Research Article

Stress and its role in the dentin hypersensitivity in rats

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\textbf{A B S T R A C T}

\textit{Introduction:} The perception of pain varies individually. Chronic stress leads to analgesia. The use of animal chronic mild stress model to mimic human condition was previously developed and now applied in the evaluation of pain perception in rats with dentin hypersensitivity (DH).

\textit{Aims:} Using DH model induced by dentin erosion (DE) mediated by acidic solution, the present study aimed the evaluation of the interaction of chronic stress and pain induced by DH in rats with DE.

\textit{Methods:} DH was induced by \textit{ad libitum} 30-day intake of acidic solution. Stress was induced by the New York subway model. Body weight was weekly taken, during treatment. Groups WO (water, no stress), WS (water and stress), EO (acidic solution, no stress) and ES (acidic solution, stress) were submitted to treatments.

\textit{Results:} After 30 days, all groups were submitted to DH test assessed by cold water stimuli in the labial surface of molars, for 5 s, and the rats responses were scored as grades 0, 0.5, 1, 2, or 3. After euthanasia, blood was taken to obtain the levels of corticosterone, stomachs were observed in fresh preparations, kidneys and livers were submitted to histological evaluation. Open field model supported stress evaluation, as did corticosterone analysis. Stressed animals showed significant increase in pain perception and a decrease in locomotion frequency, tending to be more frequent in the periphery of the arena, corroborating stressed behavior and the need of protection as a pain relief. Corticosterone levels were increased in the stressed rats with dentin erosion and also corroborate present findings. Finally, reduction in weight gain was impaired in stressed group with dentin erosion.

\textit{Conclusion:} The animal model enabled the evaluation of how chronic mild stress interfered in DH pain perception.

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1. Introduction

Stress is part of normal life and some stressful situations such as physical exercise, various emotional states and creative activity are usually considered healthy. However, prolonged and undesirable stress may induce noxious effects (Goldstein & Kopin, 2007). Clinical and pre-clinical studies about the interaction between stress and pain demonstrated complex and potent effects of stress on pain processing and response (Jennings, Okine, Roche, & Finn, 2014). Intense acute stress induces analgesia (Gibbons, Adler, Bonyhay, & Freeman, 2012) and chronic stress leads to hyperalgesia (Quintero et al., 2000). This last condition is going to be exploited in the present manuscript.

Dentin hypersensitivity (DH) can be defined as a sharp, short pain arising from exposed dentin in response to thermal, tactile, osmotic, or chemical stimuli. These stimuli are produced by the ingestion of hot, cold or acidic beverages, contact with acidic foods, tooth brushing, as can be caused by non-carious cervical lesions (Dowell & Addy, 1983). Theories which explain DH propose that the activation of dental primary afferents eventually delivers dental nociception to the central nervous system (Addy & Urquhart, 1992). Although a large number of techniques and therapeutic alternatives for relieving DH have been proposed in the literature, there is effectively a failure in the approach of this pathological process. Such treatments do not always achieve the desired result, since they are restricted to obliterating the tubules mechanically, without taking into account that the perception of pain is very
subjective and dependent on variations in the patient’s emotional state (Arteche & Casero, 2009).

Positive emotional and motivated behaviors can activate the system of central inhibition of pain in the body, which controls the stimulus of pain from the periphery through the release of endorphins by the central nervous system (Arteche & Casero, 2009). On the other hand, stress and depression can contribute negatively to the proposed treatments. The positive attitude of the professional may also contribute to a successful treatment (Higo et al., 2009).

Although emotional factors related to the patients are considered, the literature does not show experimental evidences, but an indication that the emotional aspect might interfere in the resolution of pain. Confirmation of the relationship between emotional factors and pain perception can lead to the proposal of a new protocol of care that addresses the patient holistically, technically, and psychologically, thus contributes to the resolution of dentin hypersensitivity (Sgolastra, Petrucc, Gatto, & Monaco, 2011).

Establishing animal models are an excellent way to confirm the relationship between dental erosion and emotional states. Some reports showed that sport drinks (which usually are acidic solutions) (Mistry & Grenby, 1993; Sorvari, 1989; Sorvari, Peittari, & Meurman, 1996) and Coca-Cola (Beiraghi et al., 1989; Tamura, Fujii, & Kusaba, 1979) induce dental erosion, such as gastroesophageal reflux disease (Higo et al., 2009), in rats. Previously, we showed that treatment with acidic solution for 30 days was able to induce dentin hypersensitivity after erosive challenge in rats. The pain induced by cold stimuli was consistent with the grade of dentin hypersensitivity. Close relationships between dentin hypersensitivity, response to pain, serum levels of corticosterone and anxiety behavior revealed the effects of dentin hypersensitivity at behavioral, biochemical and histological levels (Bergamini et al., 2014). Thus, the purpose of the present study was to evaluate the effect of stress on pain induced by dentin hypersensitivity after dental erosion challenge in rats to investigate the interaction between the perception of pain and increased emotionality.

2. Material and methods

2.1. Animals

Forty male Wistar rats, weighing 180–220 g, with 75 days of age at the beginning of experiments, were used. Rats were housed in polypropylene cages (38 × 32 × 16 cm, 5 rats/cage) at a controlled room temperature (22 ± 2 °C) with artificial lighting (12-h light/12-h dark cycle, lights on at 8 a.m.) with free access to Nuvilab rodent food (Nuvital Co., São Paulo, Brazil) and filtered water or acidic solution. Sterilized and residue-free wood shavings were used as animal bedding. The experiments were performed one week after the rats arrived in the laboratory. Animals were euthanised by gas chamber, as required by the Brazilian Laws. The animals used in this study were maintained in accordance with the guidelines of the Paulista University Animal Care and Use Committee, São Paulo, Brazil, under protocol No. 051/11CEP/ICCS/UNIP, 13 October 2011. These guidelines are similar to those of the National Research Council, USA.

2.2. Dental erosion

The erosion challenge was performed by giving the rats a sports drink (Gatorade®, pH 2.7). Rats of the experimental group received this solution as drinking water for 30 days (Bergamini et al., 2014).

2.3. Dentin hypersensitivity test

DH was assessed by cold water stimuli (4 °C), applied with an insulin-size syringe containing a silicon cannula inserted in the adaptor for 5 s on the labial surface of molars. Behavior response was scored (0 = no response, 0.5 = slight contraction of the body, 1 = contraction of the body, 2 = strong contraction of the body with a short vocalization; and 3 = strong contraction of the body with a prolonged vocalization), according to previous validation (Bergamini et al., 2014). The rats were independently scored by two observers and the mean score for each rat was employed in the hypersensitivity evaluation. The scores of the two observers were evaluated by the correlation test (0.97) to validate our method.

2.4. Body weight

The body weight was taken weekly and at the end of experiments, the body weight gain was calculated.

2.5. Stress induction (New York subway system)

The stress model here employed, called by the nickname “New York City Subway Stress”, was previously described (Dhabhar & McEwen, 1997), whose laboratory was located in New York (NY). It was so named it resembles the situation experienced by an individual boarding the subway time of great movement of users: restricted capacity to move the body and continuous shaking.

The methodology consists of the adaptation of a classic model of stress, the “stress of restricted movement”. This is considered as able to promote a stressful experience psychologically, through the feeling of confinement of the animal. In this methodology, animals have their movements restricted without being physically constrained and without experiencing pain. Two publications of the same group characterized this procedure as extremely constraint model useful for the study of mechanisms, of both central and peripheral stress-related disorders, as well as for the study of drugs for the treatment of these disorders (Paré & Glavin, 1986).

The apparatus to restrict movement consists of a wood laminated board (23.5 cm length) in which six polyvinyl chloride (PVC) pipes (3 cm diameter × 10 cm length) are attached to restrict the movement of subjects. The pipes have closed ends to prevent escape, but with holes at the front for rear ventilation and allowing passage of the tail. To induce the New York subway stress, the apparatus was placed on a mechanical shaker (Shaker Kline – Nova Etica, Model 108, Vargem Grande Paulista), set to one vibration/second. The rats were exposed to one hour of stress, during which the animals had no access to acid solution or feed. Animals were subjected to daily stress for 30 days (Bergamini et al., 2014). The period of one hour of stress was chosen because previous observations have shown that periods longer than this drastically reduce the body weight of rats and the consumption of acid solution.

2.6. Open field test study

Rodents in the first time of exposure to the open field apparatus (OFT) exhibits increased freezing behavior, reduced exploration and increased peripheral locomotion. These parameters were widely taken as indicative of high-stress state (Walsh & Cummins, 1976). Thus, for the evaluation of levels of emotionality, the rats were placed in the OFT apparatus, as previously described (Bernardi, de Souza, & Palermo-Neto, 1981). The apparatus consisted of a round, 96 cm diameter arena surrounded by a 25 cm high enclosure painted white and subdivided into 25 parts that were painted black. Hand-operated counters were used to score locomotion frequency (i.e., the number of floor sections entered), and a chronometer was used to measure the duration of immobility (i.e., the total time in seconds without spontaneous movements). For open-field observations, each rat was individually placed in the center of the arena, and its behavioral parameters
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