

Elucidation of Pain Perception Mechanism by Hypothalamic Neuropeptides Using Genetically Engineered Animals

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INTRODUCTION: The hypothalamus is involved in the reception and control of nociceptive stimuli input from various areas including the thalamus and cortex. The hypothalamic paraventricular nucleus (PVN) elicits anti-inflammatory effects through the secretion of several hormones, ultimately leading to the secretion of adrenocortical hormones. For the posterior pituitary hormone oxytocin (OXT), an analgesic pathway has recently been identified in which OXT directly activates the mesolimbic dopamine (DA) nervous system to produce analgesia. Specifically, when pain is perceived, dopamine (DA) is released from the ventral tegmental area (VTA), activating the descending inhibitory system to suppress pain [1]; the direct action of OXT on and activation of DA neurons in the VTA is thought to be one mechanism by which OXT suppresses pain. On the other hand, DA receptors are known to be present in OXT neurons in PVN [2], and contrary to previous explanations, it has been speculated that DA is involved in the regulation of OXT, but the details are not clear. The rat model of fibromyalgia (FM), in which rats are treated with reserpine, is frequently used to investigate *in vivo* mechanisms of DA. Administration of reserpine to rats induces fibromyalgia-like symptoms and lowers pain thresholds by irreversibly binding to vesicular monoamine transporters and depleting the nervous system of biogenic amines, including DA [3]. However, the mechanism of pain threshold lowering remains unclear. Therefore, we hypothesized that OXT may be involved in the mechanism of pain threshold lowering in the FM model.

METHODS: For the experiments, we used OXT-monomeric red fluorescent protein 1 (mRFP1) transgenic rats with visualized OXT neurons. 6- to 8-week-old male OXT-mRFP1 transgenic rats were treated with vehicle (distilled water 0.5% acetic acid with distilled water) subcutaneously in the control and reserpine (1 mg/kg, 1 mg/ml) subcutaneously in the FM model. Both groups were decapitated 6 days later. After decapitation, brains were promptly retrieved and slices containing PVN were prepared by forehead sectioning. The OXT-mRFP1 transgenic rats used in the experiments selectively visualize OXT neurons with red fluorescence, allowing for reliable identification of OXT neurons during patch clamp. Under perfusion of artificial cerebrospinal fluid, OXT-mRFP1 neurons were identified using fluorescence differential interference microscopy, and postsynaptic currents and resting membrane potentials were recorded from OXT neurons in the PVN using the slice patch clamp technique. In two groups, Control and FM models, OXT neurons were identified in fluorescent mode among neurons in the PVN, electrodes were applied, and electrical signals were recorded using the Whole-Cell patch-clamp method. Spontaneous (s) excitatory postsynaptic currents (EPSCs) were evaluated to examine excitatory synaptic transmission to OXT neurons in both groups, and miniature (m) EPSCs were evaluated to assess only single synapses in each group. In addition, miniature inhibitory postsynaptic currents (mIPSCs) were examined to investigate inhibitory synaptic transmission to OXT neurons in both groups.

RESULTS: sEPSCs did not differ in amplitude between the two groups and tended to be less frequent in the FM model (Figure 1c). mEPSCs similarly did not differ in amplitude between the two groups and tended to be less frequent in the FM model (Figure 2c). mIPSCs were not significantly different in frequency or amplitude between the two groups (Figure 1c). mEPSCs also There was no significant difference in amplitude between the two groups (Figure 2c). mIPSCs frequency and amplitude were also not significantly different between the two groups (Figure 3c).

DISCUSSION: In the present study, the amplitude of EPSCs was reduced in the FM model. In other words, excitatory neurotransmitter release in the presynapse was suppressed. Since DA was depleted in the FM model, it is possible that OXT neurons in the PVN were affected by DA with respect to the frequency of excitatory neurotransmitter release from the presynapse. In other words, we speculated that DA was depleted by reserpine, and therefore glutamate, an excitatory neurotransmitter from the presynapse, was reduced. In a previous study, it was reported that in a state of DA activity, D2 receptors, which are inhibitory among DA receptors, suppress OXT inhibitory signals, leading to OXT activity [4]. In the present study, excitatory signals to OXT neurons were suppressed in the FM model, indicating that OXT neurons were inactivated. It is possible that the suppression of D1 receptors, which are considered excitatory DA receptors, in the FM model reduced neurotransmitter release from glutamatergic interneurons; confirmation of D1 receptors in PVN OXT neurons is needed, but this is a topic for future investigation.

SIGNIFICANCE: In FM model rats treated with reserpine, excitatory presynaptic inputs of OXT neurons in the PVN, one of the centers of nociceptive modulation, were reduced.

REFERENCES: 1. López-Avila et al., Pain. 2004 Sep;111(1-2):136-43; 2. Baskerville et al., Eur J Neurosci. 2009 Dec 3;30(11):2151-64; 3. Nagakura et al., Pain. 2009 Nov;146(1-2):26-33; 4. Gamal-Eltrabily et al., Rev Neurosci. 2018 Jun 27;29(4):377-386.

Figure 1

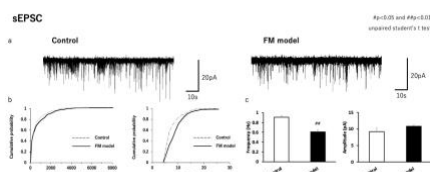


Figure 2

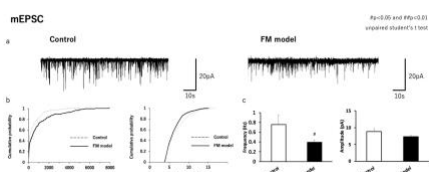


Figure 3

