



## Effect of melatonin on degranulation of dura mater mast cells in Wistar rats

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### Introduction

Migraine is a highly prevalent neurological disorder that affects about 15% of the world's population. Involved in migraine pain, the cranial dura mater is a richly vascularized and innervated membrane, where we also find mast cells, and immune cells that help in the formation of the inflammatory process. As one of the treatments for migraine, we have melatonin, a hormone produced in the pineal gland and with properties such as circadian cycle regulation and antioxidant action. Capsaicin, a bioactive compound found in peppers and with a pungent and burning effect, also has an action in the painful process and serves as a tool in the study of physiopathogenic processes.

### Objectives

To evaluate the degranulation of dura mater mast cells from Wistar rats stimulated *in situ* with capsaicin and synthetic interstitial fluid (SIF) and previously treated with melatonin.

### Methods

Twenty-five male Wistar rats were used, obtained from the Department of Antibiotics - Danti of the Federal University of Pernambuco - UFPE and with use authorized by the Ethics Committee in the Use of Animals - CEUA of UFPE, according to protocol n° 0084/2019. During the adaptation period, the animals were acclimatized under standard laboratory conditions, with water and food *ad libitum*. After this period, they were separated into two groups: a control group (CG) (n = 12) and a melatonin group (GM) (n = 13), and underwent a daily treatment for 10 days with the intraperitoneal application. The GM received a dosage of 10 mg/kg of the animal weight of melatonin diluted in a saline solution at 0.9% and the GC was treated only with the vehicle. After the treatment, the animals were anesthetized and submitted to an experimental surgery, which consisted of opening two cranial windows in the right and left parietal bones for bilateral exposure of the dura mater. After exposure, the dura mater was stimulated *in situ* with SIF on the left side and capsaicin on the right side, following the following protocol: a 10  $\mu$ l solution of SIF with 0.1% ethanol was placed on the left side and right, 10  $\mu$ l of 10<sup>-6</sup> M capsaicin diluted in 0.1% ethanol for 5 min. In sequence, two units of cotton soaked with 60  $\mu$ l of the respective solutions were placed on each side for another 10 min. At the end of the experiment, the animals were euthanized and the skulls with the dura mater were dissected and fixed in 10% buffered formalin for 24 hours. After the fixation time, the dura mater samples were detached from the skulls, washed in distilled water for 1 min, stained with toluidine blue at a concentration of 0.1%, and fixed on histological slides. The slides were photomicrographed under 400X magnification to quantify the degranulated mast cells. The collected data were submitted to Student's t-test and the results were displayed in percentage value (%) and mean  $\pm$  standard deviation, considering a significance value of p<0.05.

### Results

A comparison of mast cell degranulation rate was performed between the dura mater segments of each group. Thus, the GC, when stimulated on the right side with capsaicin, presented a percentage of degranulation of 49.2  $\pm$  15.2%. On the right side of the GM, this same stimulation showed an average of 52.6  $\pm$  34.3% of degranulated mast cells. Thus, there was no statistically significant difference in this analysis. As for the left side, stimulated with SIF, for the GC an average rate of 40.6  $\pm$  29.7% of degranulated mast cells was found. On the other hand, the observation of this same side in the group that was treated with melatonin was observed 24.2  $\pm$  16.9% of degranulated mast cells (p=0.01).

### Conclusion

Melatonin inhibited dural mast cell degranulation. These findings favor the understanding of one more of the mechanisms by which melatonin helps in the treatment of patients with migraine.

**Keywords:** Headache, Migraine, Dura mater, Melatonin, Capsaicin, Rat.