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Fosinopril and zofenopril, two angiotensin-converting enzyme (ACE) inhibitors, potentiate the anticonvulsant activity of antiepileptic drugs against audiogenic seizures in DBA/2 mice

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ABSTRACT

The renin–angiotensin system (RAS) exists in the brain and it may be involved in pathogenesis of neurological and psychiatric disorders including seizures. The aim of the present research was to evaluate the effects of some angiotensin-converting enzyme inhibitors (ACEi; captopril, enalapril, fosinopril and zofenopril), commonly used as antihypertensive agents, in the DBA/2 mice animal model of generalized tonic–clonic seizures. Furthermore, the co-administration of these compounds with some antiepileptic drugs (AEDs; carbamazepine, diazepam, felbamate, gabapentin, lamotrigine, phenobarbital, phenytoin, topiramate and valproate) was studied in order to identify possible positive interactions in the same model. All ACEi were able to decrease the severity of audiogenic seizures with the exception of enalapril up to the dose of 100 mg/kg, the rank order of activity was as follows: fosinopril > zofenopril > captopril. The co-administration of ineffective doses of all ACE inhibitors with AEDs, generally increased the potency of the latter. Fosinopril was the most active in potentiating the activity of AEDs and the combination of ACEi with lamotrigine and valproate was the most favorable, whereas, the co-administrations with diazepam and phenobarbital seemed to be neutral. The increase in potency was generally associated with an enhancement of motor impairment, however, the therapeutic index of combined treatment of AEDs with ACEi was predominantly more favorable than control. ACEi administration did not influence plasma and brain concentrations of the AEDs studied excluding pharmacokinetic interactions and concluding that it is of pharmacodynamic nature. In conclusion, fosinopril, zofenopril, enalapril and captopril showed an additive anticonvulsant effect when co-administered with some AEDs, most notably carbamazepine, felbamate, lamotrigine, topiramate and valproate, implicating a possible therapeutic relevance of such drug combinations.

1. Introduction

In recent years, the major progresses in the understanding of the mechanisms associated with epileptiform events and anticonvulsant activity of antiepileptic drugs (AEDs) have led to a clear improvement in the treatment of human epilepsies. However, approximately 20–30% of epileptic patients are refractory to standard therapy [1–4].

Angiotensin-converting enzyme (ACE), a zinc metallo-protease, catalyzes the formation of angiotensin II, a potent vasoconstrictor, from circulating angiotensin I [5]. By blocking the formation of angiotensin II in blood, ACE inhibitors significantly lower systemic vascularresistance, lower blood pressure and improve cardiac function [6]. Besides the peripheral renin–angiotensin system (RAS), all components (precursors and enzymes) of RAS are expressed in the brain [7]. In different areas of the brain, angiotensin II produces its effects acting at specific angiotensin receptors (AT1, AT2) [8]. The brain RAS is thought to participate in the control of the cardiovascular system, thirst, stress and depression [9]; a role for ACE inhibitors has been evidenced by an improvement in cognition and mood

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[10,11]. In addition, angiotensin peptides seem to be implicated in the regulation of seizure susceptibility. Namely, intracerebroventricular administration of angiotensin II increased the threshold of pentylenetetrazole-, bicuculline- or picrotoxin- induced seizures and attenuated the severity of clonic convulsions induced by agents impairing GABAergic neurotransmission (i.e. pentylenetetrazol and 3-mercaptopropionic acid) in mice [12,13]. Furthermore, in vivo studies have previously demonstrated that angiotensins III and III–IV protect mice against seizures in the pentylenetetrazol kindling model of epilepsy as well as in other experimental model of seizures[14]. Interestingly, previous data revealed that captopril was able to protect mice against convulsions induced by strychnine, although the same drug was ineffective in bicuculline-induced seizures in mice [15]. Captopril can cross the blood–brain barrier and inhibits the brain RAS after systemic administration [16,17]. There is a lack of available information about the interactions between ACE-inhibitors and antiepileptic drugs (AEDs). In one study, captopril has shown to enhance the anticonvulsant activity of carbamazepine and lamotrigine against maximal electroshock-induced seizures in mice [18]. In a second study enalapril was able to enhance the protective effects of valproate [19]. Thus, it is conceivable that the use of such ACE-inhibitors might influence the efficacy of some AEDs. Taking into consideration, the anticonvulsant-like activity of ACE inhibitors, we sought to evaluate the effect of captopril, enalapril, fosinopril and zofenopril on the generalized clonic–tonic seizures of the audiogenic sensitive DBA/2 mouse model and to assess their influence on the anticonvulsant action of various AEDs (carbamazepine, diazepam, felbamate, gabapentin, lamotrigine, phenytoin, phenobarbital, topiramate and valproate) in the same experimental model. The ACE-inhibitors used are commonly administered in humans and display different pharmacological profiles including chemical differences (sulphydrylic, carboxylic or phosphinilic group), lipophilicity and ability to cross the blood–brain barrier [16,17,20,21]. In addition to other structural differences, enalapril, fosinopril and zofenopril are prodrug esters that must be hydrolyzed in vivo to release their active ACE-inhibitory moieties [16]. The audiogenic sensible DBA/2 mouse is considered an experimental model of generalized tonic–clonic reflex epilepsy commonly used to evaluate the efficacy of new possible anticonvulsant compounds [22–24]. Based on this background, we considered it of interest to study the effects of some ACE inhibitors alone or in combination with various AEDs in the DBA/2 mouse model; doses of ACE inhibitors and AEDs were initially chosen on previous experiences [18,19,25] and adjusted during the experimentation adapting to the measured effects.

2. Materials and methods

2.1. Animals

Male and female DBA/2 mice weighing 8–12 g (22–26days old) or 20–28 g (48–56 days old) were used. Animals were purchased from Harlan Italy srl (Correzzana, Milan, Italy) and housed in groups of 8–10 in colony cages at room temperature, under a 12-h light/12-h dark cycle (lights on at 7:00 a.m.) with food pellets and water available ad libitum. Experimental groups, consisting of 10 animals were assigned according to a randomized schedule, and each mouse was used only once. Control animals were always tested on the same day as the respective experimental groups. Additionally, all efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. Procedures involving animals and their care were conducted in conformity with international and national law and policies (European Communities Council Directive of 24th November 1986, 86/609EEC).

2.2. Experimental design

DBA/2 mice were exposed to auditory stimulation, 45, 60 or 120 min following intraperitoneal (i.p.) administration of ACE inhibitors (10–100 mg/kg) or vehicle and 45 min following i.p. injection of the AEDs studied. Time of administration and doses were chosen according to previously published data in the same model [23,25–28]. Each mouse was placed under a hemispheric Perspex dome (diameter = 58 cm) and 1 min was allowed for habituation and assessment of locomotor activity. Auditory stimulation (12–16 kHz, 109 dB) was applied for 1 min or until tonic extension occurred. Seizure response was assessed using the following scale: 0 = no response, 1 = wild running, 2 = clonus, 3 = tonus, 4 = respiratory arrest, as previously described [29]. The maximum response was recorded for each animal. ED_{50} values (±95% confidence limits) for each compound and each phase of seizure response were estimated using a computer program for the method of Litchfield and Wilcoxon [30]; the relative anticonvulsant activities were determined by comparison of respective ED_{50} values; at least 32 mice were comprised within the ED_{16} and the ED_{84} to allow proper statistical analysis. Rectal temperature was recorded immediately prior to auditory testing using a digital thermometer. Animal were observed for gross behavioral changes during the period between drug administration and auditory testing.

2.3. Determination of plasma and brain levels of the antiepileptic drugs

For audiogenic testing, DBA/2 mice (20–28 g) were given i.p. either vehicle and one AED or one ACE inhibitor and one AED. The same protocol was used for behavioral and pharmacokinetic studies. However, older DBA/2mice (see Section 2.1) were used for drug level determination in order to avoid difficulties in blood sample collection from younger DBA/2 mice. No changes in pharmacokinetics were reported between 21–26 and 48–56 days old mice [23,25–28,31]. The animals were lightly anaesthetized with chloral hydrate and killed by decapitation at appropriate times to obtain blood samples of approximately 1 ml (for sample collections timing see Tables 2 and 3). The felbamate and lamotrigine assay was carried out by high-performance liquid chromatography (HPLC) as previously described [26]. Gabapentin assay was carried out using a HPLC method previously described by Tang et al. [32]. Blood samples were centrifuged at 2000 rpm for 15 min for carbamazepine, diazepam, phenytoin and phenobarbital determination. The plasma was put into an automatic system, MPS-1 (Amicon, Danvers, MA, USA), for the separation of free from protein-bound microsolutes. Plasma samples of 60 μl were transferred to special sample cups and inserted into an Automatic Clinical Analyser (ACA II, duPont, Wilmington, DE, USA) which uses a method based on the homogenous enzyme immunoassay technique. For the valproate assay, a serum sample of 50 μl was diluted twice with Tris buffer and analyzed with the same method. For brain level determination, brains were rapidly removed from skulls immediately after decapitation, weighed and homogenized by using bidistilled water (2:1, w/v). The homogenates were centrifuged at 10,000 rpm for 10 min. The supernatant samples (60 or 50-μl for valproate only) were analyzed as previously described for plasma samples. Control drug solutions were put before and after the respective antiepileptic experimental samples.

2.4. Effects on motor movements

Behavioral changes and their onset and duration were recorded after drug injection until the time of the rotarod test. In particular, two independent observers followed gross behavioral changes consisting of locomotor activity, ataxia, squatting posture and possible