Brain Research Bulletin 130 (2017) 245–250

Contents lists available at ScienceDirect

Brain Research Bulletin

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

Research report

Cerebral small vessel disease predisposes to temporal lobe epilepsy in spontaneously hypertensive rats

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ARTICLE INFO

Article history:
Received 1 January 2017
Received in revised form 30 January 2017
Accepted 13 February 2017
Available online 15 February 2017

Keywords:
SHRs
Stroke
Hypertension
Kindling
Pentylenetetrazole

ABSTRACT

The link between cerebral small vessel disease (CSVD) and epilepsy has been poorly investigated. Some reports suggest that CSVD may predispose to temporal lobe epilepsy (TLE). Aim of this study was to evaluate whether spontaneously hypertensive rats (SHRs), an established model of systemic hypertension and CSVD, have a propensity to develop TLE more than generalized seizures. To this aim, amygdala kindling, as a model of TLE, and pentylenetetrazole (PTZ)-induced kindling, as a model of generalized seizures, have been used to ascertain whether SHRs are more prone to TLE as compared to Wistar Kyoto control rats. While young SHRs (without CSVD) do not differ from their age-matched controls in both models, old SHRs (with CSVD) develop stage 5 seizures in the amygdala kindling model (TLE) faster than age-matched control rats without CSVD. At odds, no differences between old SHRs and age-matched controls was observed in the development of PTZ kindling. Enalapril pre-treatment prevented the development of CSVD and normalized kindling development to control levels in SHRs. No difference was observed in the response to pharmacological treatment with carbamazepine or losartan. Overall, our study suggests that uncontrolled hypertension leading to CSVD might represent a risk factor for TLE. Further experimental studies are needed to unravel other risk factors that, along with CSVD, may predispose to TLE.

1. Introduction

Experimental and clinical studies suggest that systemic hypertension and cerebral small vessel disease (CSVD) increase the risk of epilepsy even in the absence of prior clinically detected stroke (De Reuck et al., 2007; Li et al., 1997; Maxwell et al., 2013; Ng et al., 1993). CSVD has received little attention until modern brain imaging technologies allowed to detect small deep infarcts and white matter rarefaction (i.e. leukoaraiosis) (Pantoni, 2010). Leukoaraiosis may be observed in adults with otherwise unexplained new-onset epilepsy, but it is not clear whether leukoaraiosis represents a mere incidental radiologic finding (i.e. epiphenomenon) or plays an epileptogenic role (Ferlazzo et al., 2016; Gasparini et al., 2015).

Clinical studies suggest that temporal lobe epilepsy (TLE) predominates in epileptic patients with leukoaraiosis (Gasparini et al., 2015; Lambert et al., 2016). A link between TLE and alterations in the function of renin-angiotensin system (RAS) has been hypothesized (Lukawski et al., 2013; Mascolo et al., 2016; Passos-Silva et al., 2015). Indeed, type 1 and 2 angiotensin receptors (AT) were found to be up-regulated in the cortex and hippocampus of patients with TLE (Arganaraz et al., 2008); moreover, AT-converting enzyme inhibitors (ACEi) displayed antiepileptic activity in some animal models of seizures/epilepsy (De Sarro et al., 2012; Lukawski and Czuczwar, 2015; Lukawski et al., 2013). Finally, previous reports indicate that spontaneously hypertensive rats (SHRs) could be considered as a suitable tool to study the link between hypertension and epilepsy (Tchekalarova et al., 2011; Tchekalarova et al., 2010) and, notably, CSVD was observed in this strain (Kaiser et al., 2014). Of note, SHRs display CSVD as a consequence of uncontrolled hypertension (Chan et al., 2013) and may therefore be used to study the comorbidity between hypertension, CSVD and epilepsy (Pietranera et al., 2006).

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http://dx.doi.org/10.1016/j.brainresbull.2017.02.003
0361-9230/

The primary aim of this study was to evaluate the propensity and possible differences of SHRs to develop either TLE by inducing amygdala kindling (a TLE model) or pentylenetetrazole (PTZ) kindling (a generalized seizures model) in 16-week-old SHRs and age-matched Wistar Kyoto (WKY) control rats. The secondary aims were: 1) to assess the role of ACEi to prevent hypertension-related CSVD and epilepsy by administrating enalapril before both types of kindling in the two strains; 2) to evaluate the role of hypertension or genetic factors in TLE development before CSVD onset by applying the same protocols in 5-week-old SHR and in age-matched WKY control rats; 3) to test seizure response to losartan *vs* carbamazepine in amygdala-kindled rats of both strains.

2. Materials and methods

2.1. Animals

A total of 64 male SHRs and 64 male WKY rats of 3 weeks of age were obtained from Charles River (Milan, Italy). Rats were housed in stable conditions of humidity (60 ± 5%) and temperature (21 ± 2 °C), kept under a reversed light/dark (12/12 h) cycle (light on at 19:00 h) and given free access to food and water until the time of experiments. The experimental procedures were carried out in accordance with the guidance and general recommendations with international and national law and policies (EU Directive 2010/63/EU for animal experiments, ARRIVE guidelines and the Basel declaration including the 3R concept) and after approval by the local ethical committee. Measures were included in the protocols to minimize pain and discomfort to the animals and minimize animal usage.

2.2. Drugs

Pentylenetetrazole (PTZ; Sigma, Milan, Italy) was dissolved in 0.9% saline and injected s.c. in a volume of 1.0 ml/kg, at a subconvulsive dose of 30 mg/kg every other day until development of kindling or up to 8 weeks (De Sarro et al., 2004).

Enalapril (Germed Pharma S.p.A Cinisello Balsamo, Italy) was orally administered at a dose of 10 mg/kg/day by dissolving 20 mg in 120 ml of consumable water (calculated on the basis of the knowledge that rats drink on average 10–12 ml/100 g/day) (Citraro et al., 2015). The dose of enalapril was based on previous reports and a short preliminary experiment with two doses (10 and 20 mg/kg; n = 3 per group) administered for 10 days in which the highest dose appeared to be not well-tolerated following a behavioral evaluation by an expert veterinary (Cagalinec et al., 2006).

Losartan (Sandoz; Varese, Italy), at the dose of 50 mg/kg i.p., was dissolved in 0.9% saline. Carbamazepine (Sigma-Aldrich Milan, Italy), at the dose of 50 mg/kg i.p., was dissolved in dimethyl sulfoxide (DMSO).

2.3. Surgery and electrical kindling procedure

Thirty-two SHRs and 32 WKY rats were anesthetized by a tiletamine/zolazepam mixture (1:1; Zoletil 100®; 50 mg/kg i.p.; VIRBAC Srl, Milan, Italy); a bipolar electrode was implanted into the right hemisphere in the basolateral amygdala (AP = 2.2; L = 4.8; H = 8.5 in mm relative to bregma) according to Paxinos and Watson (Paxinos and Watson, 2005), as previously described (Loscher et al., 1998). All rats after surgery were randomly divided into 8 groups (n = 8 for all groups; see Fig. 1 for an experimental protocol scheme): 1) WKY of 5 weeks of age (5WKY); 2) WKY of 5 weeks of age treated with enalapril 10 mg/kg/day o.s. (5WKYen); 3) SHR of 5 weeks of age (5SHR); 4) SHR of 5 weeks of age treated with enalapril 10 mg/kg/day o.s. (5SHRen); 5) WKY of 16 weeks of age (16WKY); 6) WKY of 16 weeks of age treated with enalapril 10 mg/kg/day o.s. (16WKYen); 7) SHR of 16 weeks of age (16SHR); 8) SHR of 16 weeks of age treated with enalapril 10 mg/kg/day o.s. (16SHRen). The administration of enalapril was started in all treated groups at 30 days of age and continued up to end of the experimental protocol (e.g. acquisition of kindling status).

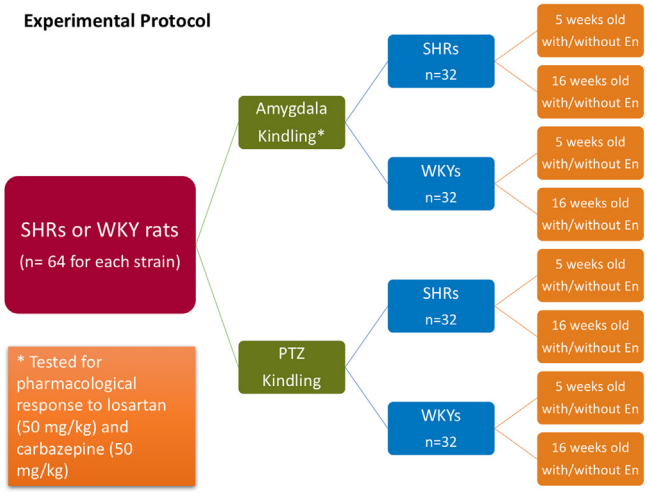

Fig. 1. Scheme of the experimental protocol used in the study. SHRs and WKY rats of the two ages considered (5 and 16 weeks) were divided in two subgroups undergoing amygdala- or PTZ-kindling and treated or not with enalapril (10 mg/kg/day; see section *Drugs*) in order to study strain predisposition to develop kindling and its relation to cerebral small vessels disease (CSVD). Pharmacological response to losartan or carbamazepine was only tested in groups undergoing amygdala kindling without enalapril treatment and of 16 weeks of age. Abbreviations: SHRs = Spontaneously hypertensive rats; WKYs = Wistar Kyoto rats; En = enalapril.

Electrical stimulation (Grass S88 K stimulator) of the amygdala was started after a recovery period of at least 1 week in all groups (range 7–10 days). Afterdischarge Threshold (ADT) was determined, using stimuli of increasing intensity (20 μA steps starting from 10 μA) presented at 1 min intervals until an AD occurred, the day before the first day of the stimulation period and it was defined as the lowest electrical stimulus that elicited an afterdischarge (AD) lasting at least 3 s (Loscher et al., 1998). From the next day on, pulsed electrical stimulation (500 μA, 1 ms, monophasic square-wave pulses, 50 Hz for 1 s) were delivered at intervals of 1 day until 5 consecutive fully kindled seizures (stage 5; seizure severity classified by Racine scale) were elicited (Loscher et al., 1998; Racine, 1972; Russmann et al., 2016). AD duration was also measured on the first day of stimulation and after 5 stage 5 seizures.

To test a putative potential difference in pharmacological response between kindled rats of the two different strains, two days after reaching a fully kindled state of 5 stage 5 seizures, the effect on the behavioral seizure score of a single dose of losartan (50 mg/kg) or carbamazepine (50 mg/kg) administered i.p. was assessed. Rats were tested at 60 min after drug administration. The doses of losartan and carbamazepine are consistent with efficacious doses of these drugs in standard kindling and other rodent seizure models (Krupp et al., 2000; Lukawski et al., 2014).

2.4. Pentylenetetrazole kindling protocol

Chemical kindling was induced in 32 SHRs and in 32 WKY rats by pentylenetetrazole (PTZ, 30 mg/kg s.c.) injected every other day at the most up to 8 consecutive weeks in the morning between 9:00 and 11:00 (De Sarro et al., 2004).

Animals were randomly divided into 8 groups (n = 8for all groups) as above described for electrical kindling and also in this case 4 out of 8 groups received enalapril (10 mg/kg/day) during the entire time-window of PTZ administration (see Fig. 1).