NOCICEPTIN/ORPHANIN FQ-INDUCED INHIBITION OF ELECTRICAL ACTIVITY IN DORSAL RAPHE NUCLEUS SLICES FROM NORMAL AND STRESSED RATS

BARBIERI MARIO, NAZZARO CRISTIANO, BEANI LORENZO, SINISCALCHI ANNA

Department of Clinical and Experimental Medicine
University of Ferrara
Via Fossato di Mortara, 17-19
44100 Ferrara

Serotonin is critically involved in the pathophysiology of mental disorders, including anxiety and depression. Nociceptin/Orphanin FQ (N/OFQ) has been reported to inhibit serotonergic neurotransmission and to induce anti-stress and anxiolytic effects. To investigate the role N/OFQ plays in regulating the response to acute stress, the electrical activity of serotonergic neurons was studied in dorsal raphe nucleus (DRN) in vitro with the "single unit" technique. DRN slices (350 µm thick) were obtained from Sprague-Dawley rats (3-5 weeks old, 80-100 g weight), taking particular care to avoid any possible acute and/or chronic stress factor. Blood samples were collected to assay corticosterone levels. A typical spontaneous electrical activity, characterized by action potentials of regular frequency (2-3 Hz), was induced by 10 µM phenylephrine applied via continuous perfusion at 34 C°. Extracellular recordings were amplified up to 1000-fold and digitalized by a specially designed software allowing single spikes (20 ms duration) to be continuously visualized over an extended period (40-50 sec); the frequency value was followed to evaluate drug effects. A first series of experiments was carried out in DRN slices prepared from control (non-stressed) rats. An inhibitory effect (up to firing blockade) was detected by adding to the bath increasing N/OFQ concentrations (1-100 nM): a sygmoidal concentration-response curve was obtained (ED50 14.56±1.43 nM, n=10). At the end of each experiment 150 µM $BaCl_2$ (which selectively and reversibly blocks calcium channels involved by NOP receptor activation) was added, to confirm that the observed changes were correlated with N/OFQ presence. Neurons which failed the test were discarded. In the presence of the selective NOP receptor antagonist UFP101, the concentration response curve to N/OFQ was parallelely shifted to the right, and the ED50 was correspondently increased: N/OFQ+ UFP 1 µM 119.8±14.6 nM (n=6). A second series of experiments was carried out in rats submitted to 15 min of forced swimming ("stressed rats"); their blood corticosterone levels were about the double than in non-stressed rats. In DRN slices prepared from stressed rats, the concentration-response curve to N/OFQ inhibition was significantly shifted to the left: ED50 2.4±054 nM (n=6). This finding show that stressful stimuli induce an increased response of DRN serotonergic neurons to N/OFQ receptor stimulation, suggesting possible interactions with stress signalling systems such as corticotropin releasing factor.