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Interactions between levetiracetam and cardiovascular drugs against electroconvulsions in mice

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ABSTRACT

Background: Hypertension and heart failure belong to common comorbid conditions with epilepsy so drug interactions between antiepileptics and cardiovascular drugs are possible in clinical practice. The aim of this study was to evaluate the effects of angiotensin AT_1 receptor antagonists (losartan potassium and candesartan cilexetil), angiotensin-converting enzyme (ACE) inhibitors (captopril and perindopril arginine) and diuretics (hydrochlorothiazide and ethacrynic acid) on the anticonvulsant activity of levetiracetam (LEV) in mice.

Methods: The protective action of LEV was examined in the maximal electroshock seizure threshold test. Drugs were administered intraperitoneally (*ip*). Additionally, combinations of cardiovascular drugs with LEV were tested for adverse effects in the passive avoidance task and the chimney test.

Results: Losartan potassium (50 mg/kg), candesartan cilexetil (8 mg/kg), captopril (50 mg/kg), hydrochlorothiazide (100 mg/kg) and ethacrynic acid (100 mg/kg) did not affect the anticonvulsant activity of LEV. Perindopril arginine (10 mg/kg) raised the convulsive threshold for LEV administered at doses of 100, 300 and 500 mg/kg. This interaction could be pharmacodynamic in nature because the brain concentration of LEV remained unchanged by perindopril. The adverse effects of the combined treatment with LEV and cardiovascular drugs were not observed in the passive avoidance task or the chimney test.

Conclusions: Although experimental data can be hardly extrapolated to clinical practice, it is suggested that perindopril arginine may positively influence the anticonvulsant action of LEV in epileptic patients. The use of losartan potassium, candesartan cilexetil, captopril, hydrochlorothiazide or ethacrynic acid in patients treated with LEV seems neutral regarding its anticonvulsant activity.

Introduction

Studies exploring the somatic comorbidity of epilepsy showed a higher prevalence of certain somatic conditions in these patients. Namely, the UK General Practice Research Database (GPRD) study identified, for example, heart failure and ischaemic heart disease as common comorbid conditions with epilepsy [1]. A Canadian study using data from two large national health surveys, the National Population Health Survey and the Community Health Survey also revealed heart disease as one of conditions with particularly high prevalence in people with epilepsy [2]. Moreover, there is a potential causal association between epilepsy and hypertension [3,4]. Severe uncontrolled hypertension increased the risk of unprovoked seizure [3]. This risk is most likely to appear through a pathway in which cerebral vascular insults result in manifest stroke, but is not confined to that pathway [4]. Because hypertension can lead to seizures in the presence or absence of stroke, antihypertensive therapy including angiotensin-converting enzyme (ACE) inhibitors or angiotensin AT_1 receptor antagonists should protect against convulsions induced by high blood pressure. Based on the above mentioned studies, it can be concluded that a concomitant use of antiepileptic drugs (AEDs) and drugs prescribed for heart failure and/or hypertension such as ACE inhibitors, AT_1 antagonists or diuretics, is likely in epileptic patients. The use of cardiovascular drugs with AEDs can lead to pharmacokinetic and pharmacodynamic drug interactions which may affect the pharmacology of AEDs.

In this study, anticonvulsant action of levetiracetam (LEV) and its effects on memory retention and motor coordination, co-administered with some cardiovascular drugs have been assessed

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in mice. LEV, an established second-generation AED, is approved as adjunctive treatment and for monotherapy of partial-onset seizures with or without secondary generalization [5,6]. Further, it belongs to a group of AEDs that are recommended to epileptic patients with heart disease [7]. It has been reported that LEV increases the threshold for electroconvulsions in the maximal electroshock seizure threshold (MEST) test in mice [8,9]. Considering this fact, we applied the MEST test in the current study. In turn, memory retention and motor coordination were evaluated in the passive avoidance task [10] and the chimney test [11], respectively. These two tests are commonly applied for the assessment of adverse effects of drugs in mice. The choice for cardiovascular drugs was based on their wide clinical use and biological activities, especially anticonvulsant-like properties reported in animal studies. Namely, ACE inhibitors, captopril and perindopril, AT_1 receptor antagonists, losartan and candesartan, and hydrochlorothiazide, a thiazide-type diuretic, have been used. They are commonly prescribed drugs for hypertension and heart failure [12–15]. Ethacrynic acid (EA), a loop diuretic that was also tested in this study, is rarer used in clinical practice because of its side-effect profile. It is employed in the treatment of congestive heart failure and other edematous states [16]. Recent experimental studies show that EA exerts the anticonvulsant activity and therefore, it was included in the current experiments. Actually, EA suppressed sound-triggered seizures in post-ischaemic audiogenic seizure-prone rats [17] and enhanced the anticonvulsant action of topiramate and valproate in the test of maximal electroshock (MES) [18,19]. As concerns anticonvulsant-like activities of other tested drugs, captopril protected mice against strychnine-induced convulsions [20] and decreased the severity of audiogenic seizures in DBA/2 mice [21]. Captopril and hydrochlorothiazide potentiated the anticonvulsant activity of carbamazepine against MES [22,23]. Losartan suppressed seizures in Wistar audiogenic rats [24] and enhanced the protective action of valproate in the MES test [25]. According to our knowledge, effects of the mentioned cardiovascular drugs on the anticonvulsant activity of LEV have not been examined in animal models of seizures.

Materials and methods

Animals

Male Swiss mice weighing 22–28 g were used. Animals were housed in colony cages with free access to food pellets and water available *ad libitum*, under standardized laboratory conditions (12-h light–dark cycle, room temperature of $21 \pm 1\ ^{\circ}C$). Experimental groups, consisting of eight animals, were assigned according to a randomized schedule. Each mouse was used only once. The experimental procedures 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).

Drugs

Angiotensin AT_1 receptor antagonists, losartan potassium (Xartan, Adamed, Pieńków, Poland) and candesartan cilexetil (Atacand, AstraZeneca AB, Sodertälje, Sweden), ACE inhibitors, captopril (Captopril Jelfa, Jelfa S.A., Jelenia Góra, Poland) and perindopril arginine (Prestarium, Servier, Neuilly-sur-Seine, France), diuretics, hydrochlorothiazide (Hydrochlorothiazidum, Polpharma S.A., Starogard Gdański, Poland) and ethacrynic acid (Ethacrynic acid, MP Biomedicals, Solon, OH, USA), and LEV (Keppra, UCB Pharma S.A., Brussels, Belgium) were used in this study. All cardiovascular drugs and LEV were suspended in a 1% solution of Tween 80 (Sigma, St. Louis, MO, USA) in distilled water and administered intraperitoneally (*ip*) in a volume of 5 ml/kg. The pretreatment times of the drugs before the tests were as follows: 120 min (losartan, candesartan, hydrochlorothiazide), 60 min (perindopril, LEV), 45 min (captopril) and 30 min (ethacrynic acid). The drugs were administered at single doses. Control animals received injections of the vehicle. The pretreatment times were based on previous reports on the biological activity of tested drugs [9,17,20,23,26,27].

Maximal electroshock seizure threshold test (MEST test)

Electroconvulsions were produced by means of an alternating current (50 Hz, 500 V, stimulus duration of 0.2 s) delivered *via* ear-clip electrodes by a generator (Rodent Shocker, Type 221, Hugo Sachs Elektronik, Freiburg, Germany). The criterion for the occurrence of seizure activity was the tonic extension of the hind limbs. 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 exposed to electroshocks of various intensities. Then, an intensity-response curve was calculated with a computer, based on a percentage of animals convulsing in experimental groups.

In order to exclude cardiovascular drugs effects, *per se*, in the MEST test, losartan, captopril, ethacrynic acid and hydrochlorothiazide were applied at subthreshold doses taken from earlier reports [22,23,25]. The influence of candesartan and perindopril on the convulsive threshold was assessed in this study. All cardiovascular drugs at subthreshold doses were examined for possible interactions with LEV administered at doses of 100, 300 and 500 mg/kg.

Passive avoidance test

The passive avoidance task is thought as a measure of long-term memory [10]. Mice pretreated with studied drugs were individually placed in an illuminated box ($12\ cm \times 20\ cm \times 15\ cm$) connected to a dark box ($24\ cm \times 20\ cm \times 15\ cm$). The dark box was equipped with an electric grid floor and a doorway ($4\ cm \times 7\ cm$) was located at floor level in the centre of the connecting wall. Entrance into the dark box was punished by an electric foot shock (0.6 mA for 2 s). Twenty-four hours after the training trial, the retention test was performed in which the same animals with no treatment were put again into the illuminated box and time that the mice took to enter the dark box was noted. The mice that avoided the dark compartment for 180 s were considered to remember the task.

Chimney test

Motor coordination was evaluated with the chimney test of Boissier et al. [11]. Mice had to climb backwards up a plastic tube (3 cm inner diameter, 25 cm in length). Motor impairment was indicated as the inability of mice to climb backward up the tube within 60 s.

Estimation of total brain concentration of levetiracetam

The measurement of total brain concentration of LEV was performed in the following groups: LEV (500 mg/kg) + vehicle and LEV (500 mg/kg) + perindopril (10 mg/kg). Brains of mice were rapidly removed from skulls after decapitation, weighed and homogenized by using distilled water (1:2, w/v) in an Ultra-Turrax T10 homogenizer (IKA, Staufen, Germany). Brain homogenate samples were prepared for analysis as follows: 200 μl of samples were pipetted into a 1.5 ml plastic tube to which 200 μl of