Pain

Assessment of the Rabbit Temporomandibular Joint after Unilateral Dental Splint Placement

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Disclosures:

Introduction: The development of effective interventions for the treatment of temporomandibular joint (TMJ) disorders (TMD) has been hindered by the dearth of data linking the changes observed in preclinical models to the development and manifestation of the pathology of the human state. For example, while it is clear that a subpopulation of TMD patients suffer from degeneration of the joint, previous histological analyses have provided little insight into the nature and extent of the degeneration, its relationship to the natural history (i.e., is there a point in which the degeneration becomes irreversible) of the disorder, let alone the relationship between the extent of degeneration and the manifestation of pain. To begin to address these issues, we have developed a reversible malocclusion model of TMD in the rabbit that enables control over the timing between the onset and resolution of the malocclusion. The objective of the present study was to determine the effects of altered occlusion on the presence of pain in a rabbit TMJ.

Methods: TMJ degeneration was induced with a metal dental splint placed unilaterally over the occlusal surface of one bridge of molars in a skeletally mature female New Zealand white rabbits. All work was approved by and performed in accordance with guidelines of the University of Pittsburgh IACUC. Behavioral testing of the mechanical nociceptive threshold was assessed with an electronic von Frey Hair (VFH) applied via a protocol similar to that developed for assessing TMJ sensitivity in the rat (1). A positive response was recorded when the rabbit flinched or pulled its head away from the application of the stimulus. Bilateral withdrawal frequency data was compared over time and between rabbits. Baseline data was collected for at least three days prior to the splint procedure. Data was collected twice during the first week after the splinting procedure, and once a week thereafter for a total of 6 weeks, on 9 splinted and 3 normal control rabbits. As a complimentary endpoint to changes in mechanical sensitivity, the presence of the immediate early gene, C-fos, was assessed in the brainstem. A standard immunohistochemical approach was employed to assess c-Fos protein on floating sections of the brainstem as previously described (2). At least three random sections per rabbit were analyzed and the number of fos positive cells were counted in two standardized regions of each slide. Counts on each slide were totaled and the cell counts from each rabbit averaged. 3 splinted rabbits were compared to 6 control rabbits. Patch clamp electrophysiological analysis of acutely dissociated retrogradely labeled TMJ afferents was used as a third measure of altered nociception in the splinted rabbits. Trigeminal ganglia from 3 control and 3 splinted rabbits, 6 weeks after splint placement were harvested and dissociated for study in vitro with protocols developed for rats and mice that were adapted to the rabbit (3). Dil labeled TMJ neurons were readily identifiable under epifluorescence illumination. Whole cell patch clamp recording was used to assess the excitability of these neurons. A standardized series of protocols was employed to assess the cell capacitance, action potential threshold, properties of the action potential waveform (overshoot, duration, after hyperpolarization magnitude, and rate of decay of the after hyperpolarization), rheobase, and the response to suprathreshold current injection. T-tests were used with significance set at p<0.05 to compare the variable values for c-fos cell counts and electrophysiology in splinted rabbits compared to control rabbits.

Results: The behavioral reactions of the splinted rabbits were varied. Some rabbits never responded at all, some responded for a time then did not respond, some responded the whole time, and some responded after a few weeks. The contralateral side appeared to be more affected than the splinted side with more rabbits reacting to the stimulus: Of the 9 splinted rabbits, 4 rabbits responded to stimuli applied to the contralateral side and 2 responded to the ipsilateral side at 6 weeks. None of the control rabbits showed responses to the VFH stimulus over the course of 6 weeks. The c-fos cell counts were statistically higher for the splinted animals after 6 weeks as compared to control (86±8 cells/section and 64±15 cells/section, p<0.05). One control brainstem was removed as an outlier from the results because the average number of cells per slide was over three standard deviations away from the overall average without the data point. For the patch clamp tests, the greatest change was seen in rheobase or the amount of current required to evoke an action potential. In contrast to the behavioral and c-fos data, rheobase (364±80 pA) and action potential threshold (-31.2±2.0 mV) were higher in rabbits with splints compared to rheobase (99±22 pA) and action potential threshold (-38.0±2.0 mV) in the control rabbits. However, there was a trend toward an increase in the
number of action potentials evoked in response to suprathreshold stimuli in neurons from splinted rabbits. The action potential duration decreased significantly from 11.5±1.7 ms to 6.1±0.9 ms and the after hyperpolarization decay time constant also significantly decreased from 16.7±1.5 to 13.4±1.2 ms in splinted animals.

Discussion: Behavioral and c-Fos data were consistent with an increase in nociceptive signaling in response to splinting and the degeneration of the TMJ. The variability in the behavioral changes observed in the rabbit model are comparable to the manifesting of TMD pain in humans were signs and symptoms of TMD (joint clicking and locking) are far more prevalent than TMD pain. This suggests that the rabbit model may be useful for identification of mechanisms that contribute to this variability. These changes in excitability and action potential waveform are consistent with compensatory changes TMJ afferents for an overall increase in afferent drive associated with joint degeneration. These changes are in contrast to clear increases in TMJ afferent excitability observed in other preclinical models of TMD, and raise the possibility that the failure to engage such compensatory mechanisms may contribute to greater pain and disability observed in a subpopulations of TMD patients.

Significance: This study was a first step towards a better understanding of a TMJ degeneration process and gaining a better understanding of how/when degeneration becomes a persistent pain condition.

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