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Research report

Effect of perinatal asphyxia on tuberomammillary nucleus neuronal density and object recognition memory: A possible role for histamine?

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HIGHLIGHTS

- Perinatal asphyxia reduces performance in the object recognition task.
- Reduced performance in the object recognition is reverted by an H3 antagonist.
- Perinatal asphyxia reduces the number of ADA-immunoreactive neurons.
- Perinatal asphyxia reduces the expression of HDC in the hypothalamus.
- The performance in memory task is correlated with ADA-immunoreactive neuronal density.

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ABSTRACT

Perinatal asphyxia (PA) is associated with long-term neuronal damage and cognitive deficits in adulthood, such as learning and memory disabilities. After PA, specific brain regions are compromised, including neocortex, hippocampus, basal ganglia, and ascending neuromodulatory pathways, such as dopamine system, explaining some of the cognitive disabilities. We hypothesize that other neuromodulatory systems, such as histamine system from the tuberomammillary nucleus (TMN), which widely project to telencephalon, shown to be relevant for learning and memory, may be compromised by PA. We investigated here the effect of PA on (i) Density and neuronal activity of TMN neurons by double immunoreactivity for adenosine deaminase (ADA) and c-Fos, as marker for histaminergic neurons and neuronal activity respectively. (ii) Expression of the histamine-synthesizing enzyme, histidine decarboxylase (HDC) by western blot and (iii) thioperamide an H3 histamine receptor antagonist, on an object recognition memory task. Asphyxia-exposed rats showed a decrease of ADA density and c-Fos activity in TMN, and decrease of HDC expression in hypothalamus. Asphyxia-exposed rats also showed a low performance in object recognition memory compared to caesarean-delivered controls, which was reverted in a dose-dependent manner by the H_3 antagonist thioperamide (5–10 mg/kg, i.p.). The present results show that the histaminergic neuronal system of the TMN is involved in the long-term effects induced by PA, affecting learning and memory.

Abbreviations: ADA, adenosine deaminase; GABA, γ-aminobutyric acid; GAD, glutamic acid decarboxylase; HDC, L-histidine decarboxylase; PA, perinatal asphyxia; TMNv, tuberomammillary nucleus, pars ventral; TMNd, tuberomammillary nucleus, pars dorsal.

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1. Introduction

Perinatal asphyxia (PA) is a severe condition associated with obstetric complications during labour and delivery, leading to high mortality among affected newborns. Survivors can suffer from severe to mild neurological disabilities, such as cerebral palsy, mental retardation, increasing further the risk factors for developing neuropsychiatric disorders and cognitive disabilities [1–4]. Models of PA have demonstrated that affected animals, show cognitive disability when evaluated by different experimental paradigms at adulthood, such as deficits in working memory, object recognition and spatial memory [1–3], which can be explained by impairments in telencephalic brain regions, such as neocortex, basal ganglia, hippocampus, and amygdala, or by deficits primarily affecting ascending pathways from the brainstem [4,5]. Indeed, deficits in dopamine release have been reported in adult PA-exposed animals [6], in agreement with the hypothesis that deficits in monoamine pathways are a signature for neuropsychiatric disorders, such as schizophrenia and depression, implying detrimental activity of cognitive abilities, including learning and memory [7,8]. The tuberomammillary nucleus (TMN) of the ventral posterior hypothalamus innervates widespread regions of the telencephalon, demonstrated by retrograde tracing and immunohistochemical studies [9]. The TMN is characterized by containing magnocellular neurons which synthesize and produce histamine in the brain, the only source of histamine in the brain. Histamine has been shown to play a critical role in learning, memory and cognitive processes [10]. To our knowledge, however, the effect of PA on histamine systems has not yet been explored.

We hypothesized that PA affects the histamine system and the alteration over this neuromodulator contributes to the alteration in learning and memory observed in asphyxia-exposed rats. To test this hypothesis, we have investigated here the effect of PA on the density and neuronal activity of TMN neurons by using ADA-immunoreactivity, as a histaminergic marker and c-Fos immunoreactivity as a neuronal activity marker. The expression of histidine decarboxylase (HDC), the histamine-synthesizing enzyme was also evaluated in the hypothalamus, as well as the performanceon an objects recognition memory task at adulthood. Thioperamide, a H3 autoreceptor antagonist [11], was used in order to increase histamine release and improve the cognitive impairment observed in PA animals.

2. Materials and methods

2.1. Perinatal asphyxia

Pregnant Wistar rats on the last day of gestation (G22) were euthanized by cervical dislocation and hysterectomized. One or two pups were immediately removed and used as sibling non-asphyxiated caesare-delivered controls (C). The uterine horns containing the remaining fetuses were immersed into a water bath at 37 °C for 21 min to induce severe asphyxia, thereafter the uterine horns were excised, the pups delivered and stimulated to breath. Asphyxia-exposed and control animals were kept on a warming pad for 1 h, and then assigned to surrogate dams for nursing, pending further experiments [12]. After weaning, thirty adult male rats (PA = 18; C = 12), weighing 270–350 g were kept in separate cages (3 per cage) at controlled temperature (21–24 °C) and 12/12 h light/dark schedule. Water and food were supplied *ad libitum.* All experiments were carried out in compliance with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised 1996), minimizing the number of animals used and their suffering. Local Institutional Bio-Safety and Bio-Ethical Committee at the Medical Faculty, University of Chile (CBA #0212 FMUCH), approved all experimental protocols.

2.2. Animals groups

Eighteen rats were used for behavioral analysis 12 of them (6C and 6PA) were used for object recognition task and immunohistochemistry analyses. Six PA rats were used for object recognition and pharmacologic blocking of H3 histamine receptor. Twelve animals (6C and 6 PA) were used for HDC Western Blot analysis.

2.3. Object recognition task

The object recognition task was performed in an 80 × 80 × 60 cm box (with a black floor) placed in a noise isolated experimental room. Behavior was recorded with a video camera in an overhead shot/view position. The animals were first habituated to the box for 15 min in the absence of any object for two consecutive days and then exposed to two identical plastic figures for 5 min letting the animals freely exploring and familiarizing with the objects. After 90 min, each animal was tested for object recognition memory by putting the animal in the box with one of the previously exposed objects, and a new one for 5 min. Offline analysis of the video recording evaluated the time spent by the animal to explore the new object, compared to that exploring the previously exposed object, as an indicator of memory recognition. This measure was recorded with an automated video-tracking Matlab routine using (Mathworks, Inc, USA).

2.4. Pharmacological treatment

A group of six PA animals was injected with saline, or the H3 histamine receptor antagonist, Thioperamide (5 mg/kg, 10 mg/kg i.p.) at different sessions, performed with the same order with one week interval among the experiments. Ten minutes after each treatments, the rats were tested for the object recognition task as described above.

2.5. Immunohistochemistry

One hour after the behavioral test, rats were anesthetized with chloral hydrate (350 mg/kg; i.p.), transcardially perfused with 300 ml of saline (NaCl, 0.9%) followed by 500 ml a phosphate buffered (PB, pH 7.4, 0.1 M) paraformaldehyde (4%) solution. The brain was then removed and postfixed in the same solution for two hours, and transferred to 30% sucrose with 0.02% sodium azide in phosphate buffered saline (PBS) until saturated and sunk. Brains were cut in the coronal plane, 50 μm thicknesses, using a sliding frozen microtome. The sections were then processed for Nissl staining and immunohistochemistry. For immunohistochemistry, free-floating sections were incubated with the primary antibody (rabbit anti-Fos polyclonal antibody, Ab-5, Oncogene, San Diego, CA, diluted 1:20,000), overnight at room temperature. Then, the samples were rinsed and incubated with the secondary antibody (Biotin-SP- conjugated AffiniPure goat anti-rabbit IgG H+L; Jackson ImmunoResearch, PA, diluted 1:1000) for 1 h, rinsed and incubated in Vectastain ABC Elite Kit (Vector Laboratories, CA, diluted 1:500), for 1 h. Finally, the sections were revealed with 0.05% 3-3′diaminobenzidine hydrochloride (DAB) and nickel chloride to get an enhanced dark blue nuclear reaction product for c-Fos. Selected, already immunostained sections were subjected to a second immunostaining, to identify ADA-ir neurons in the TMN, with rabbit anti-ADA, polyclonal antibody, (diluted 1:5000, Chemicon, CA). Revealed with DAB without nickel intensification, yielding a brown cytoplasmic precipitate, contrasting with the dark blue nuclear DAB-nickel labeling for the c-Fos-reaction. The specificity