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Influence of carbenoxolone on the anticonvulsant efficacy of phenytoin in pentylenetetrazole kindled rats

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Abnormal synchronized neuronal discharges mediated by gap junctions have an important role in epileptic seizures. The analysis of anticonvulsant drugs acting on gap junctions is still a priority in epilepsy research. Therefore, the present study was designed to investigate the effect of carbenoxolone, a gap junction blocker, on the anticonvulsant efficacy of phenytoin in pentylenetetrazole kindled rats. Male Wistar albino rats, 14 weeks of age, were used. In the first step of the study, animals were given PTZ 35 mg/kg intraperitoneally (i.p.) three times a week until kindling was produced. Then, indwelling screw electrodes – allowing EEG monitoring of conscious rats – were implanted into the crania of the kindled rats. In this way, we were able to record EEG activity and evaluate seizure stage at the same time. In the second step of the study, the interaction between carbenoxolone (40 mg/kg i.p.) and phenytoin (60 mg/kg, i.p.) was investigated. The data analysis was performed using a one-way ANOVA with LSD post-hoc test. Total spike number and the generalized seizure duration were reduced in the carbenoxolone treated group compared to the PTZ group. Phenytoin decreased generalized seizure duration, total spike number and seizure severity score. Carbenoxolone and phenytoin have anti-seizure effects in PTZ kindled rats. There was no significant difference between the carbenoxolone + phenytoin combination and phenytoin in terms of generalized seizure duration, total spike number and seizure stage. The results indicate that carbenoxolone combined with phenytoin is not more effective than the use of these drugs alone.

Key words: carbenoxolone, phenytoin, gap junction, pentylenetetrazole kindling

INTRODUCTION

A wide variety of experimental models of epilepsy have been developed in order to understand the basic mechanisms of epilepsy and to discover new drugs for seizure management. One such model is the kindling model of epilepsy. Kindling is defined as a progressive increase in vulnerability to evoked seizures (Lukasiuk et al. 2011). Two kinds of kindling model can be created: the first one is the electrical kindling model, while the second one is the chemical kindling model (Gilbert and Goodman 2006).

Pentylenetetrazole (PTZ) is one of the most preferred substances used in the production of chemical kindling. It has been reported that PTZ-kindled seizures mimic primary generalized epilepsy in terms of ILEA classification (Gilbert and Goodman 2006). The mechanism of action of PTZ is unclear, but it is thought that PTZ is a selective inhibitor of chloride channels associated with GABAA receptors and reduces the effect of GABA (Kaminska et al. 1994, Sejima et al. 1997, Hansen et al. 2004).

Gap junctions between eukaryotic cells are specialized links which provide direct intercellular communication (Goodenough and Paul 2009). Gap junctions consist of a pair of hemi-channels, called connexons. A connexon is normally made up of six similar protein subunits, called connexins (Kandel and Siegelbaum 2000).

It has been suggested that gap junctions have an essential role in neuronal synchronization and epileptogenesis (the development of epilepsy). Nassir-Asl et al. revealed that trimethylamine (a gap junction open-

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er) increased seizure severity in an acute PTZ model and inhibited the effects of the antiepileptic gap junction blocker, quinine (Nassiri-Asl et al. 2008).

There are many chemical agents and drugs that block gap junctions. The most widely-used gap junction blockers in experimental seizure models are carbenoxolone, octanol, glycyrrhetinic acid, quinine and quinidine (Juszczak and Swiergiel 2009). Studies in mice and rats demonstrated that systemic administration of carbenoxolone and quinine has anticonvulsant effects. In addition, when applied directly to the brain, octanol, quinine and carbenoxolone reduced the epileptiform activity (Bostanci and Bagirici 2006, 2007a,b). Moreover, in an earlier study, it was reported that carbenoxolone has anticonvulsant potential in DBA/2 mice (Gareri et al. 2004).

Carbenoxolone has been reported to exert cranial nerve system effects after systemic administration in rats, and it has also been reported that carbenoxolone did not block any specific type of connexins (Juszczak and Swiergiel 2009). There are however very few studies in which carbenoxolone was found to prevent seizures in kindling models of epilepsy. Some researchers revealed that carbenoxolone exhibited anticonvulsant effects in PTZ-kindled rats (Ding et al. 2006, Lan et al. 2007b). Furthermore, Sayyah et al. administrated carbenoxolone to the basolateral nucleus of the amygdala, and found that carbenoxolone increased after-discharge duration and shortened the generalized seizure duration (Sayyah et al. 2007).

Phenytoin is one of the most preferred treatments for partial seizures (Tunnicliff 1996). Chemically, phenytoin is a lipophilic organic acid, and easily penetrates through the cell membrane. It has been shown that phenytoin binds voltage-gated sodium channels, and prevents seizures by extending the inactivation duration of these channels (Shorvon 2005).

Krug et al. originally investigated the effects of phenytoin against the PTZ-kindling model in rats, and suggested that phenytoin was not effective in this model (Krug et al. 1998). However, phenytoin has been found to have anticonvulsant effects in amygdaloid-kindled guinea pigs (Gilbert et al. 2001).

There is very little information in the literature concerning the effect of carbenoxolone in the kindling models of epilepsy. To the best of our knowledge this is the first study investigating the effect of carbenoxolone and phenytoin in combination on PTZ kindled rats. Therefore the purpose of the present study was to explore the effects of carbenoxolone and phenytoin and the influence of carbenoxolone on the anticonvulsant efficacy of phenytoin in PTZ-kindled rats.

METHODS

Animals

Male albino rats of the Wistar strain (12–16 weeks of age, weighing 200 ± 50 g) were used in the study. The animals were provided by the Center for Surgical Research, University of Ondokuz Mayis at Samsun. Before the experiments, this study was approved by the Ethical Committee for Animal Experiments at the University of Ondokuz Mayis (OMU HAYDEK/2008-48). The experiments were carried out in accordance with the guidelines of the European Community Council for experimental animal care. Rats were housed individually in a 12-h light: 12-h dark cycle (lights on at 08.00 am–08:00 pm), at a temperature of 22 ± 2°C and 50–55% humidity. Water and food were given ad libitum.

Kindling procedure

For PTZ-kindling, in the first step of the study, animals were given PTZ 35 mg/kg intraperitoneally (i.p.) three times a week (Monday, Wednesday, Friday) until kindling was produced. Animals were placed individually into transparent cages (dimensions 35 × 35 × 35 cm) and observed for 30 min after each PTZ injection. Seizure behaviors were evaluated in terms of Fischer and Kittner's study (Fischer and Kittner 1998) on a seizure severity scale of 1–5.

(Stage 0) no seizure; (Stage 0.5) weak nodding; (Stage 1) ear, face and eyelid twitching; (Stage 1.5) mild forelimb clonus; (Stage 2) myoclonic body jerks, clonic convulsions of fore-limb (no rearing); (Stage 2.5) partial rearing and rapid clonic seizures of forelimb; (Stage 3) powerful bilateral fore-limb clonus with complete rearing (≥ 10 s); (Stage 3.5) rearing and falling with intense bilateral fore-limb clonus; (Stage 4) generalized clonic seizures with rearing-falling down episodes, or jumping; (Stage 4.5) generalized clonic-tonic seizures with failure of rightning reflex; (Stage 5) generalized clonic-tonic seizures and status epilepticus (≥ 2 min).

If the seizure behavior was stage 3 or more severe, it was accepted as a generalized seizure. Kindling was

considered to be completed when an animal had experienced a total of five generalized seizures (grade 3 or more severe). To induce complete kindling 14.0 ± 1 injections were performed. The non-kindled rats were not included in the study.

Surgical operation

Indwelling screw electrodes, which allow EEG monitoring of conscious animals, were fixed into the skulls of the kindled rats. In this way, we were able to record EEG and evaluate the seizure stage at the same time. The kindled rats were anesthetized with intraperitoneally (i.p.) 90 mg/kg ketamine hydrochloride and 10 mg/kg xylasine combination, and then positioned in a stereotaxic apparatus (Harvard). After a midline scalp incision, electrodes were fixed into the skull at the following coordinates: first electrode: 3 mm lateral to sagittal suture and 4 mm anterior to Bregma: (primary motor cortex, M1) and second electrode: 3 mm lateral to sagittal suture and 4 mm posterior to Bregma (medial parietal association cortex, MPtA) (Paxinos and Watson 2007). A reference electrode was positioned in the skull at 4 mm posterior to the Bregma. All electrodes were attached to a connecting socket. Finally, the electrodes were implanted by using cold-cured dental acrylic. After the surgery, the animals were allowed to recuperate for a week.

Electrophysiological recordings

Totally awake kindled rats were connected to a computerized EEG recording system (PowerLab/4SP, AD Instruments) by a cable. All EEG recordings were stored on an individual computer for off-line analysis and spike numbers for each animal were automatically calculated by using a software program (Chart v.5.1.1).

Drugs and routes

Pentylenetetrazole (PTZ) was dissolved in saline and injected i.p. at a dose of 35 mg/kg.

Carbenoxolone (CBX) was dissolved in saline and given i.p. at a dose of 40 mg/kg 60 min before PTZ injection. Phenytoin (PHT) was dissolved in 3% dimethyl-sulfoxide (DMSO), and administrated i.p. at a dose of 60 mg/kg 30 min prior to PTZ injection.

Pentylenetetrazole, carbenoxolone, and phenytoin were obtained from Sigma-Aldrich Co.

Experimental groups

Only kindled-rats were involved in groups. After kindling, the animals were divided into six groups. PTZ dosage, which was used to complete the kindling process for each group, was homogeneous. Seizure stages in all groups were evaluated simultaneously with the EEG recordings for 30 min.

(1) Control group (n=6) – The EEGs of kindled animals were recorded for 30 min without administration of any drugs; (2) Pentylenetetrazole group (n=6) – 35 mg/kg i.p. PTZ was injected into rat; (3) Carbenoxolone group (n=6) – Carbenoxolone was given i.p. at a dose of 40 mg/kg 60 min before PTZ injection; (4) Phenytoin group (n=6) – Phenytoin was administrated i.p. at a dose of 60 mg/kg 30 min before PTZ administration; (5) Carbenoxolone + phenytoin group (n=6): Carbenoxolone was given i.p. at a dose of 40 mg/kg 60 min before PTZ injection. Phenytoin was administrated i.p. at a dose of 60 mg/kg 30 min before injection of PTZ; (6) DMSO group (n=6) – 3% DMSO (a volume of 0.1 ml) was administrated i.p. 30 min prior to PTZ injection.

Statistical analysis

The data obtained were analyzed using the SPSS 15.0 package program for Windows. Data are expressed as means ± SEM. Statistical significance was determined as P values less than 0.05. Normal distribution was assessed in accordance with the Shapiro Wilk's test. T test was used for comparisons of two groups that meet the normal distribution. One-way analysis of variance (ANOVA) was used to test for statistical significance, and if the difference was significant, LSD *post-hoc* test was used. Mann-Whitney U test was performed if variables did not show a normal distribution.

RESULTS

The effects of PTZ on EEG activity and seizure score in the PTZ-kindled rats

Thirty-five mg/kg PTZ was administrated by i.p. in the PTZ group. Then the animals were observed and their EEGs were recorded during the course of 30 min. In the PTZ group, the generalized seizures were observed in all animals. The average seizure stage was 3.5 ± 0.2 in the PTZ group (Figs 1, 2).

The effects of carbenoxolone on EEG activity and seizure score in the PTZ-kindled rats

Carbenoxolone shortened the generalized seizure duration ($P<0.05$) and reduced the total average spike numbers ($P<0.01$) compared to the PTZ group. Carbenoxolone did not reduce the seizure stage ($P>0.05$) (Figs 1, 2).

The effects of phenytoin on EEG activity and seizure score in the PTZ-kindled rats

Phenytoin diminished the seizure stage, reduced the total average spike numbers and shortened the generalized seizure duration in comparison with the PTZ and DMSO groups ($P<0.05$) (Figs 1, 2).

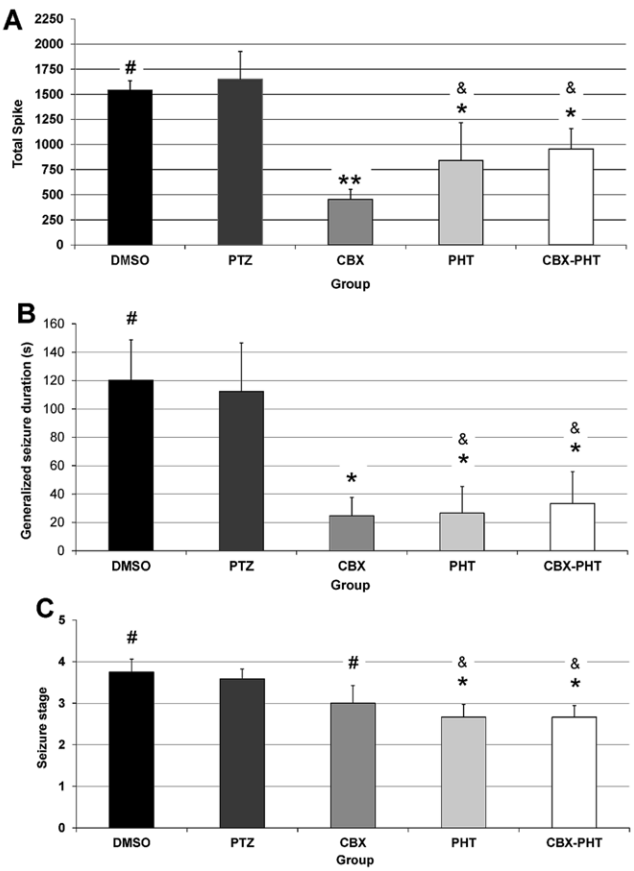

Fig. 1. (A) Spike numbers in each treatment group (Means ± SEM). One-way ANOVA with LSD *post-hoc* test was used; (B) Generalized seizure durations in each treatment group (Means ± SEM). T test was used; (C) Seizure stages for each treatment group (Means ± SEM). Mann-Whitney U test was used. Indications: In comparison with the PTZ group: $^{\#}P>0.05$, $^{*}P<0.05$, $^{**}P<0.01$; In comparison with the DMSO group: $^{\&}P<0.05$. (PTZ) Pentylenetetrazole; (CBX) Carbenoxolone; (PHT) Phenytoin.

The effects of carbenoxolone and phenytoin combination on EEG activity and seizure score in the PTZ-kindled rats

The carbenoxolone + phenytoin combination shortened the generalized seizure duration ($P<0.05$), and reduced the total average spike numbers ($P<0.05$) and the seizure stage ($P<0.05$) compared to the PTZ and DMSO groups. However, the carbenoxolone-phenytoin combination did not result in any significant change in the seizure stage or total average spike number, compared to the phenytoin and carbenoxolone groups (Figs 1, 2).

The effects of DMSO on EEG activity and seizure score in the PTZ-kindled rats

DMSO did not lead to any significant change in all seizure parameters when compared with PTZ group ($P>0.05$) (Figs 1, 2).

DISCUSSION

In the present study, the effects of carbenoxolone and phenytoin were monitored in PTZ-kindled rats. The influence of carbenoxolone on the anticonvulsant efficacy of phenytoin was then investigated in the study.

We applied PTZ three times a week to develop the kindling model since intermittent administration of PTZ has been confirmed as more appropriate than daily injections (Gilbert and Goodman 2006).

Carlen et al. has suggested that gap junctions play an important role in neuronal synchronization and that neuronal synchronization is responsible for the spreading of epileptic discharges (Carlen et al. 2000). Gajda et al. showed that gap junctions provide for the synchronization of cortical activity and may be responsible for epileptogenesis. It has been suggested that the blockade of glial gap junctions could influence neuronal excitability (Gajda et al. 2005).

Carbenoxolone in doses of 200 and 300 mg/kg was reported to prolong the onset time of seizures and to decrease the duration of seizures in an acute PTZ model in mice (Hosseinzadeh and Nassiri-Asl 2003). Carbenoxolone was also was found to prevent seizures in a refractory epilepsy model (Nilsen

et al. 2006). In a previous study, Lan and coworkers (2007b) reported that 10 mg/kg i.p. carbenoxolone decreased spike waves in PTZ-kindled rats and in another study it was found that NMDAR2-Li and Fos-Li neuron numbers were decreased in the carbenoxolone-treated group, but not in a PTZ-kindled group (Lan et al. 2007a). Furthermore, it was revealed that 20 mg/kg i.p. carbenoxolone reduced seizure stages (Ding et al. 2006), and 40 mg/kg i.p. carbenoxolone shortened the wild running phase of audiogenic seizures (Gareri et al. 2004). Similarly, in our study, 40 mg/kg i.p. carbenoxolone decreased spike numbers and reduced the seizure stage and shortened the generalized seizure duration. The difference between the study of Ding and colleagues (2006) and our study is that they have administrated carbenoxolone in lower doses and not reported any EEG monitoring. Lan and coauthors (2007b) have reported EGG monitoring but they have administrated carbenoxolone in lower doses.

However, Voss and others (2009) showed that carbenoxolone had pro-convulsant effects and gap junctions did not promote seizure activity *in vitro*. In contrast, results of some *in vivo* experiments indicated that gap junction blockers, including carbenoxolone reduced seizure activity (Gareri et al. 2004, Bostanci and Bagirici 2007a). In addition, Nassir-Asl and coworkers (2008) reported that a gap junction opener, which is called trimethylamine increased seizure severity *in vitro* and they suggested that gap junctions may have a pro-convulsant potential.

It has been suggested that phenytoin exerts variable effects in the kindling model. Ebert and colleagues (1999) showed that this variation is due in part to the existence of subgroups of kindled rats with different sensitivities to phenytoin; they reported that some animals consistently responded while others never responded to phenytoin. It was also hypothesized that these variable effects, in reference to increases in after-discharge threshold in some kindled rats, may be due to genetic factors (Cramer et al. 1998, Ebert and Loscher 1999).

Some researchers have reported that phenytoin had no anticonvulsant effect in kindling models (McNamara et al. 1989, Rundfeldt et al. 1990, Lothman et al. 1991, Rundfeldt and Loscher 1993). However, others have shown that phenytoin exhibited anticonvulsant effects in kindling models of epilepsy (Morimoto et al. 1997, Otsuki et al. 1998). Gilbert and coauthors (2001) suggested that variable effects of phenytoin arose from the administration route, gender, intensity of stimulus and amount of kindling.

Phenytoin does however show dose-dependent anticonvulsant effects on amygdaloid and hippocampal kindling models of epilepsy in rats. Otsuki et al. administrated phenytoin by i.p.at doses of 30, 60, 120, and 180 mg/kg 30 min before the kindling stimulations, and reported that phenytoin prolonged after-discharge durations at doses of 60 and 120 mg/kg, but significantly shortened after-discharge durations and seizure stages significantly at 180 mg/kg. They also reported that phenytoin was ineffective in terms of reducing seizure stages at lower doses (Otsuki et al. 1998). Similarly, in a previous study, Krug and others (1998) showed that phenytoin was not effective in preventing PTZ kindling. In their study, phenytoin

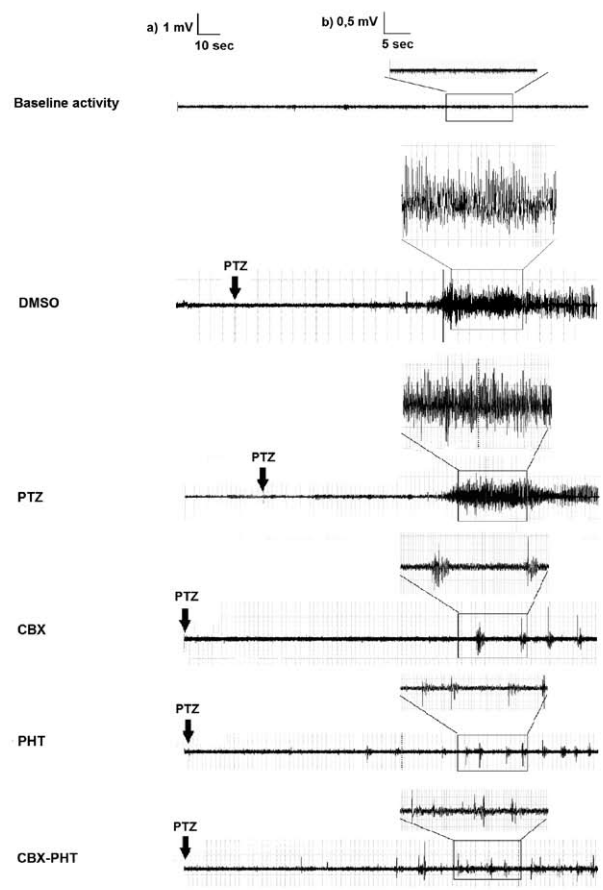

Fig. 2. Representative EEGs for each treatment group. Arrows indicate pentylenetetrazole administrations. (a) 1 mV, 10 seconds; (b) 0,5 mV, 10 seconds

was administrated 60 min before each PTZ injection for PTZ kindling and that phenytoin was unable to prevent kindling (Krug et al. 1998). In contrast, at single i.p. doses of 50 and 70 mg/kg phenytoin was found to be effective in terms of increasing the after-discharge threshold and reducing after-discharge duration and seizure severity in amygdaloid-kindling (Gilbert et al. 2001). In that study they also measured plasma levels of phenytoin following single i.p. injections of 50 and 75 mg/kg phenytoin, reporting that both doses had statically equivalent plasma levels. Indeed, the main difference between the studies of Gilbert and colleagues (2001) and Otsuki and coauthors (1998) was the model. Gilbert and colleagues (2001) administrated single phenytoin injections, and used kindled guinea pigs. However, Otsuki and others (1998) injected phenytoin 30 min before each kindling stimulation in rats. Moreover, in another study using Sprague Dawley rats, phenytoin has been administrated by i.p. 180 mg/kg 30 min prior to each kindling stimulation, and it was reported that phenytoin ameliorated amygdaloid kindled seizures (Morimoto et al. 1997). It can thus be concluded that at lower doses phenytoin prevents seizures in kindled animals, but it is unable to stop the kindling process. At the higher doses, phenytoin can also prevent the kindling process.

Gareri and coworkers (2004) suggested that carbenoxolone enhanced the efficacy of some antiepileptic drugs due to the blockage of selective connexins, and showed that the degree of potentiation induced by carbenoxolone was greater for some antiepileptic drugs and less for phenytoin than some other drugs in DBA/2 mice. However, in our study, carbenoxolone did not have any potentiation for phenytoin in PTZ kindled rats.

It has been reported that carbenoxolone inhibited the junctional transfer and increased the level of Cx43 expression in bovine aortic endothelial cells (Sagar and Larson 2006). After recurrent seizures, the expression levels of Cx 32, Cx43 and Cx46 mRNA were shown to increase (Gajda et al. 2003). In addition, Ding and colleagues (2006) have suggested that Cx32 mRNA expression has been increased in PTZ kindled rats. It has been suggested that phenytoin may have effects on gap junctions. Phenytoin is a 2-aminoethoxydiphenyl borate analog, and has been shown to block the Cx26 and Cx32 subtypes in liver cell cultures (Tao and Harris 2007). The antiepileptic mechanism of carbenoxolone involves the blockade of gap junctions. The similar anticonvulsant effects of carbenoxolone and phenytoin may due to the use of the same pathways and this may be why potentiation was not observed when they were combined.

CONCLUSION

Carbenoxolone and phenytoin have anti-seizure effects in PTZ kindled rats. However, our results indicate that carbenoxolone combined with phenytoin is no more effective than the use of these two drugs alone. Further investigations are needed to explore the effects of these drugs on gap junctions or ion channels.

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REFERENCES

Bostanci MO, Bagirici F (2006) The effects of octanol on penicillin induced epileptiform activity in rats: an in vivo study. Epilepsy Res 71: 188–194.

Bostanci MO, Bagirici F (2007a) Anticonvulsive effects of carbenoxolone on penicillin-induced epileptiform activity: an in vivo study. Neuropharmacology 52: 362–367.

Bostanci MO, Bagirici F (2007b) Anticonvulsive effects of quinine on penicillin-induced epileptiform activity: an in vivo study. Seizure 16: 166–172.

Carlen PL, Skinner F, Zhang L, Naus C, Kushnir M, Perez Velazquez JL (2000) The role of gap junctions in seizures. Brain Res Brain Res Rev 32: 235–241.

Cramer S, Ebert U, Loscher W (1998) Characterization of phenytoin-resistant kindled rats, a new model of drug-resistant partial epilepsy: comparison of inbred strains. Epilepsia 39: 1046–1053.

Ding M-P, Xu L-L, Bao Y-Y (2006) Effects of carbenoxolone on upregulation of connexin 32 in hippocampus of pentylenetetrazole kindled rats. Chinese Pharmaceutical Journal 41: 182–185.

Ebert U, Loscher W (1999) Characterization of phenytoin-resistant kindled rats, a new model of drug-resistant partial epilepsy: influence of genetic factors. Epilepsy Res 33: 217–226.

Ebert U, Rundfeldt C, Lehmann H, Loscher W (1999) Characterization of phenytoin-resistant kindled rats, a new model of drug-resistant partial epilepsy: influence of experimental and environmental factors. Epilepsy Res 33: 199–215.

Fischer W, Kittner H (1998) Influence of ethanol on the pentylenetetrazol-induced kindling in rats. J Neural Transm 105: 1129–1142.

Gajda Z, Gyengesi E, Hermesz E, Ali KS, Szente M (2003) Involvement of gap junctions in the manifestation and control of the duration of seizures in rats in vivo. Epilepsia 44:1596–1600.

Gajda Z, Szupera Z, Blazso G, Szente M (2005) Quinine, a blocker of neuronal cx36 channels, suppresses seizure activity in rat neocortex in vivo. Epilepsia 46: 1581–1591.

Gareri P, Condorelli D, Belluardo N, Gratteri S, Ferreri G, Donato Di Paola E, De Sarro A, De Sarro G (2004) Influence of carbenoxolone on the anticonvulsant efficacy of conventional antiepileptic drugs against audiogenic seizures in DBA/2 mice. Eur J Pharmacol 484: 49–56.

Gilbert ME, Goodman JH (2006) Chemical Kindling. In: Models of Seizures and Epilepsy (Pitkänen A, Philip A, Solomon LM, Eds). Elsevier Academic Press, Burlington, MA. p 379–391.

Gilbert TH, Bharadia V, Teskey GC (2001) Conventional anticonvulsant drugs in the guinea-pig kindling model of partial seizures: effects of acute phenytoin. Exp Brain Res 140: 469–478.

Goodenough DA, Paul DL (2009) Gap junctions. Cold Spring Harb Perspect Biol 1: a002576.

Hansen SL, Sperling BB, Sanchez C (2004) Anticonvulsant and antiepileptogenic effects of GABAA receptor ligands in pentylenetetrazole-kindled mice. Prog Neuropsychopharmacol Biol Psychiatry 28: 105–113.

Hosseinzadeh H, Nassiri Asl M (2003) Anticonvulsant, sedative and muscle relaxant effects of carbenoxolone in mice. BMC Pharmacol 3: 3.

Juszczak GR, Swiergiel AH (2009) Properties of gap junction blockers and their behavioral, cognitive and electrophysiological effects: animal and human studies. Prog Neuropsychopharmacol Biol Psychiatry 33: 181–198.

Kaminska B, Lukasiuk K, Kaczmarek L (1994) Seizures-evoked activation of transcription factors. Acta Neurobiol Exp (Wars) 54: 65–72.

Kandel ER, Siegelbaum SA (2000) Overview of synaptic transmission. In: Principles of Neural Science (Kandel ER, Schwartz JH, Jessell TM, Eds). McGraw-Hill, New York, NY, p. 179.

Krug M, Becker A, Grecksch G, Pfeiffer A, Matthies R, Wagner M (1998) Effects of anticonvulsive drugs on pentylenetetrazol kindling and long-term potentiation in freely moving rats. Eur J Pharmacol 356: 179–187.

Lan L, Wang BX, Zhao X, Duan L, Cao R, Liu Y, Rao ZR (2007a) Effect of carbenoxolone on expression of Fos, NMDAR2 and GFAP in the hippocampus of pentylenetetrazol-kindled epileptic rats (in Chinese). Zhongguo Dang Dai Er Ke Za Zhi 9: 465–468.

Lan L, Zhao X, Lan Y, Duan L, Cao R, Liu Y, Rao Z (2007b) Effect of carbenoxolone on behavior and electroencephalogram of PTZ-kindled epileptic rats (n Chinese). Journal of Xi'an Jiaotong University (Medical Sciences) 28: 627–630.

Lothman EW, Williamson JM, VanLandingham KE (1991) Intraperitoneal phenytoin suppresses kindled responses: effects on motor and electrographic seizures. Epilepsy Res 9: 11–18.

Lukasiuk K, Wilczynski GM, Kaczmarek L (2011) Extracellular proteases in epilepsy. Epilepsy Res 96: 191–206.

McNamara JO, Rigsbee LC, Butler LS, Shin C (1989) Intravenous phenytoin is an effective anticonvulsant in the kindling model. Ann Neurol 26: 675–678.

Morimoto K, Sato H, Sato K, Sato S, Yamada N (1997) BW1003C87, phenytoin and carbamazepine elevate seizure threshold in the rat amygdala-kindling model of epilepsy. Eur J Pharmacol 339: 11–15.

Nassiri-Asl M, Zamansoltani F, Zangivand AA (2008) The inhibitory effect of trimethylamine on the anticonvulsant activities of quinine in the pentylenetetrazole model in rats. Prog Neuropsychopharmacol Biol Psychiatry 32: 1496–1500.

Nilsen KE, Kelso AR, Cock HR (2006) Antiepileptic effect of gap-junction blockers in a rat model of refractory focal cortical epilepsy. Epilepsia 47: 1169–1175.

Otsuki K, Morimoto K, Sato K, Yamada N, Kuroda S (1998) Effects of lamotrigine and conventional antiepileptic drugs on amygdala- and hippocampal-kindled seizures in rats. Epilepsy Res 31: 101–112.

Paxinos G, Watson C (2007) The Rat Brain in Stereotaxic Coordinates. Elsevier, Amsterdam, NL.

Rundfeldt C, Honack D, Loscher W (1990) Phenytoin potently increases the threshold for focal seizures in amygdala-kindled rats. Neuropharmacology 29: 845–851.

Rundfeldt C, Loscher W (1993) Anticonvulsant efficacy and adverse effects of phenytoin during chronic treatment in amygdala-kindled rats. J Pharmacol Exp Ther 266: 216–223.

Sagar GD, Larson DM (2006) Carbenoxolone inhibits junctional transfer and upregulates Connexin43 expression by a protein kinase A-dependent pathway. J Cell Biochem 98:1543–1551.

Sayyah M, Rezaie M, Haghighi S, Amanzadeh A (2007) Intra-amygdala all-trans retinoic acid inhibits amygdala-kindled seizures in rats. Epilepsy Res 75: 97–103.

Sejima H, Ito M, Kishi K, Tsuda H, Shiraishi H (1997) Regional excitatory and inhibitory amino acid concentrations in pentylenetetrazol kindling and kindled rat brain. Brain Dev 19: 171–175.

Shorvon SD (2005) Forms, causes and therapy in children and adults. In: Handbook of Epilepsy Treatment. Blackwell Publishing, Oxford, UK.

Tao L, Harris AL (2007) 2-aminoethoxydiphenyl borate directly inhibits channels composed of connexin26 and/or connexin32. Mol Pharmacol 71: 570–579.

Tunnicliff G (1996) Basis of the antiseizure action of phenytoin. Gen Pharmacol 27: 1091–1097.

Voss LJ, Jacobson G, Sleigh JW, Steyn-Ross A, Steyn-Ross M (2009) Excitatory effects of gap junction blockers on cerebral cortex seizure-like activity in rats and mice. Epilepsia 50: 1971–1978.