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Drug/nutrition interaction in the developing brain: Dipyrone enhances spreading depression in rats

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ABSTRACT

The abuse of pharmaceutical drugs and the inadequate ingestion of nutrients constitute external factors that can alter brain development, both individually and in combination. We used cortical spreading depression (CSD) as a neurophysiological parameter to investigate the combined effects of the antipyretic/analgesic/anti-inflammatory drug dipyrone and malnutrition (M) in the developing rat brain. Suckling malnourished rats (M; $n=69$) and well nourished controls (W; $n=76$) received dipyrone (300 mg/kg/day) or saline per gavage for 7 consecutive days during the 2nd, 3rd, or 4th postnatal week. At 35–45 days, CSD was recorded at 2 points in the parietal region. In both groups, dipyrone increased CSD propagation velocities compared to respective saline controls ($P<0.05$). This effect was intensified when dipyrone application during the 4th postnatal week intensified the increase compared to the 2nd and 3rd weeks. In saline-treated groups, the velocities (mean ± s.d., in mm/min) were 3.70 ± 0.11, 3.77 ± 0.16, and 3.78 ± 0.13 (W) and 4.13 ± 0.10, 4.16 ± 0.10, and 4.14 ± 0.09 (M), for animals treated in the 2nd, 3rd and 4th postnatal weeks. In dipyrone-treated groups, the respective values were 3.99 ± 0.14, 4.03 ± 0.16, and 4.30 ± 0.19 (W) and 4.47 ± 0.17, 4.70 ± 0.31, and 5.01 ± 0.28 (M). Results support the hypothesis that dipyrone has a CSD-facilitating effect, which is more intense at a late brain developmental stage and is facilitated by malnutrition. This may help explain the developmental brain excitability changes that are associated with pharmacological and nutritional factors.

Introduction

Dipyrone is a pyrazolone-derived, non-steroidal antipyretic/analgesic/anti-inflammatory drug that is largely used in clinical therapeutics, both in adults and in children (Mao et al., 2006; Reis et al., 2003). In particular, it has been used in children as an antipyretic, helping to prevent seizures induced by fever (Doretto et al., 1998). Its pediatric use is common in several countries (Ergün et al., 2000; Vasquez et al., 2005), despite the possibility of deleterious side-effects on the developing brain. Although most non-steroidal antipyretic/anti-inflammatory drugs have mechanisms of action based primarily on the inhibition of the enzyme cyclooxygenase (COX; Abbott and Hellemans, 2000; Alves and Duarte, 2002), the mechanisms of dipyrone are poorly understood and there is controversy regarding the drug's sites of action (Collares and Vinagre, 2003).

Insufficient intake of nutrients can lead to malnutrition, which has more severe effects on the brain when occurring early in life during the "brain growth spurt" period (Dobbing, 1968). In the rat, this phase corresponds to the suckling period during the first three postnatal weeks. During this time window, the brain is highly vulnerable not only to nutritional deficiency (Dobbing and Smart, 1974), but also to other external factors such as pharmacological agents (Amâncio-dos-Santos et al., 2006). However, the electrophysiological effects of dipyrone on the developing brain under normal or deficient nutritional conditions have not been subjected to much systematic study.

There is evidence that the critical time window for brain development is inhomogeneous with respect to distinct neural structures and developmental processes (Levitsky and Strupp, 1995; Morgane et al., 1993). Evidence concerning brain electrical activity, implies that a deleterious factor like malnutrition would have the most important impact when acting at a certain point in time within the critical period (Rocha-de-Melo and Guedes, 1997). This evidence allows one to predict a similar heterogeneous pattern of brain developmental effects in the case of drugs like dipyrone.

Experimental models devoted to studying the developmental consequences of pharmacological and nutritional factors on brain function may improve general understanding about the developmental processes of the nervous system, as well as its strategies for adaptation to external insults, which may be clinically relevant. When occurring early in life, such insults can modify the patterns of

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developmental processes in the brain, influencing brain functions as well as mechanisms of neural plasticity (Buonomano and Merzenich, 1998; Guedes et al., 1996; Morgane et al., 1978; 1993; Rema et al., 2006).

To analyze the possible influence of dipyrone on neuronal excitability, we investigated the electrophysiological effects of 7 consecutive days of dipyrone treatment on the propagation of the phenomenon known as cortical spreading depression (CSD) in the developing rat brain. CSD is a slow propagating excitability-related neural response that has been electrophysiologically demonstrated on the cortical tissue of experimental animals (Bures et al., 1974; Leão, 1944), and also in the human brain (Berger et al., 2008; Fabricius et al., 2008; Gorji and Speckmann, 2004; Mayevsky et al., 1996). It is a fully reversible phenomenon produced by electrical, mechanical, or chemical stimulation of one point in brain tissue, from which it spreads concentrically to remote cortical regions (Leão 1944).

As indicated by experimental evidence, the neural tissue normally offers a certain degree of resistance to CSD propagation. This resistance can be increased or decreased by experimental manipulations, resulting in lower or higher CSD velocities of propagation, respectively (Guedes and Do Carmo, 1980; Rocha-de-Melo et al., 2006). Therefore, the determination of CSD velocity along the cortical tissue is a sound and easy way to estimate susceptibility of the brain to CSD. Experimental conditions that either facilitate or impair the brain's ability to produce and propagate CSD may be helpful to the understanding of electrophysiological brain phenomena and of the diseases related to them, such as epilepsy (Guedes and Cavalheiro, 1997; Guedes et al., 2009; Leão, 1944, 1972).

In three models of experimental epilepsy, an anticonvulsive effect has been demonstrated for dipyrone (Doretto et al., 1998). Considering that the mechanisms underlying CSD and epilepsy may share some common features (Guedes and Do Carmo, 1980; Leão, 1944, 1972), we postulate that susceptibility of the brain to CSD could be altered by dipyrone treatment early in life. Using electrophysiological CSD recording, three questions are addressed in the brains of weaned young rats that have been, either well nourished or previously subjected to malnutrition during lactation. First, how does daily administration of dipyrone during three distinct periods of 7 consecutive days within the critical period of brain development affect CSD propagation? Second, in which of the three treatment periods would dipyrone have more of an impact in terms of altering the susceptibility of the brain to CSD? Third, how would this effect be influenced by the previous nutritional condition of the developing brain?

Materials and methods

Animals

Wistar rat pups of both genders ($n = 145$, of which 75 were male) were randomly distributed into two groups at birth according to nutritional status due to the lactation conditions: well nourished and malnourished (groups respectively designated W and M). The W group originated from litters with six pups, whereas 12 pups comprised litters in the M condition during the entire lactation period (0–25 postnatal days), as described previously (Rocha-de-Melo et al. 2006). Litters were reared in polyethylene cages (51 cm × 35.5 cm × 18.5 cm) in a room maintained at 22 ± 2 °C with a 12:12-h light–dark cycle (lights on at 7 h a.m.). During the suckling period, mothers were fed a lab chow diet (Purina do Brasil Ltd.) with 23% protein. After weaning, pups were housed 4–6 per cage, and fed the same maternal lab chow diet until the day of the electrophysiological CSD recording (postnatal days 35–45). The body weights were determined on postnatal days 7, 14, 21, 28, and 38. The animals in this study were handled in accordance with the "Principles of Laboratory Animal Care" (National Institutes of Health, Bethesda, USA), and with the norms of the Ethics Committee for Animal Research of the Universidade Federal de Pernambuco, Brazil.

Dipyrone treatment

The pups subjected to both nutritional conditions were treated by gavage, for 7 days, with 300 mg/kg/day of dipyrone (D, from Sigma Co.; 36 W and 34 M rats) or with an equivalent volume of saline solution (S; 40 W and 35 M rats). Both D- and S-treated pups, in each of the two nutritional conditions, were subdivided into three groups, according to the postnatal week in which the gavage was carried out: 2nd (21 W and 23 M rats), 3rd (26 W and 22 M rats), or 4th week (29 W and 24 M rats). A total of 12 groups were studied (Table 1). The dipyrone was dissolved in distilled water immediately before administration. The volume of the gavage solutions ranged from 0.5 mL/day (in the 2nd postnatal week) to 1.0 mL/day (in the 3rd and 4th postnatal weeks). The gavage was always performed between 12:00 h and 14:00 h. The dose of dipyrone used was chosen based on the work of Doretto et al. (1998). In each group, the proportion of males was approximately equal to that of females.

CSD recording

When the animals were 35 to 45 days old, they were submitted to CSD recording for a 4-hour period. Under anesthesia (1 g/kg urethane plus 40 mg/kg chloralose, ip), three trephine holes (2–3 mm in diameter) were drilled on the right side of the skull (two at the parietal bone and one at the frontal bone). The three holes were aligned in the anteroposterior direction and were also parallel to the midline.

CSD was elicited at 20 min intervals by applying a cotton ball (1–2 mm diameter), soaked in 2% KCl solution (approximately 0.27 M) to the anterior hole drilled at the frontal region for 1 min. The electrocorticogram (ECoG) and the slow DC potential change accompanying CSD were recorded simultaneously at the two parietal points on the cortical surface by using a pair of Ag-AgCl agar-Ringer electrodes. These electrodes consisted of plastic conic pipettes (5 cm length, 0.5 mm tip inner diameter), filled with Ringer solution and solidified with the addition of 0.5% agar, into which a chlorided silver wire was inserted. The pipettes were fixed together pair-wise with cyanoacrylate glue, so that the interelectrode distance was kept constant for each pair (range: 4–5.5 mm). Each pair of electrodes was connected to a lever that could be vertically moved by turning around a screw, so that the recording electrodes could be gently placed on the intact dura-mater, under low-power microscope control, without any excessive pressure on the cortical surface. A common reference electrode, of the same type, was placed on the nasal bones. The

Table 1
Distribution of the 12 experimental groups according to nutritional condition (W, well nourished; M, early malnourished), gavage treatment (D, dipyrone treatment; S, saline treatment) and the week of life in which gavage was applied (2nd, 3rd, and 4th weeks of life, respectively).

Groups	Nutritional condition	Gavage treatment	Week of life
1	W (76)	S (40)	2nd (11)
2			3rd (13)
3			4th (16)
4		D (36)	2nd (10)
5			3rd (13)
6			4th (13)
7	M (69)	S (35)	2nd (11)
8			3rd (11)
9			4th (13)
10		D (34)	2nd (12)
11			3rd (11)
12			4th (11)

Numbers in parentheses indicate the number of rats studied.