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Long term osmotic stress exposure outcomes on rat dopaminergic innervations and the associated motor behavior

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ABSTRACT

The osmotic stress is a powerful stimulus that elicits profound peripheral and central disturbances. In the mammalian brain, osmotic stress has been associated to several glial and neuronal changes. The lack of data regarding the impact on the dopaminergic system and locomotion led us to investigate the effect of prolonged water deprivation in rat on the midbrain dopaminergic system and locomotor performance by dehydrating rats for one and two weeks. Locomotor activity and tyrosine hydroxylase (TH) expression were assessed using the open field test and immunohistochemistry respectively. Water deprivation was accompanied with a significant increment of TH expression within substantia nigra compacta (SNc) and ventral tegmental area (VTA) gradually as the duration of dehydration increases. While locomotor activity showed the inverse tendency manifested by a drop of crossed boxes number following one and two weeks of water deprivation. Our data suggest a substantial implication of midbrain dopaminergic system in the central response to the osmotic stimuli accompanied with locomotor deficiencies.

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1. Introduction

Dehydration of the body results from water deprivation, excessive sweating, and various disease states such as polyuria, severe diarrhea and hyperthermia (Bakar and Niazi, 1983). Water deprivation is generally associated to may cause significant hormonal, physiological and biochemical changes of the whole body (Ha et al., 1996). Such symptoms are due to alteration of numerous endocrine (Dupoué et al., 2014), neurotransmitter and neuropeptidergic systems such as serotoninergic (Elgot et al., 2009; Chatoui et al., 2012), vasopressinergic (Gamrani et al., 2011) and dopaminergic systems (Elgot et al., 2012). It is well known that dopamine ensures an important role as a modulator of peripheral as well as central physiological functions in both humans and animals. Implication of the nigro-striatal dopaminergic pathway in the control of motor activity is well established (Crocker et al., 2001). The effect of water deprivation on locomotor activity has been investigated in several animal species. Indeed, dehydration decreases locomotor performance in amphibians, lizards (Prates et al., 2013) and Children’s pythons (Antaresia childreni); a species of snake in the family Pythonidae (Dupoué et al., 2014). In addition, water deprivation reduces performances, including anti-predator responses including locomotion and defensive behaviors (Angilletta, 2009). Otherwise, deprived rats from water during 23 h resulted in increased locomotor activity (Hall, 1955). However, when rectangular stabilimeters are used, an opposite tendency is noted (Campbell and Cicala, 1962). Such discrepancies could depend on the type of device used to assess locomotion (Campbell, 1964).
At the level of the central nervous system, beside its role in locomotion, dopamine has an essential role in the regulation of water balance, at several brain regions (Sulyok, 1988; Frankmann et al., 1994). Previous works showed an increased dopamine levels within substantia nigra compacta (SNc) and ventral tegmental area (VTA) which was concomitant with hyperactivity depending on the severity of water deprivation in a special rodent: Meriones shawi (Shaw’s Jird), a desert rodent characterized by its resistance to long periods of thirst (Elgot et al., 2012). Moreover, the hypothalamic vasopressinergic system which consists primarily of magnocellular neurons in the paraventricular (PVN) and supraoptic (SON) nuclei projecting into the neurohypophysis is under a dopaminergic control originating from the arcuate nucleus (Swanson and Sawchenko, 1983).

These observations demonstrate somehow the implication of dopamine in the hydromineral balance, both at central and peripheral levels. To our knowledge, the effect of prolonged dehydration on the midbrain dopaminergic system in rat has never been examined before. In this paper, we assessed the effect of prolonged water deprivation for 1 and 2 weeks on TH-immunoreactivity in DAergic neurons at the level of SNc and VTA as well as locomotor activity in Wistar rat.

2. Material and methods

2.1. Animals

The present study is carried out in adult male Wistar obtained from animal facilities of the Faculty of Sciences, Cadi Ayyad University, Marrakech. Rats were housed in the at a constant room temperature (25 °C), with a 12 h dark-light cycle and ad libitum access to food to all studied groups. All animals were treated according to the European decree, related to the ethical evaluation and authorization of projects using animals for experimental procedures, 1st February 2013, NOR: AGRG1238767A. Thus, all efforts were made to minimize the number and suffering of animals used.

2.2. Experimental design

Animals were divided into three groups: Group 1 of controls (n = 6; 279.25 ± 24.55) with free access to food and water. Group 2: dehydrated rats (n = 6; 270.30 ± 22.46) by complete water deprivation during 1 week. Group 3: dehydrated rats (n = 6; 266.27 ± 25.75) with water deprivation during 2 weeks.

2.3. Locomotor activity assessment

Locomotor activity was assessed using the “Open-Field” test which consists to a wooden apparatus with 25 squares of identical dimensions (20 cm/side) (100 cm × 100 cm × 40 cm) made out of wood. Before the test, each animal was exposed, three consecutive days, to the open field for 10 min for acclimation. At the day of the experiment, each animal is placed in the middle of the field and the total number of crossed boxes were recorded for 5 min as indicator of locomotor performance (El Hiba et al., 2013).

2.4. Immunofluorescence

Each animal of each group rats were anesthetized intraperitoneally with urethane (40 mg/kg i.p.), then, they were intracardially perfused with 4% paraformaldehyde. After their removal, brains are postfixed overnight in the same fixative solution. Coronal sections of 80 µm thickness were made with microtome and selected sections were then processed for floating immunohistochemistry. They were first incubated for 2 h, at room temperature, with a mixture of 2.5% bovine serum albumin (BSA) with 2.5% normal goat serum (NGS) and 0.3% Triton in PBS. Then, incubation with anti-tyrosine hydroxylase antibody (rabbit; dilution 1/1000; Pel Freez, USA) diluted in PBS with BSA 0.5% and NGS 0.5% is performed during 12 h at 4 °C. After rinsing in PBS, sections were incubated with the fluorescent-labeled secondary antibody Alexa 543 (dilution 1/500) for 1 h at room temperature. Controls were performed by omitting the first antibody. Images were taken with a Zeiss confocal (Wetzlar, Germany). The intensity of immunoreactivity was finally analyzed.

2.5. Statistical analysis

Data are reported as mean ± SEM, and were subjected to a one way analysis of variance (ANOVA). Post hoc differences between group means were tested with the Tukey test. Values of p lower than 0.05 was considered significant. Statistical analyses were performed using the computer software SPSS 10.0 for windows.

3. Results

3.1. Effect of prolonged water deprivation on locomotor activity in rat

Using the open-field apparatus to assess the effect of water deprivation of 1 and 2 weeks in rat on the locomotor performance, our data demonstrated that dehydrated animals exhibited a significant loss (p < 0.05) of number of crossed boxes reflecting a deficit of locomotor performance following 1 week of total water deprivation as compared to controls (Fig. 1), this decrease continue even more after 2 weeks of dehydration.

3.2. Effect of prolonged water deprivation on TH-immunoreactivity within VTA and SNc

Our data showed in control rats, the presence of many TH-immunoreactive (TH-IR) neurons within both VTA and SNc (Fig. 2). After 1 week of dehydration, an obvious and significant (p < 0.05) enhancement of the TH-IR was observed in the two structures; VTA and SNc (Fig. 2B). Dopaminergic neurons appear strongly immunoreactive and very dense; the neuronal processes showed a rise of their immunoreactivity corresponding to a possible increase of the TH level. This increase becomes much more significant (p < 0.05) after 2 weeks of water deprivation, both in perikarya and dendrites of dopaminergic neurons (Fig. 2C).

Fig. 1. Histogram showing the number of crossed boxes in the Open Field test during 5 min observation in control and dehydrated animals for 1 (1 W) and 2 weeks (2 W). A significant reduction of locomotor performance is observed in dehydrated rats. **p < 0.01 a highly significant difference in dehydrated rats vs. controls. *p < 0.05 a significant difference between dehydrated groups.
4. Discussion

The present investigation was focused on the assessment of the brain dopaminergic and locomotor behavior impairments occurring after chronic water restriction in rat. Interestingly, water deprivation for 1 and 2 weeks was accompanied with a significant increment of TH-IR within the midbrain dopaminergic structures such as SNc and VTA. These neuronal modifications were concomitant with a progressive loss of locomotor activity. In our knowledge, the present study is the first to investigate the effect of prolonged dehydration on dopaminergic system involved in the locomotor behavior control in rat. Although, previous data sustains our finding and demonstrate in the desert rodent *Meriones shawi* under osmotic stress by complete water restriction for 1 month, a central increment of TH levels in SNc and VTA (Elgot et al., 2012). Moreover, disruption of water balance have been shown to alter the metabolism of dopamine (DA) in the rat hypothalamus (Klemfuss and Seiden, 1986). Indeed, it is well established that the posterior pituitary and the infundibular stalk are terminated by a group of DA neurons; the tuberohypophyseal, originating from the arcuate and periventricular nuclei (Palkovits, 1981). Activation of these nuclei can be induced by dehydration or hyperosmotic solutions (Alper et al., 1982). Consequently, dehydration enhances the electrically evoked dopamine release from the neural and intermediate lobes of the rat hypophysis.

Otherwise, dopamine is mainly recognized as the main neurotransmitter involved in locomotor activity control (Beninger, 1983). Based on our data, dehydrated rats exhibited a continuous loss of locomotor activity depending upon the severity of dehydration. The last one has previously been shown to decrease locomotor performance in amphibians, lizards (Prates et al., 2013) and Children's pythons (Antaresia childreni) (Dupoué et al., 2014), as well as anti-predator responses (Angilleta, 2009). Moreover, previous finding have shown, using running wheels, that locomotor activity increases when rats were deprived of water for 23 h (Hall, 1955), while a decreased locomotor activity can be observed when rectangular stabilimeters were used (Campbell and Cicala, 1962). Such differential responses could depend on the type of device used for locomotion assessment (Campbell, 1964).

The present finding seems to be in discordance with our knowledge on the role of dopamine in the regulation of locomotor activity, since this monoamine contributes positively to locomotor activity control (Beninger, 1983). In the present study, increased TH-IR observed during dehydration does not automatically mean a subsequent increase in DA in the nigrostriatal projection. Indeed, the osmotic stress, mediated by vasopressin, is known to affect dopamine release at the level of projections rather than nuclei of origin. In fact, Arg-vasopressin known to be enhanced during the osmotic stress (Gamrani et al., 2011; Elgot et al., 2012), seems to have an influence upon DA release in the nucleus caudatus rather than DA synthesis (Beninger, 1983). Indeed, some finding showed that local microinjections of Arg⁸-vasopressin into the nucleus caudatus suppressed the release of DA by the terminal nerves, suggesting that AVP may act as modulator by reducing terminal DA release, rather than modulating DA-biosynthesis on the nucleus of origin which seems to be unchanged (Van Heuven-Nolsen et al., 1985).

In addition, the vasopressinergic system of the hypothalamus which is responsible for the systemic secretion of AVP formed by magnocellular neurons in the paraventricular (PVN) and supraoptic (SON) nuclei (Gamrani et al., 2011), is known to project to the neurohypophysis and innervated by dopaminergic terminals originated from arcuate nucleus (Swanson and Sawchenko, 1983). Thus, experimental osmotic stimuli normally associated to increased AVP release, also resulted in elevated levels of DA in the neurohypophysis able to inhibit AVP secretion (Lightman et al., 1982). Therefore, DA contributes to regulation of the AVP
release into blood circulation which then participates to the homeostatic regulation by equilibrating the hydric balance (Gamrani et al., 2011; Elgot et al., 2012).

Through the present study, we have elucidated the possible implication of DA in the osmotic stimulation by water deprivation in rat. Increased DA level within SNc and VTA could be considered as one of the neurophysiological adaptations of the brain to water restriction and may contribute in the regulation of the hydric balance.

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