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Different response to antiepileptic drugs according to the type of epileptic events in a neonatal ischemia-reperfusion model

Luc Morin [a], Julie Enderlin [a], Pierre-Louis Leger [a,b], Gaëtan Perrotte [a], Philippe Bonnin [c], Nina Dupuis [a], Olivier Baud [a,d], Christiane Charriaut-Marlangue [a,1], Stéphane Auvin [a,e,*,1]

[a] PROTECT, INSERM, Unversité Paris Diderot, Sorbonne Paris Cité, Paris, France
[b] UPMC, Paris Universitas, AP-HP, Hôpital Armand Trousseau, Service de Réanimation pédiatrique, Paris, France
[c] Univ Paris Diderot, Sorbonne Paris Cité, INSERM, U965, 75010 Paris, France
[d] Univ Paris Diderot, Sorbonne Paris Cité, AP-HP Service de Réanimation Néonatale, Hôpital Robert Debré, 75019 Paris, France
[e] AP-HP, Hôpital Robert Debré, Service de Neurologie Pédiatrique, 75019 Paris, France

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ABSTRACT

Perinatal arterial stroke is the most frequent form of cerebral infarction in children. Neonatal seizures are the most frequent symptom during the neonatal period. The current management of perinatal stroke is based on supportive care. It is currently unknown if treatment of the seizures modifies the outcome, and no clinical studies have focused on seizures during neonatal stroke. We studied the effect of phenobarbital and levetiracetam on an ischemic-reperfusion stroke model in P7 rats using prolonged electroencephalographic recordings and a histologic analysis of the brain (24 h after injury). The following two types of epileptic events were observed: 1) bursts of high amplitude spikes during ischemia and the first hours of reperfusion and 2) organized seizures consisting in discharges of a 1–2 Hz spike-and-wave. Both phenobarbital and levetiracetam decreased the total duration of the bursts of high amplitude spikes. Phenobarbital also delayed the start of seizures without changing the total duration of epileptic discharges. The markedly limited efficacy of the antiepileptic drugs studied in our neonatal stroke rat model is frequently observed in human neonatal seizures. Both drugs did not modify the stroke volume, which suggests that the modification of the quantity of bursts of high amplitude spikes does not influence the infarct size. In the absence of a reduction in seizure burden by the antiepileptic drugs, we increased the seizure burden and stroke volume by combining our neonatal stroke model with a lithium-pilocarpine-induced status epilepticus. Our data suggest that the reduction of burst of spikes did not influence the stroke volume. The presence of organized seizure with a pattern close to what is observed in human newborns seems related to the presence of the infarct. Further research is required to determine the relationship between seizure burden and infarct volume.

1. Introduction

With an incidence of 1/2800 to 1/5000 live births, perinatal arterial stroke is the most frequent form of cerebral infarction in children. Approximately 60% of newborns with arterial stroke exhibit symptoms, mainly neonatal seizures, while 40% of newborns do not show any symptoms during the neonatal period. In the latter case, the patients are subsequently diagnosed with motor impairment, developmental delay or epilepsy (Lynch, 2009). After the occurrence of neonatal seizures during a stroke, there is a nearly 3-fold increased risk of later epilepsy. In these cases, the cumulative incidence of epilepsy by age 10 years is approximately 70% (Fox et al., 2016). Currently, there is no specific treatment for neonatal stroke. The current strategy is mainly based on supportive care, including the management of neonatal seizures.

Phenobarbital (PB) enhances inhibition in the brain by allosterically modulating the permeability of chloride channels coupled to $GABA_A$ receptors (Czapinski et al., 2005) and is currently the first-line treatment for neonatal seizures. Only two randomized and controlled studies with adequate methodology are available (Painter et al., 1999; Boylan et al., 2004). Both of these studies indicated that treatment with phenobarbital was only effective in approximately 40–50% of babies. Levetiracetam (LEV) is a pyrrolidine that binds to the synaptic vesicle protein SV2a, which is highly expressed throughout the brain, and LEV has been increasingly used as an off-label therapy for neonatal seizures in humans

* Corresponding author at: Service de Neurologie Pédiatrique et des Maladies Métaboliques, CHU Hôpital Robert Debré, 48, boulevard Sérurier, 75935 PARIS CEDEX 19, France.
E-mail address: stephane.auvin@inserm.fr (S. Auvin).
[1] C. Charriaut-Marlangue and S. Auvin share senior authorship.
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(Silverstein and Ferriero, 2008; Donovan et al., 2016). Preclinical data has showed the efficacy of LEV to suppress neonatal hypoxic-induced seizures (Talos et al., 2013). Despite the limited efficacy of antiepileptic drugs on neonatal seizures, seizure suppression is believed to play a role in the long-term prognosis of the underlying cause of the seizure, such as anoxo-ischemic encephalopathy (McBride et al., 2000). However, there is no data focusing on the anti-seizure activity and outcome of phenobarbital-treated cases of perinatal arterial stroke.

Before the design of clinical trials in human newborns, preclinical studies must provide insights on the treatment of neonatal seizures in cases of arterial stroke. However, few models are currently available. In P12 rats, a carotid ligation combined with hypoxia results in acute seizures and significant tissue damage (Cuaycong et al., 2011). Using P12 CD1 mice, a unilateral carotid ligation alone also results in acute seizure and brain injury (Comi et al., 2009). In this latter model, the reduction in brain injury by gabapentin and phenobarbital may be due to a decrease in seizures (Traa et al., 2008; Markowitz et al., 2011). The link between seizure burden during the acute phase of neonatal stroke and stroke volume is crucial because of the possible treatment consequences. However experimental studies used usually few hours of electroencephalogram (EEG) recordings. Furthermore, experimental studies have shown that the long-term epileptic outcome is related to the level of brain injury (Kadam et al., 2010).

The aim of the current study was to establish the occurrence of epileptic events during both the ischemic and reperfusion phases of our P7 rat stroke model (Bonnin et al., 2011). We evaluated the effects of antiepileptic drugs (PB and LEV) on neonatal seizures during the acute phase of stroke and the outcome for perinatal arterial stroke. We also evaluated the relationship between seizure quantity, based on electroencephalographic recordings, and infarct volume.

2. Materials and methods

2.1. Animals

Wistar rats (Janvier, Le Genest St-Isle, France, 17–20 g, both sexes) on postnatal day 7 (P7) were used in this study. Animals were housed under standard laboratory conditions with controlled temperature/humidity. The animal ethical institutional review committee approved this study. All animal procedures complied with the ethical guidelines of the Robert Debré Hospital Research Council Review Board (A75-19-01) and the INSERM and ARRIVE guidelines, including the recommendations for reduction, refinement and replacement (known as the 3Rs, (Scholz et al., 2013)). Table 1 summarizes the experimental design and the number of animals per group for each experiment reported here.

2.2. Ischemia-reperfusion

Thermoregulated (37.0 ± 0.5 °C) and anesthetized rats (1% isoflurane in air via a facemask) were subjected to focal ischemia by left middle cerebral artery (MCA) electrocoagulation (pMCAo: permanent middle cerebral artery occlusion) combined with a transient (50 min) occlusion of both common carotid arteries (pMCAo + bi-tCCAo (transient common carotid arteries occlusion), Bonnin et al., 2011) to evaluate seizure type and duration using electroencephalography (EEG). We also recorded EEG in P7 rat pups exposed to transient ischemia only (50 min, bi-tCCAo (transient common carotid arteries occlusion)). Animals were euthanized 24 h after ischemia, and lesion volumes were measured with edema-correction. Two groups were also euthanized at P68 to measure lesion volume. Two investigators, who were blinded to the treatment groups, determined the size of the lesion (stroke volume) in each animal as previously described (Leger et al., 2016).

Table 1
Experimental design.

Experiment	Ischemic model	n
EEG recordings to characterize our model		
EEG recording pilot study 2 electrodes	pMCAo + bi-tCCAo	11
EEG recording + saline i.p. 1 electrode (right)	pMCAo + bi-tCCAo	11[a]
EEG recording 1 electrode (right)	bi-tCCAo	6
Histology (infarct volume/TUNEL/Fluoro-Jade-B)		
Saline injection	pMCAo + bi-tCCAo	16
	pMCAo + bi-tCCAo	12 (histology at P68)
PB injection		
Before ischemia 10 mg/kg i.p.	pMCAo + bi-tCCAo	10
Before ischemia 20 mg/kg i.p.	pMCAo + bi-tCCAo	13
Before ischemia 20 mg/kg i.p	pMCAo + bi-tCCAo	8 (histology at P68)
Before ischemia 40 mg/kg i.p.	pMCAo + bi-tCCAo	7
3 h after; 20 mg/kg i.p.	pMCAo + bi-tCCAo	10
Li-Cl 18 h before	pMCAo + bi-tCCAo	8
Li-Cl + Pilo 100 mg/kg i.p.	pMCAo + bi-tCCAo	12
EEG recordings of AED treatment		
EEG recording + PB i.p. 1 electrode (right)	pMCAo + bi-tCCAo	12[a]
EEG recording + LEV i.p. 1 electrode (right)	pMCAo + bi-tCCAo	10[a]
Echo Doppler & laser speckle		
Doppler + saline	pMCAo + bi-tCCAo	8
Doppler + PB	pMCAo + bi-tCCAo	8
Laser speckle + saline	pMCAo + bi-tCCAo	6
Laser speckle + PB	pMCAo + bi-tCCAo	6

[a] Animals recorded for EEG were also used for histology and/or TUNEL assay.

2.3. Electroencephalographic (EEG) recording

Under 1% isoflurane anesthesia, a cortical bipolar electrode (Plastics One Inc., Roanoke, VA, U.S.A.) was implanted on the dura of the right side of the brain (P7: 2.5 mm AP, 3 mm ML and 0 mm DV from Bregma) before the surgery to induce the stroke model. Eleven 11 pups were implanted with two electrodes on both sides of the cortex (Supplementary material 1). The animals were connected to an MP100/EEG100B acquisition system (BIOPAC, Santa Barbara, CA, U.S.A.) after the stroke. EEG recordings were acquired in conscious rat pups using the ACQKNOWLEDGE 4.1 software (Biopac Systems Inc., Goleta, CA, U.S.A.) under basal conditions, during ischemia (50 min), and during the first 24 h after reperfusion. To account for the absence of feedings with the dam, animals were supplemented with 1 ml of 5% glucose twice during the EEG recording. The animals were euthanized after 24 h.

2.4. EEG analysis

All EEG recordings were analyzed in a double-blind manner with an analysis of the background and the occurrence of epileptic discharges. The mean duration of epileptic events during the 24-hour recording was evaluated in both control (PBS) and treated animals. In addition, we determined the mean duration per hour and the seizure onset, defined by the time between the start of the EEG recording to the first organized discharge of spikes lasting >10 s.

2.5. Assessment of cell damage

Brain sections collected 24 h after injury were processed for terminal dUTP nick-end labeling (TUNEL) according to the manufacturer's instructions (In situ Cell Death Detection Kit, AbCys, Paris, France). Additional sections were stained with Fluoro-Jade® B (Merck-Millipore, France). Positive cells were counted (in a blind manner) as previously described (Villapol et al., 2009).