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Combined treatment with gabapentin and drugs affecting the renin–angiotensin system against electroconvulsions in mice

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ABSTRACT

Recent experimental data suggest that certain angiotensin-converting enzyme (ACE) inhibitors and angiotensin AT_1 receptor antagonists may possess anticonvulsant activity. The purpose of this study was to examine the effects of two ACE inhibitors, captopril and enalapril, and two AT_1 receptor antagonists, losartan and telmisartan, on the protective action of gabapentin in the maximal electroshock seizure threshold test in mice. Additionally, the effects of the combined treatment with gabapentin and antihypertensive drugs on memory retention in the passive avoidance task and motor coordination in the chimney test were assessed. All drugs were injected intraperitoneally. Losartan (50 mg/kg) significantly increased the convulsive threshold for gabapentin. The other antihypertensive drugs, captopril (50 mg/kg), enalapril (30 mg/kg) and telmisartan (30 mg/kg), did not affect the anticonvulsant activity of gabapentin. The observed interaction between gabapentin and losartan could be pharmacokinetic in nature. Losartan increased plasma and total brain concentrations of gabapentin. In the chimney test, losartan (50 mg/kg) administered with gabapentin (50 mg/kg) caused motor impairment. In the passive avoidance test, memory retention was not affected by the combined treatment with gabapentin and antihypertensive drugs. It is suggested that the use of captopril, enalapril and telmisartan in epileptic patients receiving gabapentin is presumed neutral upon its anticonvulsant action. The utmost caution is advised when combining losartan and gabapentin in clinical practice due to the appearance of pharmacokinetic interactions between losartan and gabapentin as well as motor impairment evoked by these drugs in mice.

1. Introduction

The existence of the renin–angiotensin system with all components of this system, such as angiotensinogen, processing enzymes, angiotensins and specific receptors, in the brain is established (Paul et al., 2006). Angiotensinogen is cleaved by renin to form the decapeptide angiotensin I, which is hydrolyzed at its carboxyterminus by the angiotensin-converting enzyme (ACE). This leads to the generation of the octapeptide angiotensin II (von Bohlen und Halbach and Albrecht, 2006). The physiological actions of angiotensin II are mediated predominantly by angiotensin AT_1 and AT_2 receptors (von Bohlen und Halbach and Albrecht, 2006). The brain renin–angiotensin system can affect various physiological and behavioral responses including cardiovascular control, thirst, stress, memory and depression (Wright et al., 2008). This system may be also implicated in the control of seizure susceptibility. Angiotensin II has been demonstrated to elevate the threshold of pentylenetetrazol, bicuculline or picrotoxin-induc9ed seizures in mice (Tchekalarova and Georgiev, 2005). On the other hand, drugs affecting the renin–angiotensin system such as ACE inhibitors and angiotensin AT_1 receptor antagonists that are normally used for the treatment of hypertension and heart failure (Farsang, 2011; Thind, 1990) may also influence the seizure susceptibility. Namely, enalapril, a non-sulphydryl ACE inhibitor and losartan, an AT_1 receptor antagonist, impaired the triggering and maintenance of seizures in the rat audiogenic model of epilepsy (Pereira et al., 2010). Captopril, an ACE inhibitor that contains an active sulfhydryl group, protected mice against convulsions induced by strychnine (Minano et al., 1987). Further, captopril along with fosinopril, the non-sulphydryl ACE inhibitor, and zofenopril, the sulphydryl ACE inhibitor, was able to decrease the severity of audiogenic seizures in DBA/2 mice (De Sarro et al., 2012). Also, studies on possible drug interactions against maximal electroshock, confirmed an anticonvulsant-like activity of some ACE inhibitors and AT_1 receptor antagonists. Specifically, enalapril, losartan and telmisartan, another AT_1

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receptor antagonist, potentiated the protective action of valproate, one of the classical antiepileptic drugs (Łukawski et al., 2010b, 2011). Captopril enhanced the anticonvulsant activity of carbamazepine and lamotrigine in this test (Łukawski et al., 2010a).

Based on this background, we sought to evaluate the effects of enalapril, captopril, losartan and telmisartan on the anticonvulsant action of gabapentin, an antiepileptic drug which is used in the adjunctive treatment of partial seizures, with or without secondary generalization (Czuczwar and Patsalos, 2001). It belongs to a group of antiepileptic drugs that are recommended to epileptic patients with cardiovascular diseases (Ruiz-Giménez et al., 2010). Thus, a concomitant use of gabapentin and ACE inhibitors or AT_1 receptor antagonists is likely in clinical practice. Noteworthy, cardiovascular disorders occur more frequently in people with epilepsy (Gaitatzis et al., 2004). In this study, we applied the maximal electroshock seizure threshold test that is considered as an experimental model of tonic–clonic seizures (Löscher et al., 1991). Adverse effects of the combined treatment with antihypertensive drugs and gabapentin in the passive avoidance task (Venault et al., 1986) or the chimney test (Boissier et al., 1960) have been assessed too.

2. Materials and methods

2.1. Animals

The experiments were conducted on adult male Swiss mice weighing 20–26 g. They were purchased from a licensed dealer and housed in colony cages at room temperature (21±1 °C), under a 12:12 h light/dark cycle. Animals had free access to food and tap water ad libitum. Experimental groups consisting of eight animals, were assigned according to a randomized schedule. Each mouse was used only once. The experimental protocols and procedures run in this study were approved by the Local Ethics Committee for Animal Experiments and complied with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Drugs

Captopril (Captopril, Jelfa S.A., Poland), enalapril (Enarenal, Polpharma S.A., Poland), losartan (Xartan, Adamed, Poland), telmisartan (Micardis, Boehringer Ingelheim, Germany) and gabapentin (Neurontin, Parke-Davis, Germany) were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water. All drugs were injected intraperitoneally (i.p.) in a volume of 5 ml/kg body weight and administered 120 min (losartan and telmisartan), 60 min (gabapentin) or 45 min (captopril and enalapril) before the tests; control animals received injections of the vehicle. The pretreatment times of the drugs were based upon the literature (Borowicz et al., 2002; Gohlke et al., 2001; Minano et al., 1987; Raghavendra et al., 1998). The ACE inhibitors and angiotensin AT_1 receptor antagonists were used at the doses that did not affect the convulsive threshold as reported earlier (Łukawski et al., 2010b, 2011).

2.3. Maximal electroshock seizure threshold test

Electroconvulsions were produced by a generator (Rodent Shocker, Type 221, Hugo Sachs, Freiburg, Germany). The alternating current (50 Hz, 500 V, 0.2 s stimulus duration) was delivered via ear-clip electrodes. Full tonic extension of both hind limbs was taken as the criterion for the occurrence of seizure activity. The convulsive threshold was evaluated as CS_{50}, which is the current strength (in mA) required to produce tonic hindlimb extension in 50% of the animals tested. To calculate the convulsive threshold, at least three groups of mice (eight animals per group) were challenged with electroshocks of various intensities. An intensity–response curve was calculated with a computer, based on a percentage of animals convulsing in experimental groups.

2.4. Passive avoidance test

On the first day, the training trial was performed. During the trial, the pretreated animals were placed individually in an illuminated box ($12 \times 20 \times 15$ cm) connected to a dark box ($24 \times 20 \times 15$ cm) that was equipped with an electric grid floor. Entrance into the dark box through the doorway (4×7 cm) located at floor level in the centre of the connecting wall between the boxes, was punished by an electric foot shock (0.6 mA for 2 s). On the next day (24 h later), the retention test was conducted in which the same animals with no treatment, were put into the illuminated box and the retention time to enter the dark box was recorded. Animals avoiding the dark compartment for 180 s were considered to remember the task. The passive avoidance test may be regarded as a measure of long-term memory (Venault et al., 1986).

2.5. Chimney test

Motor performance was quantified with the chimney test (Boissier et al., 1960). The pretreated animals had to climb backwards up a plastic tube (25-cm length, 3-cm inner diameter) with a rough inner surface. Motor impairment was indicated as the inability of mice to climb backward up the tube within 60 s.

2.6. Chromatographic determination of plasma and brain concentrations of gabapentin

The measurement of plasma and total brain concentrations of gabapentin was performed in mice that received either the single injection of gabapentin (50 mg/kg i.p.) or the combination of gabapentin (50 mg/kg i.p.) and losartan (50 mg/kg i.p.). Mice were decapitated at times scheduled for the maximal electroshock seizure threshold test, and blood samples of approximately 1 ml were rapidly collected into heparinized Eppendorf tubes. Simultaneously, the brains of mice were removed from their skulls, weighed and homogenized using an original Abbott buffer (1:2 weight/volume) in an Ultra-Turrax T8 homogenizer (IKA, Staufen, Germany). The homogenates were centrifuged at $6708 \times g$ for 10 min. Samples of blood were centrifuged at $6708 \times g$ for 5 min, and both plasma and brain supernatant samples of 200 μl were stocked in the deep freeze at −80 °C. Then, plasma and supernatant samples of 100 μl were added to 200 μl of acetonitrile. The samples were mixed and centrifuged at $6708 \times g$ for 5 min. Next, 50 μl of supernatant was mixed with 100 μl of *O*-phthaldialdehyde reagent and derivatized at room temperature for 1 min. Samples of 20 μl were injected into the chromatograph. The chromatograph (HP 1050) was equipped with a fluorescence detector (HP 1046A). For HPLC, a stainless-steel HP Hypersil ODS column (200×4.6 mm) was used at an ambient temperature of 22 °C. The mobile phase consisted of acetonitrile: methanol: acetate buffer (20 mM acetic acid and 250 mM sodium acetate); 285:320:395 vol/vol/vol (BAKER HPLC grade). The mobile phase flow rate was 1 ml/min. Determination of gabapentin was achieved by comparing its peak area obtained from plasma and supernatant samples with the peak area of the external standard (naphthalene), and these were linearly related over the range 0.6–60.0 μg/ml of gabapentin. Plasma and total brain concentrations of gabapentin were expressed in μg/ml of plasma or brain