Salivary proteome changes in response to social anxiety and sensory stimuli

Lorenzo Zallocco [1,5], Maurizio Ronci[2], Antonio Lucacchini[3], Maria Rosa Mazzoni[1], Laura Giusti[3,4], Laura Sebastiani[6]*

[1] *Dept. of Pharmacy, University of Pisa, Italy*
[2] *Dept. of Pharmacy, University G. d'Annunzio of Chieti-Pescara, Italy*
[3] *Dept. of Clinical and Experimental Medicine, University of Pisa, Italy*
[4] *School of Pharmacy, University of Camerino, Italy*
[5] *Dept. of Biotechnology, Chemistry and Pharmacy, University of Siena, Italy*
[6] *Dept. of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Italy*

[*] *CORRESPONDING AUTHOR: lorenzo.zallocco@student.unisi.it*

Abstract

Aim: Social anxiety is a stress condition in which the psychophysiological balance is altered and the body reacts to adapt, through the activation of different neuronal pathways, including the hypothalamic-pituitary axis and the autonomic system.
This study was aimed to highlight the variations of the salivary proteome produced in a sample of students by a social stress condition which simulated an "exam".

Materials and Methods: A group of 34 healthy students (age: 18-30; 14 females) with scores in the Leibowitz Social Anxiety Scale below the cut-off for social phobia, were enrolled in the study. Half of the students (group A) underwent an initial relaxation phase facilitated by an auditory stimulus (sea waves) followed by a simulated oral exam (stress test). The other participants (Group AO) underwent a similar experimental session with the exception that a pleasant olfactory stimulus was diffused in the room. During the whole session, electrocardiogram, and skin conductance (SC) were monitored. Whole saliva samples were collected both at the end of the relaxation phase (T0) and the stress phase (T1), immediately processed and stored at -80°C until use. Salivary proteins (200 μg) were separated by two-dimensional electrophoresis, resulting gels stained with the ruthenium complex (RuBPS), images captured by ImageLAS4010 and analysed with the Same Spot software. The differentially expressed protein spots were trypsin digested and analysed by LC-MS/MS for protein identification. To validate results we performed a western blot analysis of α-Amylase and immunoglobulin-α chain 1 using specific antibodies.

Results: All the participants showed a stress-related reduction of the vagal activity and an increase of the sympathetic one. The proteomic results showed a significant differential expression of 65 spots (T1 vs T0) in the A group and 28 spots (T1 vs T0) in AO group. An increase of α-amylase secretion and a decrease of different kind of immunoglobulin chains and polymeric immunoglobulin receptor were observed in both groups. Moreover, a peculiar difference of expression was observed for calgranulin B, cystatin S/SA, LDHA and 14-3-3 proteins in the A group, only, as a consequence of acute stress.

Conclusion: The proteomic analysis of saliva can be a useful approach to evaluate the rapid responses induced by a social acute stress. Also, our findings indicate that olfactory stimuli differentially modulate the stress-induced proteome changes.
Our results shed new light in the salivary proteome change correlated to variations of psycho-sensory area which are mainly assessed by autonomic nervous systems changes.