Early malnutrition attenuates the impairing action of naloxone on spreading depression in young rats

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Objectives: Malnutrition early in life can disrupt neurotransmitter systems in the brain, affecting its electrophysiological function. The opioid receptor antagonist naloxone can affect the electroencephalogram (EEG) and behavior in animals and humans, and patients under drug-abuse treatment use it as a therapy. The goal of this work in the rat is to determine whether malnutrition early in life modulates the action of naloxone on the excitability-related phenomenon known as cortical spreading depression (CSD).

Methods: Malnutrition was induced by feeding the dams during the gestation and lactation with a low-protein diet (8% protein). Their male pups received a single daily subcutaneous injection of naloxone (10 mg/kg/day) from the 7th to the 28th postnatal day, and were subsequently (30–40 days of life) submitted to a 4-hours CSD recording session, with electrodes at two points at a fixed distance apart on the parietal cortical surface.

Results: Compared to well-nourished rats receiving a 23% protein diet, malnourished animals displayed lower body weights and higher CSD velocities of propagation, confirming the facilitating effect of malnutrition on CSD. Naloxone treatment reduced in well-nourished rats the CSD propagation velocity, as compared to saline-injected controls. In contrast, the naloxone effect was less intense in the malnourished condition, and the CSD velocity difference between malnourished-naloxone and malnourished-saline groups did not reach statistical significance.

Discussion: Data strongly support the involvement of opioid-based mechanisms in excitability-related neural processes, which probably influence CSD propagation, and indicate that early malnutrition attenuates the impairing action of naloxone on CSD.

Keywords: Malnutrition, Brain development, Opioid system, Cortical spreading depression

Introduction

Several brain developmental effects of endogenous and exogenous opiates have been investigated in laboratory animals. Some early studies have provided evidence of changes in cell proliferation and outgrowth, as well as cell density, suggesting neuronal and glial altered development with consequent physiological changes in brain electrical activity. Opioid-mediated effects on brain electrical activity and behavior have been studied in humans and in laboratory animals. It has been demonstrated that the opioid antagonist naloxone can antagonize such neural opioid effects and naloxone-like compounds can treat drug abuse.

Concerning the electrophysiological effects of opioids, we have previously tested, in the rat cortex, the action of naloxone on the phenomenon known as cortical spreading depression (CSD). Leão first described CSD as a neural phenomenon, characterized by massive alterations in cerebrocortical ion homeostasis, in response to the electrical, chemical, or mechanical stimulation of a point on the brain. Once elicited in one cortical point, CSD concentrically propagates for the entire cortical surface, with propagation velocities of the order of a few mm/minute. CSD has been studied in vivo in several animal species, and has already been demonstrated in the human brain both in vitro and in vivo. One condition that surely enhances the cortical susceptibility to CSD is malnutrition early in life.
In mammals, normal brain development during the initial phase of life requires, as an essential factor, an adequate intake of nutrients. Nutritional deficiency early in life can negatively modify structural, biochemical, and electrophysiological parameters of the brain, and these effects can eventually last until adulthood. Early malnutrition decreases the brain CSD responses to pharmacologically induced alterations in certain neurotransmitter systems. In well-nourished animals, naloxone was shown to block CSD. No information is available, however, on the possible action of early malnutrition in modulating the effects of naloxone on CSD.

In this study, we aimed to characterize naloxone-mediated CSD effects in the malnourished rat cerebral cortex. We compared CSD propagation in weaned well-nourished and malnourished rats previously treated with naloxone during the suckling period. We hypothesized that malnutrition early in life modulates the action of naloxone on the CSD. Data indicated that early malnutrition attenuated the decelerating effect of naloxone on CSD.

Methods

Wistar newborn male rats, born at the same day from different mothers, were randomly distributed to form litters composed of six pups. During the gestation and lactation the dams were divided into two groups, one receiving a commercial laboratory rat chow diet (Purina do Brazil Ltd, Paulínia, Brazil) with 23% protein (well-nourished group) and the other receiving a low-protein diet with 8% protein (malnourished group). After weaning (at postnatal day 21) pups had free access to water and to the commercial laboratory rat chow diet. Animals were maintained in a room at a temperature of 23 ± 1°C with a 12 hours light:12 hours dark cycle (lights on at 6:00 AM). The animals were handled in accordance with the norms of the Ethics Committee for Animal Research of the Universidade Federal de Pernambuco, Brazil, which complies with the ‘Principles of Laboratory Animal Care’ (NIH, Bethesda, MD, USA). From the 7th to the 28th postnatal day, two well-nourished and two malnourished groups were treated daily with single subcutaneous injections. One group received 10 mg/kg/day naloxone (Sigma, St. Louis, MI, USA) and the other received an equivalent volume (10 ml/kg) of saline. Injections were applied between 12 AM and 2 PM. When the pups were 30–40 days old, they were submitted to a 4-hour-long CSD recording session. On the day of the electrophysiological recording, the animal was anesthetized with a mixture of 1 g/kg urethane plus 40 mg/kg chloralose injected intraperitoneally. A tracheal cannula was inserted and three trephine holes were made on the right side of the skull. These holes were aligned in the anteroposterior direction and parallel to the midline (see insert in Fig. 2). One hole was positioned on the frontal bone (2 mm in diameter) and was used to apply the stimulus (KCl) to elicit CSD. The other two holes were drilled on the parietal bone (3–4 mm in diameter) and were used to record the propagating CSD wave. Recital temperature was continuously monitored and maintained at 37 ± 1°C by means of a heating pad. CSD was elicited at 20 minutes intervals by a 1-minute application of a cotton ball (1–2 mm in diameter) soaked with 2% KCl solution (approximately 270 mM) to the anterior hole drilled at the frontal region. Both the reduction of the spontaneous cortical electrical activity (electrocorticogram (ECoG)) and the direct current (DC) slow potential change accompanying CSD were recorded for 4 hours, by using two Ag–AgCl agar-Ringer electrodes (one in each hole) against a common reference electrode of the same type, placed on the nasal bones. The CSD velocity of propagation was calculated from the time required for a CSD wave to pass the distance between the two cortical electrodes. In the two cortical recording places, the initial point of each DC negative rising phase was used as the reference point to calculate the CSD velocities. After the electrophysiological recording, the animal was killed with an overdose of anesthetic. Data in all groups were expressed as means ± standard deviations. CSD propagation rates were compared between groups by using the analysis of variance followed by the Tukey test, where indicated. P values of less than 0.05 were considered significant.

Results

The body weights of both malnourished and well-nourished groups are shown in Fig. 1. From postnatal days 7–30, the mean values were significantly lower in the malnourished groups, as compared with the well-nourished groups. The mean ± SEM weights in the saline- and naloxone-treated well-nourished rats ranged from 6.32 ± 0.30 to 6.82 ± 0.17 g in 1-day-old rats, and from 100.76 ± 4.54 to 103.04 ± 3.74 g in 30-day-old animals. In the malnourished groups, the weights ranged from 5.88 ± 0.26 to 5.46 ± 0.22 g in 1-day-old rats, and from 39.67 ± 3.66 to 35.87 ± 2.63 g in 30-day-old animals. In the same nutritional condition, no drug-associated weight difference was observed.

Stimulation of a point on the frontal cortex for 1 minute with 2% KCl was effective in eliciting a single CSD wave that propagated, and was recorded
Examples of electrophysiological recordings representative of the four groups of this study (two well nourished and two malnourished) are presented in Fig. 2. The ECoG depression and the slow potential change confirmed the presence of CSD after KCl stimulation. CSD velocities in the malnourished group treated with saline were higher ($P < 0.05$) than in the well-nourished saline group, confirming the previously reported facilitating effect of early malnutrition on CSD propagation (see the Discussion section). In the well-nourished condition, the 21-day naloxone treatment during the lactation period reduced the CSD velocity of propagation as compared with the respective saline-treated controls ($P < 0.05$). This effect was less intense in the malnourished condition, so that the naloxone versus saline velocity difference did not reach statistical significance. For the well-nourished and malnourished saline-treated groups, the mean $\pm$ SEM velocities (in mm/minute) were $3.90 \pm 0.10$ (well-nourished group) and $4.56 \pm 0.11$ (malnourished group). For the naloxone-treated groups, the corresponding mean velocities were $3.52 \pm 0.05$ (well-nourished) and $4.33 \pm 0.14$ (malnourished). Fig. 3 illustrates these data.

**Discussion**

In the rat, the most important period for the brain development occurs during the lactation period, when the synaptogenic activity is very intense. It represents a very important phase for the functional maturation of the brain. For that reason, we have chosen the lactation period to treat the animals with naloxone. We have previously documented in the well-nourished rat that naloxone, applied during the lactation period, impairs CSD propagation, as evaluated by the lower CSD velocity in comparison with the saline-treated controls ($P < 0.05$). The present findings on the well-nourished group confirmed those previously reported data. Taken together, they strongly suggest the involvement of opioid-based mechanisms in the excitability-related neural processes, which probably influence CSD propagation. One may postulate that opioid mechanisms, which naloxone can block, are involved in the lasting effects of this opioid antagonist observed on CSD. However, it must be kept in mind that pharmacological
manipulation of other neurotransmitter systems might also influence CSD.\textsuperscript{22,23} Furthermore, we know from the literature that opioid-based mechanisms participate in excitability-related neural processes such as those involved in the processing of selective auditory information,\textsuperscript{24} generation of cortical EEG activity,\textsuperscript{25} kindling,\textsuperscript{26} and epilepsy.\textsuperscript{27,28} Interestingly, compelling evidence demonstrates that environmental conditions that influence neural excitability can also interfere with brain susceptibility to CSD.\textsuperscript{29–31} It appears that the cortical tissue naturally resists to CSD propagation\textsuperscript{32} so that, when the brain CSD-resistance decreases, CSD propagation velocity increases, as compared with normal conditions, and vice versa. Therefore, the experimental alteration of the brain ability to counteract CSD constitutes an interesting way of getting valuable clues to the comprehension of the phenomenon and hopefully of the neurological disorders related to it, such as classical migraine,\textsuperscript{33} brain ischemia,\textsuperscript{34} and epilepsy.\textsuperscript{35,36}

Concerning the question of how the opiate system might influence the cortical susceptibility to CSD, one possibility would be that toxic and/or structural alterations of the cerebral cortex consequent to the pharmacological manipulation of the opioid system during development is causally involved in the effects on CSD. As examples of such toxic and structural alterations, it has been shown that morphine causes apoptosis in cultured human neurons,\textsuperscript{36} and recently an impairment of white matter has been described in human heroin addicts submitted to methadone treatment.\textsuperscript{37} In the developing rat submitted to unfavorable lactation conditions, neural structural modifications have been associated to enhancement in CSD propagation.\textsuperscript{15,38,39}

Brain developmental processes can be considerably altered by malnutrition early in life.\textsuperscript{11,13} We know from our previous work that early malnutrition decreases the brain CSD responses to changes in blood glucose levels.\textsuperscript{40,41} We also know from the work of others that previously malnourished adult rats display reduced behavioral reactions to GABAergic compounds like diazepines.\textsuperscript{42} Interestingly, early malnutrition also reduces the brain CSD responses to diazepam,\textsuperscript{19} suggesting that the gamma-aminobutyric acid (GABA)-system is modulated by early malnutrition. Based on the present results, we suggest a similar malnutrition-related modulation on the effects of the opioid system on CSD (note the absence of statistical significance in the CSD velocities of malnourished rats treated with naloxone, as compared with their saline-treated controls, in Fig. 3). This is in agreement with a previous report, suggesting a reduction of opioid receptors in malnourished rats.\textsuperscript{43} It is also possible that two independent phenomena (CSD acceleration by malnutrition and deceleration by naloxone) exist, and occur independently. Further investigation shall clarify this possibility. On the other hand, malnutrition does not seem to modulate the responsiveness of the serotoninergic system to the CSD phenomenon.\textsuperscript{16,44} The implication is that early malnutrition can differentially modulate distinct neurotransmitter systems.

Our findings suggest that the nutritional status early in life was able to modulate the CSD effects of naloxone, which did not significantly change the CSD propagation in the malnourished group (Fig. 3). The distinct effects of naloxone in the two nutritional conditions can be attributed to the developmental impact of the dietary deficiency.\textsuperscript{15} In the malnourished brain, processes like synapse formation, dendritic development, cell packing density, and myelination can be affected,\textsuperscript{12,13} and we postulate that this may be involved in the present facilitating CSD effects associated with malnutrition, which is in accordance with previous reports.\textsuperscript{15,20,39} Recent evidence on the role of brain myelination in CSD propagation\textsuperscript{45} deserves comment. Those authors demonstrated an inverse correlation between the degree of brain myelination and propagation of CSD, indicating a dichotomous modulation of the myelin involvement in the CSD effects. Similar dichotomous modulation has also been observed comparing overfed and malnourished rats, with CSD deceleration in overfed rats and acceleration in malnourished rats, as compared with the well-nourished controls.\textsuperscript{39}

In conclusion, this study documents in rats the modulating action of early malnutrition on the CSD effects of the opioid antagonist naloxone. Data support previous evidence\textsuperscript{21} in favor of an opioid-mediated antagonistic action on brain susceptibility to CSD, and suggest that early malnutrition reduces this effect.

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References
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