ABSTRACT

Background: Temporomandibular disorders (TMDs) often manifest as masticatory muscle pain (myalgia). Nerve growth factor (NGF) and glutamate injection into healthy human masseter muscle induced signs and symptoms mimicking those of TMD myalgia and have therefore been suggested as pain models to study the neurobiological mechanisms of this type of chronic pain conditions. Previous animal studies have demonstrated that certain pain biomarkers are involved in the development of pain and mechanical muscle sensitisation induced by NGF and/or glutamate or by inflammation. Examples of such biomarkers are substance P (SP), N-methyl-D-aspartate receptor (NMDA), and NGF. However, it is unknown if NGF and/or glutamate-induced masseter muscle pain and sensitisation in humans share the same pain-pathways suggested to be involved in animals. Therefore, the main aim of this thesis was to investigate the effect of NGF and/or glutamate on the expression of putative pain biomarkers by nerve fibres within the human masseter muscle.

Materials and methods: The thesis consisted of two experiments involving injection of NGF and/or glutamate into a pain-free masseter muscle. Participants included 60 healthy volunteers (30 in each experiment). For the first experiment, sterile NGF (0.4 ml, 25 µg/ml) was injected into the left masseter muscle (experimental side). On the other hand, in the second experiment, 0.2 ml of sterile glutamate (1.0 M) was injected in a muscle that was pre-treated with NGF. For both experiments, microbiopsies were obtained from the right masseter muscle (contralateral side, i.e., baseline) as well as from the experimental side. Moreover, pain characteristics were assessed before and after the injections. Biopsy sections were analysed via immunohistochemistry, where PGP9.5 was used to identify nerve fibres, while primary antibodies against each substance and their corresponding secondary antibodies were used to identify the putative biomarkers of interest. Sections were visualised with a confocal microscope (Leica TCS SPE).

Results: NGF administration alone did not cause an increase in the frequency of nerve fibre expression ($P>0.05$). In contrast, the combined injection of NGF and glutamate increased the expression of SP alone ($F=13.713$, $P=0.002$), with NR2B ($F=10.599$, $P=0.006$) or with NGF ($F=5.151$, $P=0.040$), and all together ($F=4.774$, $P=0.046$). This increase was also greater in women than in men ($P<0.05$). Pain characteristics correlated positively with the expression of NR2B alone or together with SP by nerve fibres ($P<0.05$). In other words, a greater expression of NR2B by nerve fibres or by putative afferent fibres (expressing SP) was associated with increased pain.

Conclusion: It appears that, in humans, muscle pain occurrence and sensitisation depend significantly on peripheral presumptive afferent fibres expressing NMDA-receptors and NGF. It also appears that the expression by these afferent fibres account for variation in pain characteristics between males and females in the context of experimental induction of myalgia. Nevertheless, additional research must be conducted to determine whether such findings are related to TMD myalgia mechanisms.