48.3 (34.1–68.4)	19.3	*All data are expressed as mg/kg and were calculated according to the method of Litchfield and Wilcoxon (1949). Vehicle, PEA 90 min , WIN 20 min , ACEA 60 min or GW6471 30 min before rotarod test, respectively. AEDs: lamotrigine, oxcarbazepine and gabapentin 45 min , carbamazepine, diazepam, levetiracetam and felbamate 60 min , phenobarbital and valproate 60 min , phenytoin 120 min , topiramate 90 min before rotarod test. TI=therapeutic index, represents the ratio between TD_{50} and ED_{50} from the clonic phase of audiogenic seizures. Significant differences among groups are marked by $^{a}P < 0.05$ and $^{aa}P < 0.01$.*		
Phenytoin + PEA	37.2 (28.3–48.6)[a]	17.7			
Phenytoin + NIDA 41020	48.1 (32.7–70.7)	20.9			
Phenytoin + WIN	41.1 (30.5–55.4)	18.7			
Phenytoin + ACEA	33.2 (25.6–43.1)[a]	15.1			
Phenytoin + GW64716	47.3 (34.2–65.4)	18.2			

3.6. Effects of PEA, ACEA and WIN on the total/free plasma and brain concentrations of AEDs in DBA/2 mice

Plasma and brain levels of AEDs given alone or together with PEA (5 mg/kg, i.p.), ACEA (2 mg/kg, i.p.), NIDA 41020 (1 mg/kg, i.p.; only for plasma determination) or WIN (5 mg/kg, i.p.) are reported in Tables 4 and 5. At the doses investigated both AEDs' plasma and brain concentrations were not significantly altered by the administration of any of these drugs; however, PEA, WIN, NIDA and/or ACEA slightly modified free plasma levels of felbamate, phenytoin, phenobarbital (Table 4). Brain levels of AEDs were not significantly modified and slight not significant modification were observed for carbamazepine, diazepam, felbamate, oxcarbazepine, phenytoin, phenobarbital and valproate (Table 5).

4. Discussion

The results of this study suggest that the endogenous non cannabimimetic compound PEA and the synthetic cannabinoids ACEA and WIN, administered before auditory test, are able to prevent reflex audiogenic seizures in DBA/2 mice, a widely investigated model of audiogenic generalized seizures (De Sarro et al., 2015; Donato Di Paola et al., 2007; Gitto et al., 2007; Italiano et al., 2016). While efficacy against clonus and tonus was comparable for all three drugs; ACEA was the most potent followed by PEA and then WIN and PEA was the longer acting of the three. The second group of experiments demonstrated that the anticonvulsant properties of PEA, ACEA and WIN were antagonized by NIDA-41020 (a selective CB_1 receptor antagonist) and therefore supports the fact that these drugs directly or indirectly exert their anticonvulsant action through the endocannabinoid system. Accordingly, the present results further suggest that the activation on the cannabinoid system in the brain has anticonvulsant effects and might contribute to enhance the anticonvulsant activity of AEDs used considering the pharmacodynamics potentiation observed in this study. The anticonvulsant properties of cannabinoid system activation might derive from their direct antagonism or impairment of glutamate neurotransmission, which has been suggested by some authors (Maier et al., 2012; Polissidis et al., 2013; Ruehle et al., 2013; Sanchez-Blazquez et al., 2014). However, other studies have shown that neuroprotection could be also mediated by a modulation of GABAergic tone (Albayram et al., 2011; Antonucci et al., 2012; Blair et al., 2009; Karlocai et al., 2011).

Previously, some authors have demonstrated that WIN or ACEA alone do not influence the threshold for electroconvulsions and that both drugs, acutely administered, increase brain level of carbamazepine, phenobarbital and valproate and potentiate the anticonvulsant effects of various AEDs without significantly modifying plasma and brain levels (Luszczki et al., 2011a, 2011b, 2006, 2010, 2013; Luszczki and Florek-Luszczki, 2012).

The efficacy of PEA to reduce seizures is consistent with several reports demonstrating the antiseizure properties of this compound against PTZ- and electroshock-induced convulsions in mice (Aghaei et al., 2015; Lambert et al., 2001), against kindled amygdaloid seizures in rats (Sheerin et al., 2004) and absence seizures in WAG/Rij rat (Citraro et al., 2013b). The microinjection of GW6471 alone did not induce significant modification in the severity of reflex seizures in DBA/2 mice, or influence ACEA and WIN effects, while antagonized the anticonvulsant properties of PEA. This suggests that PEA properties at PPAR-α receptors are involved in the control of audiogenic seizures in DBA/2 mice. Furthermore, NIDA-41020 antagonized the anticonvulsant effects of PEA, ACEA and WIN; therefore, the above-described results show that PEA plays an anticonvulsant role in DBA/2 mice acting on both CB_1 receptors and PPAR-α, whereas ACEA and WIN act prevalently through CB_1 receptors.

Table 4
Influence of PEA, NIDA 41020, WIN or ACEA on total and free plasma levels of some antiepileptic drugs in DBA/2 mice.

Drug (time) (dose mg/kg)	Vehicle+AED		PEA+AED		NIDA+AED		WIN+AED		ACEA+AED	
	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free
Carbamazepine (60 min) (15 mg/kg)	6.5 ± 0.9	0.77 ± 0.2	6.4 ± 0.7	0.82 ± 0.2	6.5 ± 0.78	0.80 ± 0.2	6.4 ± 0.19	0.84 ± 0.2	6.4 ± 0.7	0.84 ± 0.18
Diazepam (60 min) (5 mg/kg)	0.28 ± 0.03	0.02 ± 0.007	0. 27 ± 0.03	0.025 ± 0.006	0.28 ± 0.03	0.020 ± 0.009	0.27 ± 0.03	0.025 ± 0.006	0.27 ± 0.026	0.024 ± 0.005
Felbamate (60 min) (100 mg/kg)	4.6 ± 0.4	3.0 ± 0.26	4.5 ± 0.4	2.9 ± 0.23	4.4 ± 0.33	3.1 ± 0.25	4.6 ± 0.38	2.9 ± 0.23	4.3 ± 0.34	2.9 ± 0.22
Gabapentin (45 min) (70 mg/kg)	10.2 ± 1.5	NA	10.3 ± 1.4	NA	10.2 ± 1.2	NA	10.3 ± 1.3	NA	10.2 ± 1.4	NA
Lamotrigine (45 min) (10 mg/kg)	1.8 ± 0.2	0.67 ± 0.07	1.9 ± 0.2	0.68 ± 0.1	1.9 ± 0.2	0.68 ± 0.1	1.8 ± 0.2	0.69 ± 0.12	1.9 ± 0.2	0.68 ± 0.11
Levetiracetam (60 min) (100 mg/kg)	39.2 ± 4.5	NA	39.4 ± 4.3	NA	39.4 ± 4.4	NA	39.3 ± 4.1	NA	39.5 ± 4.5	NA
Oxcarbazepine (45 min) (15 mg/kg)	12.8 ± 1.1	NA	12.9 ± 1.2	NA	13.0 ± 1.2	NA	13.1 ± 1.3	NA	12.9 ± 1.1	NA
Phenytoin (120 min) (10 mg/kg)	4.54 ± 0.09	0.82 ± 0.07	4.46 ± 0.09	0.84 ± 0.08	4.52 ± 0.08	0.83 ± 0.07	4.51 ± 0.09	0.85 ± 0.08	4.56 ± 0.09	0.87 ± 0.08
Phenobarbital (60 min) (20 mg/kg)	39.2 ± 3.5	4.9 ± 0.3	39.1 ± 3.6	5.2 ± 0.4	38.9 ± 3.5	5.3 ± 0.5	39.5 ± 3.5	3.8 ± 0.3	39.0 ± 3.6	5.2 ± 0.5
Topiramate (90 min) (30 mg/kg)	7.43 ± 0.58	4.51 ± 0.39	7.48 ± 0.61	4.32 ± 0.36	7.39 ± 0.49	4.23 ± 0.38	7.42 ± 0.49	4.40 ± 0.4	7.46 ± 0.61	4.35 ± 0.34
Valproate (60 min) (200 mg/kg)	276 ± 24	44.2 ± 4.2	274 ± 27	54.9 ± 4.8	275 ± 26	45.1 ± 4.1	275 ± 26	48.2 ± 4.5	274 ± 27	53.5 ± 4.7

Drugs were administered i.p. Vehicle, PEA (5 mg/kg) 90 min before auditory test, NIDA 41020 (1 mg/kg, i.p.) 45 min before auditory test, WIN (5 mg/kg) 20 min before auditory test or ACEA (2 mg/kg) 60 min before+lamotrigine, oxcarbazepine and gabapentin 45 min, carbamazepine, diazepam, levetiracetam, felbamate, phenobarbital and valproate 60 min, phenytoin 120 min, topiramate 90 min before sampling. Values are means (μg/ml) of at least eight determinations ± S.E.M. Student's *t*-test was used for statistical analysis of the data. No significant differences were observed. NA=not applicable.

Table 5
Influence of PEA, WIN or ACEA on brain levels of some antiepileptic compounds in DBA/2 mice.

Drug (time) (dose mg/kg)	+Vehicle	+PEA	+WIN	+ACEA
Carbamazepine (60 min) (15 mg/kg)	3.65 ± 0.49	4.66 ± 0.44	3.92 ± 0.42	3.96 ± 0.42
Diazepam (60 min) (5 mg/kg)	0.214 ± 0.018	0.289 ± 0.024	0.264 ± 0.021	0.272 ± 0.019
Felbamate (60 min) (100 mg/kg)	3.57 ± 0.39	3.75 ± 0.42	3.59 ± 0.34	3.61 ± 0.33
Gabapentin (45 min) (70 mg/kg)	9.8 ± 1.5	9.9 ± 1.5	9.9 ± 1.4	9.9 ± 1.3
Lamotrigine (45 min) (10 mg/kg)	1.5 ± 0.18	1.60 ± 0.19	1.58 ± 0.18	1.57 ± 0.19
Levetiracetam (60 min) (100 mg/kg)	31.52 ± 4.54	31.85 ± 4.36	31.68 ± 4.26	31.50 ± 4.32
Oxcarbazepine (45 min) (15 mg/kg)	0.80 ± 0.15	0.88 ± 0.17	0.86 ± 0.16	0.85 ± 0.16
Phenytoin (120 min) (10 mg/kg)	1.54 ± 0.22	1.89 ± 0.20	1.69 ± 0.19	1.71 ± 0.20
Phenobarbital (60 min) (20 mg/kg)	9.04 ± 0.72	11.09 ± 0.74	10.72 ± 0.74	10.55 ± 0.77
Topiramate (90 min) (30 mg/kg)	4.41 ± 0.31	4.48 ± 0.25	4.46 ± 0.30	4.47 ± 0.29
Valproate (60 min) (200 mg/kg)	91.29 ± 7.89	108.32 ± 8.54	103.38 ± 8.36	102.67 ± 8.24

Drugs were administered i.p. Vehicle, PEA (5 mg/kg) 90 min before, WIN (5 mg/kg) 20 min before or ACEA (2 mg/kg) 60 min+lamotrigine, oxcarbazepine and gabapentin 45 min, carbamazepine, diazepam, levetiracetam, felbamate, phenobarbital and valproate 60 min, phenytoin 120 min, topiramate 90 min before sampling. Values are means (μg/ml) of at least eight determinations ± S.E.M. Student's *t*-test was used for statistical analysis of the data. No significant differences were observed.

4.1. Cannabinomimetic compounds and seizures

The anticonvulsant properties of CB_1 receptors agonists in several models, as above described, are likely linked to the ability of this receptor to activate multiple secondary mechanisms of actions: for instance ACEA and WIN by activation of CB_1 receptors lead to a decrease in cAMP production through activation of Gi proteins. This effect is linked to an inhibition of protein kinase A (Bidaut-Russell and Howlett, 1991). Moreover, in the brain, the inhibition of cAMP pathway decreases intracellular Ca^{2+} load through voltage-activated N and P/Q type Ca^{2+} channels. This inhibition seems to be responsible of a reduction of presynaptic neurotransmitter release (Mackie et al., 1993). CB_1 receptor activation also increases the conductance of presynaptic A type (Hampson et al., 1995) and G-protein-coupled inward rectifying K^+ channels (Mackie et al., 1995). Moreover, CB_1R are also involved in the reduction of glutamate release both from spinal cord (Richardson et al., 1998) and hippocampal neurons (Shen et al., 1996), as well as in the inhibition of GABA reuptake in the globus pallidus. All these effects could be responsible for their anticonvulsant features (Engler et al., 2006; Lutz, 2004; Maneuf et al., 1996; Sieradzan et al., 2001). In particular, it has been shown that CB_1 receptors are found on the nerve terminals of GABAergic interneurons localized in neocortex, hippocampus and amygdala (Falenski et al., 2007; Freund et al., 2003) and on the nerve terminals of excitatory amino acids localized in cerebellum and striatum (Armstrong et al., 2009; Huang et al., 2001; Marsicano and Lutz, 1999). All these mechanisms of anticonvulsant action associated with the activation of CB_1 receptors could be linked to the anticonvulsant properties against reflex seizures in DBA/2 mice and for the increase of anticonvulsant activity of AEDs observed in the present study (see below).

4.2. Interactions among AEDs, PEA and cannabinomimetics

The anticonvulsant effect of carbamazepine, diazepam, gabapentin, felbamate, phenobarbital, and valproate against audiogenic seizures was increased by the co-administration of all compounds studied (PEA, WIN and ACEA) administered at doses that did not influence the frequency of audiogenic seizures per se. At odds, the potency of levetiracetam, oxcarbazepine, topiramate and phenytoin was generally not significantly increased by WIN and ACEA while PEA appeared to have a broader spectrum of potentiation also improving the effectiveness of more drugs in comparison to WIN and ACEA. Antagonism at either CB_1 or PPAR-α receptors was not able to affect AEDs effects indicating that at least in this model their effects are not mediated by any of these systems. The mechanisms causing the pharmacological potentiation of AEDs seem to be based only on pharmacodynamic interactions since no significant alterations in AEDs plasma or brain concentrations were observed. Several mechanisms may be involved in the anticonvulsant potentiation observed with PEA, ACEA and WIN. The favorable effects observed might be categorized according to the mechanism of action possessed by AEDs. Considering their molecular mechanisms, it is possible to hypothesize that ACEA, PEA and WIN might enhance the anticonvulsant effect of gabapentin by interfering with inhibition of P/Q-type and L-type high-voltage-activated calcium channels, and enhanced the anticonvulsant activity of phenobarbital and diazepam through a positive interaction with GABA neurotransmission (De Sarro et al., 2002). However, it is not possible to exclude that ACEA, PEA and WIN, used at different doses, could enhance the anticonvulsant activity of AEDs through other mechanisms.

4.3. PEA and PPAR-α interactions

PEA has low affinity for CB_1 and CB_2 receptors even if the CB_2 antagonist SR144528 reverses some of its pharmacological properties (Calignano et al., 1998; Citraro et al., 2013b). It was reported that PEA possesses effects that might not be correlated to the activation of CBRs. Conversely, PEA's effects are currently suggested to be mediated by the nuclear receptor PPAR-α (D'Agostino et al., 2007; Lo Verme et al., 2005; Mattace Raso et al., 2014; Raso et al., 2011). Evidence reported that PEA as well as its structural analogue oleoylethanolamide (Fu et al., 2003) by activating on PPAR-α, are able to decrease pain and inflammation (D'Agostino et al., 2007; Lo Verme et al., 2005; O'Sullivan and Kendall, 2010; Re et al., 2007). PPAR-α is mostly distributed in several peripheral organs (O'Sullivan and Kendall, 2010); however, it is also localized in some areas of the nervous system (Kainu et al., 1994; Moreno et al., 2004). Furthermore, PEA by activating on PPAR-α in microglial cells and hippocampal neurons is able to exert neuroprotective properties (Koch et al., 2011; Scuderi et al., 2012). Moreover, as demonstrated by Sasso et al. (2010), administration of PEA increased pentobarbital induced loss of righting reflex (LORR) duration in mice, enhancing neurosteroidogenic pathway, and this effect disappears in PPAR-α knockout mice. Based on this background and on the effects observed in our experiments, it possible to confirm that PPAR-α are implicated in the anticonvulsant effects of PEA against audiogenic seizures in DBA/2 similarly to what was previously observed in WAG/Rij rats (Citraro et al., 2013b). In this latter model, we demonstrated that PEA effects are mediated by CB1 receptors, which are indirectly activated through the action of PEA on PPAR-α receptors (Citraro et al., 2013b). However, PEA anticonvulsant effects might also be related to the enhancement of the action of endogenous AEA (Ben-Shabat et al., 1998; Jonsson et al., 2001; Lambert and Di Marzo, 1999; Petrosino et al., 2010). Therefore, our results confirm PEA antiepileptic properties while its mechanism

remains to be completely clarified since several systems are probably involved further than PPAR-α mediated effects such as transient vanilloid 1 channel (TRPV1) receptors (De Petrocellis et al., 2001; Ho et al., 2008).

4.4. Conclusions

In conclusion, these results suggest that PEA and cannabinoid analogues (ACEA and WIN) could be a potential pharmacological treatment against audiogenic seizures. However, additional studies are needed to better clarify the antiseizure effects of PPAR-α, in this and other animal models. Nevertheless, PEA acts through several mechanisms of action and most of them might contribute to its anticonvulsant properties. Furthermore, we reported that PEA and the cannabinomimetics studied are able to potentiate the antiseizure effects of several AEDs indicating that this drugs and their mechanisms of action might be relevant for the development of potentially new antiepileptic drugs.

Conflict of interest

None of the authors has any conflict of interest to disclose.

Role of funding source

This work was supported by the Italian Ministry of Education, University and Research (MIUR, Cofin 2007, prot. 2007WRJNMX Rome) and the National Research Council (CNR, Rome). PON01_02512 Progetto "Ricerca e Sviluppo di bioregolatori attivi sui meccanismi epigenetici dei processi infiammatori nelle malattie croniche e degenerative (BIAM-EPI)" CUP RICERCA N.B21H11000730005. Both were not involved in study design, collection, analysis and interpretation of data.

Acknowledgments

This work was supported by the Italian Ministry of Education, University and Research (MIUR, Cofin 2007, Rome) and the National Research Council (CNR, Rome) is gratefully ack.

References

Aghaei, I., Rostampour, M., Shabani, M., Naderi, N., Motamedi, F., Babaei, P., Khakpour-Taleghani, B., 2015. Palmitoylethanolamide attenuates PTZ-induced seizures through CB1 and CB2 receptors. Epilepsy Res. 117, 23–28.

Albayram, O., Alferink, J., Pitsch, J., Piyanova, A., Neitzert, K., Poppensieker, K., Mauer, D., Michel, K., Legler, A., Becker, A., Monory, K., Lutz, B., Zimmer, A., Bilkei-Gorzo, A., 2011. Role of CB1 cannabinoid receptors on GABAergic neurons in brain aging. Proc. Natl. Acad. Sci. USA 108, 11256–11261.

Andres-Mach, M., Zolkowska, D., Barcicka-Klosowska, B., Haratym-Maj, A., Florek-Luszczki, M., Luszczki, J.J., 2012. Effect of ACEA–a selective cannabinoid CB1 receptor agonist on the protective action of different antiepileptic drugs in the mouse pentylenetetrazole-induced seizure model. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 39, 301–309.

Antonucci, F., Alpar, A., Kacza, J., Caleo, M., Verderio, C., Giani, A., Martens, H., Chaudhry, F.A., Allegra, M., Grosche, J., Michalski, D., Erck, C., Hoffmann, A., Harkany, T., Matteoli, M., Hartig, W., 2012. Cracking down on inhibition: selective removal of GABAergic interneurons from hippocampal networks. J. Neurosci. 32, 1989–2001.

Armstrong, C., Morgan, R.J., Soltesz, I., 2009. Pursuing paradoxical proconvulsant prophylaxis for epileptogenesis. Epilepsia 50, 1657–1669.

Ben-Shabat, S., Fride, E., Sheskin, T., Tamiri, T., Rhee, M.H., Vogel, Z., Bisogno, T., De Petrocellis, L., Di Marzo, V., Mechoulam, R., 1998. An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. Eur. J. Pharmacol. 353, 23–31.

Bidaut-Russell, M., Howlett, A.C., 1991. Cannabinoid receptor-regulated cyclic AMP accumulation in the rat striatum. J. Neurochem. 57, 1769–1773.

Blair, R.E., Deshpande, L.S., Sombati, S., Elphick, M.R., Martin, B.R., DeLorenzo, R.J., 2009. Prolonged exposure to WIN55,212-2 causes downregulation of the CB1 receptor and the development of tolerance to its anticonvulsant effects in the hippocampal neuronal culture model of acquired epilepsy. Neuropharmacology 57, 208–218.

Calignano, A., La Rana, G., Piomelli, D., 2001. Antinociceptive activity of the endogenous fatty acid amide, palmitylethanolamide. Eur. J. Pharmacol. 419, 191–198.

Calignano, A., La Rana, G., Giuffrida, A., Piomelli, D., 1998. Control of pain initiation by endogenous cannabinoids. Nature 394, 277–281.

Citraro, R., Scicchitano, F., De Fazio, S., Raggio, R., Mainardi, P., Perucca, E., De Sarro, G., Russo, E., 2011. Preclinical activity profile of alpha-lactoalbumin, a whey protein rich in tryptophan, in rodent models of seizures and epilepsy. Epilepsy Res. 95, 60–69.

Citraro, R., Russo, E., Ngomba, R.T., Nicoletti, F., Scicchitano, F., Whalley, B.J., Calignano, A., De Sarro, G., 2013a. CB1 agonists, locally applied to the cortico-thalamic circuit of rats with genetic absence epilepsy, reduce epileptic manifestations. Epilepsy Res. 106, 74–82.

Citraro, R., Russo, E., Scicchitano, F., van Rijn, C.M., Cosco, D., Avagliano, C., Russo, R., D'Agostino, G., Petrosino, S., Guida, F., Gatta, L., van Luijtelaar, G., Maione, S., Di Marzo, V., Calignano, A., De Sarro, G., 2013b. Antiepileptic action of N-palmitoylethanolamine through CB1 and PPAR-alpha receptor activation in a genetic model of absence epilepsy. Neuropharmacology 69, 115–126.

Costa, B., Comelli, F., Bettoni, I., Colleoni, M., Giagnoni, G., 2008. The endogenous fatty acid amide, palmitoylethanolamide, has anti-allodynic and anti-hyperalgesic effects in a murine model of neuropathic pain: involvement of CB(1), TRPV1 and PPARgamma receptors and neurotrophic factors. Pain 139, 541–550.

D'Agostino, G., Russo, R., Avagliano, C., Cristiano, C., Meli, R., Calignano, A., 2012. Palmitoylethanolamide protects against the amyloid-beta25-35-induced learning and memory impairment in mice, an experimental model of Alzheimer disease. Neuropsychopharmacol.: Off. Publ. Am. Coll. Neuropsychopharmacol. 37, 1784–1792.

D'Agostino, G., La Rana, G., Russo, R., Sasso, O., Iacono, A., Esposito, E., Raso, G.M., Cuzzocrea, S., Lo Verme, J., Piomelli, D., Meli, R., Calignano, A., 2007. Acute intracerebroventricular administration of palmitoylethanolamide, an endogenous peroxisome proliferator-activated receptor-alpha agonist, modulates carrageenan-induced paw edema in mice. J. Pharmacol. Exp. Ther. 322, 1137–1143.

De Petrocellis, L., Davis, J.B., Di Marzo, V., 2001. Palmitoylethanolamide enhances anandamide stimulation of human vanilloid VR1 receptors. FEBS Lett. 506, 253–256.

De Sarro, G., Gareri, P., Falconi, U., De Sarro, A., 2000. 7-Nitroindazole potentiates the antiseizure activity of some anticonvulsants in DBA/2 mice. Eur. J. Pharmacol. 394, 275–288.

De SarroG.RussoE.CitraroR.MeldrumB.S., 2015. Genetically epilepsy-prone rats (GEPRs) and DBA/2 mice: two animal models of audiogenic reflex epilepsy for the evaluation of new generation AEDs. Epilepsy Behav.

De Sarro, G., Di Paola, E.D., Ferreri, G., De Sarro, A., Fischer, W., 2002. Influence of some beta-adrenoceptor antagonists on the anticonvulsant potency of antiepileptic drugs against audiogenic seizures in DBA/2 mice. Eur. J. Pharmacol. 442, 205–213.

De Sarro, G., Rizzo, M., Sinopoli, V.A., Gitto, R., De Sarro, A., Zappala, M., Chimirri, A., 1998. Relationship between anticonvulsant activity and plasma level of some 2,3-benzodiazepines in genetically epilepsy-prone rats. Pharmacol. Biochem. Behav. 61, 215–220.

De Sarro, G., Ibbadu, G.F., Marra, R., Rotiroti, D., Loiacono, A., Donato Di Paola, E., Russo, E., 2004. Seizure susceptibility to various convulsant stimuli in dystrophin-deficient mdx mice. Neurosci. Res. 50, 37–44.

DevinskyO.CilioM.R.CrossH.Fernandez-RuizJ.FrenchJ.HillC.KatzR.Di MarzoV.Jutras-AswadD.NotcuttW.G.Martinez-OrgadoJ.RobsonP.J.RohrbackB.G.ThieleE.WhalleyB.FriedmanD., 2014. Cannabidiol: pharmacology and potential therapeutic role in epilepsy and other neuropsychiatric disorders. Epilepsia.

Di Cesare Mannelli, L., D'Agostino, G., Pacini, A., Russo, R., Zanardelli, M., Ghelardini, C., Calignano, A., 2013. Palmitoylethanolamide is a disease-modifying agent in peripheral neuropathy: pain relief and neuroprotection share a PPAR-alpha-mediated mechanism. Mediat. Inflamm. 2013, 328797.

Di Marzo, V., Melck, D., Orlando, P., Bisogno, T., Zagoory, O., Bifulco, M., Vogel, Z., De Petrocellis, L., 2001. Palmitoylethanolamide inhibits the expression of fatty acid amide hydrolase and enhances the anti-proliferative effect of anandamide in human breast cancer cells. Biochem. J. 358, 249–255.

Donato Di Paola, E., Gareri, P., Davoli, A., Gratteri, S., Scicchitano, F., Naccari, C., De SarroG., G., 2007. Influence of levetiracetam on the anticonvulsant efficacy of conventional antiepileptic drugs against audiogenic seizures in DBA/2 mice. Epilepsy Res. 75, 112–121.

Engler, B., Freiman, I., Urbanski, M., Szabo, B., 2006. Effects of exogenous and endogenous cannabinoids on GABAergic neurotransmission between the caudate-putamen and the globus pallidus in the mouse. J. Pharmacol. Exp. Ther. 316, 608–617.

Falenski, K.W., Blair, R.E., Sim-Selley, L.J., Martin, B.R., DeLorenzo, R.J., 2007. Status epilepticus causes a long-lasting redistribution of hippocampal cannabinoid type 1 receptor expression and function in the rat pilocarpine model of acquired epilepsy. Neuroscience 146, 1232–1244.

Freund, T.F., Katona, I., Piomelli, D., 2003. Role of endogenous cannabinoids in synaptic signaling. Physiol. Rev. 83, 1017–1066.

Friedman, D., Devinsky, O., 2015. Cannabinoids in the treatment of epilepsy. N. Engl. J. Med. 373, 1048–1058.

Fu, J., Gaetani, S., Oveisi, F., Lo Verme, J., Serrano, A., Rodriguez De Fonseca, F.,

Rosengarth, A., Luecke, H., Di Giacomo, B., Tarzia, G., Piomelli, D., 2003. Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-alpha. Nature 425, 90–93.
Gitto, R., Ficarra, R., Stancanelli, R., Guardo, M., De Luca, L., Barreca, M.L., Pagano, B., Rotondo, A., Bruno, G., Russo, E., De Sarro, G., Chimirri, A., 2007. Synthesis, resolution, stereochemistry, and molecular modeling of (R)- and (S)-2-acetyl-1-(4′-chlorophenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline AMPAR antagonists. Bioorg. Med. Chem. 15, 5417–5423.
Goffin, K., Van Paesschen, W., Van Laere, K., 2011. In vivo activation of endocannabinoid system in temporal lobe epilepsy with hippocampal sclerosis. Brain: J. Neurol. 134, 1033–1040.
Hampson, R.E., Evans, G.J., Mu, J., Zhuang, S.Y., King, V.C., Childers, S.R., Deadwyler, S.A., 1995. Role of cyclic AMP dependent protein kinase in cannabinoid receptor modulation of potassium "A-current" in cultured rat hippocampal neurons. Life Sci. 56, 2081–2088.
Hansen, H.S., 2010. Palmitoylethanolamide and other anandamide congeners. Proposed role in the diseased brain. Exp. Neurol. 224, 48–55.
Hill, A.J., Mercier, M.S., Hill, T.D., Glyn, S.E., Jones, N.A., Yamasaki, Y., Futamura, T., Duncan, M., Stott, C.G., Stephens, G.J., Williams, C.M., Whalley, B.J., 2012. Cannabidivarin is anticonvulsant in mouse and rat. Br. J. Pharmacol. 167, 1629–1642.
Ho, W.S., Barrett, D.A., Randall, M.D., 2008. 'Entourage' effects of N-palmitoylethanolamide and N-oleoylethanolamide on vasorelaxation to anandamide occur through TRPV1 receptors. Br. J. Pharmacol. 155, 837–846.
Howlett, A.C., Breivogel, C.S., Childers, S.R., Deadwyler, S.A., Hampson, R.E., Porrino, L.J., 2004. Cannabinoid physiology and pharmacology: 30 years of progress. Neuropharmacology 47 (Suppl. 1), S345–S358.
Huang, C.C., Lo, S.W., Hsu, K.S., 2001. Presynaptic mechanisms underlying cannabinoid inhibition of excitatory synaptic transmission in rat striatal neurons. J. Physiol. 532, 731–748.
Italiano, D., Striano, P., Russo, E., Leo, A., Spina, E., Zara, F., Striano, S., Gambardella, A., Labate, A., Gasparini, S., Lamberti, M., De Sarro, G., Aguglia, U., Ferlazzo, E., 2016. Genetics of reflex seizures and epilepsies in humans and animals. Epilepsy Res. 121, 47–54.
Jones, N.A., Hill, A.J., Weston, S.E., Burnett, M.D., Stephens, G.J., Whalley, B.J., Williams, C.M., 2011. WITHDRAWN: cannabidiol exerts anti-convulsant effects in animal models of temporal lobe and partial seizures. Seizure.
Jonsson, K.O., Vandevoorde, S., Lambert, D.M., Tiger, G., Fowler, C.J., 2001. Effects of homologues and analogues of palmitoylethanolamide upon the inactivation of the endocannabinoid anandamide. Br. J. Pharmacol. 133, 1263–1275.
Kainu, T., Wikstrom, A.C., Gustafsson, J.A., Pelto-Huikko, M., 1994. Localization of the peroxisome proliferator-activated receptor in the brain. Neuroreport 5, 2481–2485.
Karlocai, M.R., Toth, K., Watanabe, M., Ledent, C., Juhasz, G., Freund, T.F., Magloczky, Z., 2011. Redistribution of CB1 cannabinoid receptors in the acute and chronic phases of pilocarpine-induced epilepsy. PloS One 6, e27196.
Koch, M., Kreutz, S., Bottger, C., Benz, A., Maronde, E., Ghadban, C., Korf, H.W., Dehghani, F., 2011. Palmitoylethanolamide protects dentate gyrus granule cells via peroxisome proliferator-activated receptor-alpha. Neurotox. Res. 19, 330–340.
Kozan, R., Ayyildiz, M., Agar, E., 2009. The effects of intracerebroventricular AM-251, a CB1-receptor antagonist, and ACEA, a CB1-receptor agonist, on penicillin-induced epileptiform activity in rats. Epilepsia 50, 1760–1767.
Lambert, D.M., Di Marzo, V., 1999. The palmitoylethanolamide and oleamide enigmas: are these two fatty acid amides cannabimimetic? Curr. Med. Chem. 6, 757–773.
Lambert, D.M., Vandevoorde, S., Jonsson, K.O., Fowler, C.J., 2002. The palmitoylethanolamide family: a new class of anti-inflammatory agents? Curr. Med. Chem. 9, 663–674.
Lambert, D.M., Vandevoorde, S., Diependaele, G., Govaerts, S.J., Robert, A.R., 2001. Anticonvulsant activity of N-palmitoylethanolamide, a putative endocannabinoid, in mice. Epilepsia 42, 321–327.
Leo, A., Russo, E., Elia, M., 2016. Cannabidiol and epilepsy: rationale and therapeutic potential. harmacol. Res.: Off. J. Ital. Pharmacol. Soc.
Litchfield Jr., J.T., Wilcoxon, F., 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96, 99–113.
Lo Verme, J., Fu, J., Astarita, G., La Rana, G., Russo, R., Calignano, A., Piomelli, D., 2005. The nuclear receptor peroxisome proliferator-activated receptor-alpha mediates the anti-inflammatory actions of palmitoylethanolamide. Mol. Pharmacol. 67, 15–19.
Luszczki, J.J., Florek-Luszczki, M., 2012. Synergistic interaction of pregabalin with the synthetic cannabinoid WIN 55,212-2 mesylate in the hot-plate test in mice: an isobolographic analysis. Pharmacol. Rep.: PR 64, 723–732.
Luszczki, J.J., Czuczwar, P., Cioczek-Czuczwar, A., Czuczwar, S.J., 2006. Arachidonyl-2′-chloroethylamide, a highly selective cannabinoid CB1 receptor agonist, enhances the anticonvulsant action of valproate in the mouse maximal electroshock-induced seizure model. Eur. J. Pharmacol. 547, 65–74.
Luszczki, J.J., Misiuta-Krzesinska, M., Florek, M., Tutka, P., Czuczwar, S.J., 2011b. Synthetic cannabinoid WIN 55,212-2 mesylate enhances the protective action of four classical antiepileptic drugs against maximal electroshock-induced seizures in mice. Pharmacol., Biochem., Behav. 98, 261–267.
Luszczki, J.J., Wlaz, A., Karwan, S., Florek-Luszczki, M., Czuczwar, S.J., 2013. Effects of WIN 55,212-2 mesylate on the anticonvulsant action of lamotrigine, oxcarbazepine, pregabalin and topiramate against maximal electroshock-induced seizures in mice. Eur. J. Pharmacol. 720, 247–254.
Luszczki, J.J., Czuczwar, P., Cioczek-Czuczwar, A., Dudra-Jastrzebska, M., Andres-Mach, M., Czuczwar, S.J., 2010. Effect of arachidonyl-2′-chloroethylamide, a selective cannabinoid CB1 receptor agonist, on the protective action of the various antiepileptic drugs in the mouse maximal electroshock-induced seizure model. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 34, 18–25.
Luszczki, J.J., Andres-Mach, M., Barcicka-Klosowska, B., Florek-Luszczki, M., Haratym-Maj, A., Czuczwar, S.J., 2011a. Effects of WIN 55,212-2 mesylate (a synthetic cannabinoid) on the protective action of clonazepam, ethosuximide, phenobarbital and valproate against pentylenetetrazole-induced clonic seizures in mice. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 35, 1870–1876.
Lutz, B., 2004. On-demand activation of the endocannabinoid system in the control of neuronal excitability and epileptiform seizures. Biochem. Pharmacol. 68, 1691–1698.
Mackie, K., Devane, W.A., Hille, B., 1993. Anandamide, an endogenous cannabinoid, inhibits calcium currents as a partial agonist in N18 neuroblastoma cells. Mol. Pharmacol. 44, 498–503.
Mackie, K., Lai, Y., Westenbroek, R., Mitchell, R., 1995. Cannabinoids activate an inwardly rectifying potassium conductance and inhibit Q-type calcium currents in AtT20 cells transfected with rat brain cannabinoid receptor. J. Neurosci. 15, 6552–6561.
Maier, N., Morris, G., Schuchmann, S., Korotkova, T., Ponomarenko, A., Bohm, C., Wozny, C., Schmitz, D., 2012. Cannabinoids disrupt hippocampal sharp wave-ripples via inhibition of glutamate release. Hippocampus 22, 1350–1362.
Maneuf, Y.P., Nash, J.E., Crossman, A.R., Brotchie, J.M., 1996. Activation of the cannabinoid receptor by delta 9-tetrahydrocannabinol reduces gamma-aminobutyric acid uptake in the globus pallidus. Eur. J. Pharmacol. 308, 161–164.
Marsicano, G., Lutz, B., 1999. Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. Eur. J. Neurosci. 11, 4213–4225.
Mattace Raso, G., Russo, R., Calignano, A., Meli, R., 2014. Palmitoylethanolamide in CNS health and disease. Pharmacol. Res. 86, 32–41.
Moreno, S., Farioli-Vecchioli, S., Ceru, M.P., 2004. Immunolocalization of peroxisome proliferator-activated receptors and retinoid X receptors in the adult rat CNS. Neuroscience 123, 131–145.
Naderi, N., Aziz Ahari, F., Shafaghi, B., Najarkolaei, A.H., Motamedi, F., 2008. Evaluation of interactions between cannabinoid compounds and diazepam in electroshock-induced seizure model in mice. J. Neural Transm. 115, 1501–1511.
O'Sullivan, S.E., Kendall, D.A., 2010. Cannabinoid activation of peroxisome proliferator-activated receptors: potential for modulation of inflammatory disease. Immunobiology 215, 611–616.
Payandemehr, B., Ebrahimi, A., Gholizadeh, R., Rahimian, R., Varastehmoradi, B., Gooshe, M., Aghaei, H.N., Mousavizadeh, K., Dehpour, A.R., 2015. Involvement of PPAR receptors in the anticonvulsant effects of a cannabinoid agonist, WIN 55,212-2. Prog. neuro-Psychopharmacol. Biol. Psychiatry 57, 140–145.
Petrosino, S., Iuvone, T., Di Marzo, V., 2010. N-palmitoyl-ethanolamine: biochemistry and new therapeutic opportunities. Biochimie 92, 724–727.
Polissidis, A., Galanopoulos, A., Naxakis, G., Papahatjis, D., Papadopoulou-Daifoti, Z., Antoniou, K., 2013. The cannabinoid CB1 receptor biphasically modulates motor activity and regulates dopamine and glutamate release region dependently. Int. J. Neuropsychopharmacol. 16, 393–403.
Raso, G.M., Esposito, E., Vitiello, S., Iacono, A., Santoro, A., D'Agostino, G., Sasso, O., Russo, R., Piazza, P.V., Calignano, A., Meli, R., 2011. Palmitoylethanolamide stimulation induces allopregnanolone synthesis in C6 Cells and primary astrocytes: involvement of peroxisome-proliferator activated receptor-alpha. J. Neuroendocrinol. 23, 591–600.
Re, G., Barbero, R., Miolo, A., Di Marzo, V., 2007. Palmitoylethanolamide, endocannabinoids and related cannabimimetic compounds in protection against tissue inflammation and pain: potential use in companion animals. Vet. J. 173, 21–30.
Richardson, J.D., Aanonsen, L., Hargreaves, K.M., 1998. Hypoactivity of the spinal cannabinoid system results in NMDA-dependent hyperalgesia. J. Neurosci. 18, 451–457.
Ruehle, S., Remmers, F., Romo-Parra, H., Massa, F., Wickert, M., Wortge, S., Haring, M., Kaiser, N., Marsicano, G., Pape, H.C., Lutz, B., 2013. Cannabinoid CB1 receptor in dorsal telencephalic glutamatergic neurons: distinctive sufficiency for hippocampus-dependent and amygdala-dependent synaptic and behavioral functions. J. Neurosci. 33, 10264–10277.
Russo, E., Donato di Paola, E., Gareri, P., Siniscalchi, A., Labate, A., Gallelli, L., Citraro, R., De Sarro, G., 2013. Pharmacodynamic potentiation of antiepileptic drugs' effects by some HMG-CoA reductase inhibitors against audiogenic seizures in DBA/2 mice. Pharmacol. Res. 70, 1–12.
Sanchez-Blazquez, P., Rodriguez-Munoz, M., Garzon, J., 2014. The cannabinoid receptor 1 associates with NMDA receptors to produce glutamatergic hypofunction: implications in psychosis and schizophrenia. Front. Pharmacol. 4, 169.
Sarro, G.D., Paola, E.D., Gratteri, S., Gareri, P., Rispoli, V., Siniscalchi, A., Tripepi, G., Gallelli, L., Citraro, R., Russo, E., 2012. Fosinopril and zofenopril, two angiotensin-converting enzyme (ACE) inhibitors, potentiate the anticonvulsant activity of antiepileptic drugs against audiogenic seizures in DBA/2 mice. Pharmacol. Res. 65, 285–296.
Sasso, O., La Rana, G., Vitiello, S., Russo, R., D'Agostino, G., Iacono, A., Russo, E., Citraro, R., Cuzzocrea, S., Piazza, P.V., De Sarro, G., Meli, R., Calignano, A., 2010. Palmitoylethanolamide modulates pentobarbital-evoked hypnotic effect in mice: involvement of allopregnanolone biosynthesis. Eur. Neuropsychopharmacol.: J. Eur. Coll. Neuropsychopharmacol. 20, 195–206.
Scuderi, C., Valenza, M., Stecca, C., Esposito, G., Carratu, M.R., Steardo, L., 2012. Palmitoylethanolamide exerts neuroprotective effects in mixed neuroglial cultures and organotypic hippocampal slices via peroxisome proliferator-activated

receptor-alpha. J. Neuroinflamm. 9, 49.
Shafaroodi, H., Moezi, L., Bahremand, A., Dehpour, A.R., 2013. The role of alpha(2)-adrenoceptors in the anti-convulsant effects of cannabinoids on pentylenetetrazole-induced seizure threshold in mice. Eur. J. Pharmacol. 714, 1–6.
Sheerin, A.H., Zhang, X., Saucier, D.M., Corcoran, M.E., 2004. Selective antiepileptic effects of N-palmitoylethanolamide, a putative endocannabinoid. Epilepsia 45, 1184–1188.
Shen, M., Piser, T.M., Seybold, V.S., Thayer, S.A., 1996. Cannabinoid receptor agonists inhibit glutamatergic synaptic transmission in rat hippocampal cultures. J. Neurosci. 16, 4322–4334.
Sieradzan, K.A., Fox, S.H., Hill, M., Dick, J.P., Crossman, A.R., Brotchie, J.M., 2001. Cannabinoids reduce levodopa-induced dyskinesia in Parkinson's disease: a pilot study. Neurology 57, 2108–2111.
Sugiura, T., Kondo, S., Kishimoto, S., Miyashita, T., Nakane, S., Kodaka, T., Suhara, Y., Takayama, H., Waku, K., 2000. Evidence that 2-arachidonoylglycerol but not N-palmitoylethanolamine or anandamide is the physiological ligand for the cannabinoid CB2 receptor. Comparison of the agonistic activities of various cannabinoid receptor ligands in HL-60 cells. J. Biol. Chem. 275, 605–612.
Vilela, L.R., Medeiros, D.C., Rezende, G.H., de Oliveira, A.C., Moraes, M.F., Moreira, F. A., 2013. Effects of cannabinoids and endocannabinoid hydrolysis inhibition on pentylenetetrazole-induced seizure and electroencephalographic activity in rats. Epilepsy Res. 104, 195–202.
Wallace, M.J., Martin, B.R., DeLorenzo, R.J., 2002. Evidence for a physiological role of endocannabinoids in the modulation of seizure threshold and severity. Eur. J. Pharmacol. 452, 295–301.
Wallace, M.J., Blair, R.E., Falenski, K.W., Martin, B.R., DeLorenzo, R.J., 2003. The endogenous cannabinoid system regulates seizure frequency and duration in a model of temporal lobe epilepsy. J. Pharmacol. Exp. Ther. 307, 129–137.