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Cerebellar oxidative stress and fine motor impairment in adolescent rats exposed to hyperthermia-induced seizures is prevented by maternal caffeine intake during gestation and lactation

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ABSTRACT

Febrile seizures (FS) is one of the most common convulsive disorders in infants and young children that only occurs during the first years of life in humans, when the cerebellum is still developing. Several works have shown that maternal caffeine consumption during gestation and lactation can exert protective effects on developing brain under pathological conditions. Here, we have used an animal model of FS to know whether maternal caffeine consumption during gestation and lactation exhibited protective effects on rat cerebellum.

Pregnant rats were allowed to freely drink water or caffeine (1 g/l) during gestation and lactation. At PD13, neonates were submitted to hyperthermia-induced seizures (HIS) whereas pups not subject to hyperthermic stimulus were used as controls. 48 h, 5 and 20 days after HIS, rats were killed and plasma membranes and cytosolic fractions were isolated from cerebella. The enzymatic activities of glutathione reductase, glutathione S-transferase, caspase-3, 5´-nucleotidase and the levels of thiobarbituric acid reacting substances, adenosine A_1 and A_{2A} receptors were studied in these preparations. Furthermore, rats were tested in balance beam test and footprint test 20 days after HIS (PD33) in order to investigate the effect on fine motor coordination and gait patterns.

Results obtained suggest that maternal caffeine consumption during gestation and lactation exerts two kinds of beneficial effects on cerebellum from rats submitted to HIS: a) at short term, maternal caffeine abolishes hyperthermic seizures induced-oxidative stress and caspase-3 activation and b) in adolescent rats (PD33), maternal caffeine prevents fine motor coordination impairment and gait disturbances.

1. Introduction

Caffeine that belongs to a family of compounds known as methylxanthines is commonly consumed during gestation and lactation. This substance can easily reach fetal and neonatal brains because is metabolized more slowly by the mother during gestation and by the foetuses and neonates, it can cross blood-brain and placental barriers and it is excreted in the breast milk (Adén, 2011). The effects that maternal caffeine consumption might have on the human offspring have been widely investigated through epidemiological studies. However, it has been difficult to draw clear conclusions (Doepker et al., 2016). Interestingly, experimental studies using rodent models have revealed that maternal oral caffeine consumption during lactation exerts protective effects on neonatal brain under pathological conditions (Back et al., 2006; Bona et al., 1995).

Caffeine can act on different biochemical targets but only antagonism of adenosine receptor occurs at physiological concentrations (Fredholm et al., 1999). At present, there are four types of adenosine receptor (A_1, A_{2A}, A_{2B} and A_3) although in the Central Nervous System (CNS) adenosine actions are mainly mediated by adenosine A_1 and A_{2A} receptors which are widely expressed in the brain including cerebellum. Upon activation, adenosine A_1 receptor exerts potent pre-synaptic and post-synaptic actions providing inhibition of neurotransmitter release by decreasing presynaptic calcium flows and stabilization of post-synaptic membrane potential by opening G protein-coupled, inwardly rectifying potassium channels (Fredholm et al., 2005). Both actions support, therefore, that adenosine A_1 receptor play an anticonvulsive role. Concerning A_{2A} receptor, its role in convulsive disorders is more controversial. Thus, different A_{2A} agonists have been shown to suppress seizures in an animal model of epilepsy (De Sarro et al., 1999) whereas

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Table 1
Table showing the number of litters and pups used in the study and the corresponding group allocation.

Oral solution: water										
litter	C1		C2		C3		C4		C5	
# pups	7		7		8		7		9	
treatment	C(3)	HIS(4)	C(4)	HIS(3)	C(4)	HIS(4)	C(4)	HIS(3)	C(5)	HIS(4)
48 H	1	2	1	1	1	2	1	1	1	1
5 days	1	1	1	1	1	1	1	1	2	1
20 days	1	1	2	1	2	1	2	1	2	2
Oral solution: caffeine										
litter	C1		C2		C3		C4		C5	
# pups			7		6		7		6	
treatment			C(3)	HIS(4)	C(3)	HIS(3)	C(3)	HIS(4)	C(3)	HIS(3)
48 H			1	1	1	1	1	1	1	1
5 days			1	1	1	1	1	1	1	1
20 days			1	2	1	1	1	2	1	1

other works have revealed that adenosine A_{2A} receptor has a pro-convulsive effect (El Yacoubi et al., 2009; Hosseinmardi et al., 2007).

Febrile seizures (FS) is one of the most common convulsive disorders in infants and young children that occurs in response to a fever but without evidence of invasive infection of the central nervous system (Shinnar and Glauser, 2002; Stafstrom, 2002). It is estimated that between 2% and 4% of children, under the age of 5 years, suffer, at least, one seizure along with fever (Hauser, 1994). FS are classified into simple and prolonged based upon the duration of seizures. Simple FS are short in duration (less than 15 min) and are generally regarded as benign. Prolonged FS last more than 15 min and may produce neurological sequelae and/or increase the risk of developing epilepsy later in life (Dubé et al., 2006; Patterson et al., 2014). Since the beginning, hippocampus and cortex have been the brain structures that have attracted most of the attention. However, there are several evidences that suggest that FS might also affect the cerebellum. Thus, FS only occurs during the first years of life in humans, when the cerebellum is still developing (ten Donkelaar et al., 2003). Furthermore, cerebellum is especially vulnerable to hyperthermia (Walter and Carraretto, 2016). In that sense, Lomoio et al. (2011) using a rodent model have shown that a single episode of neonatal seizures alters the cerebellum of immature rats. More evidences showing an alteration of cerebellum after hyperthermic seizures were obtained by McCaughran et al. (1984) who found that HIS had marked effects on the development of the cholinergic system in the cerebellum.

In the last decades, several animal models of FS have been developed to study the pathogenesis and consequences of FS (Bender and Baram, 2007; Feng and Chen, 2016). In the present work, we have used a widely used animal model of febrile seizures in which hyperthermia was induced by a regulated stream of mildly heated air (Baram et al., 1997; Dubé et al., 2006) in order to know whether maternal caffeine consumption during gestation and lactation exhibited protective effects on rat cerebellum.

2. Materials and methods

2.1. Materials

Cyclopentyl-1,3-dypropylxanthine,8-[dipropyl-2,3-^{3}H(N)]-(DPCPX) 120 Ci/mmol was from Perkin Elmer (Madrid, Spain) and [2–3 H] − 4-(2-[7-amino-2-(2-furyl)-[1,2,4]traizolo-[2,3-a]-[1,3,5]-triazin-5-ylamino]ethyl)phenol ([^{3}H]ZM241385 50 Ci/mmol) from ARC (St. Louis, MO). Calf intestine adenosine deaminase, theophylline, N^{6}-cyclopentyladenosine, adenosine 5´-monophosphate (5´-AMP), malachite green, 1-chloro-2,4-dinitrobenzene (CDNB), L-Glutathione oxidized, NADPH, L-Glutathione reduced, 2´,7´-dichlorofluorescein, 5'-N-ethylcarboxamidoadenosine (NECA) and thiobarbituric acid were from Sigma (Madrid, Spain). Ammonium molybdate was from Merck (Madrid, Spain). All other reagents were of analytical grade and obtained from commercial sources.

2.2. Animals

The care and use of animals were carried out accordingly with the European Directive 2010/63/EU and with Spanish laws (Real Decreto 53/2013 and Ley 32/2007) for the use of laboratory animals. All experiments were according to the Animal Experimental Committee of University of Castilla-La Mancha. Every effort was made to minimize animal suffering and to reduce the number of animals used. Animals were maintained in a 12 h light / 12 h dark cycle (lights on at 07:00 h) in a temperature- controlled room (25 °C) with constant humidity (40–50%) and with free access to food and drinking water.

Ten pregnant Wistar rats were used in this study. Five pregnant rats were treated with caffeine (1 g/l) in the drinking water from gestational day 2 onwards during gestation and lactation whereas the others five pregnant rats received tap water. This caffeine dose was selected because previous works had shown that maternal oral intake of caffeine (1 g/l) during gestation and lactation reach neonatal brain and modulated adenosine receptors (León et al., 2002, 2005; Lorenzo et al., 2010). One caffeine-treated rat was discarded because it showed cannibalism behavior. We do not know whether this behavior could be evoked by caffeine treatment because we have also observed a similar behavior in control rats in other occasions but, in any case, we cannot exclude it.

At postnatal day 13, half of each litter was subjected to hyperthermia-induced seizures whereas the other half was used as controls. 48 h (PD15) and 5 days (PD18) after hyperthermic insult, groups of neonates (see distribution in the Table 1) were killed and the cerebella removed in order to investigate the effect of maternal caffeine consumption and hyperthermia-induced seizures on oxidative stress parameters and adenosinergic system. The remaining pups were weaned at postnatal day 21 and maintained without additional intervention until postnatal day 34 when balance beam test and footprint test were carried out. At the end of behavioral studies rats were killed and the cerebella removed, frozen in liquid N_2 and stored at −80 °C until experiments were performed. In total, 64 rats were distributed as follows:

PD15: 5 control-water, 7 HIS-water, 4 control-caffeine and 4 HIS-caffeine.
PD18: 6 control-water, 5 HIS-water, 4 control-caffeine and 4 HIS-caffeine.
PD33: 9 control-water, 6 HIS-water, 4 control-caffeine and 6 HIS-caffeine.

As can be seen, the variation in the number of animals in each