Published in final edited form as:

Eur J Pain. 2013 May ; 17(5): 649–653. doi:10.1002/j.1532-2149.2012.00230.x.

“Obesity increases nociceptive activation of the trigeminal system”

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Abstract

Background—Obesity is a risk factor associated with several pain syndromes. However, the mechanisms underlying the association between obesity and pain are not known. The aim of this study is to test the hypothesis that obesity enhances neuronal responses to nociceptive stimulation within the trigeminal nucleus caudalis (TNC).

Methods—Male and female C57BL/6J mice were fed a high fat or regular diet from the time of weaning until 20 weeks of age. We then quantified neuronal activation by measuring Fos immunoreactivity within the TNC in response to a facial injection of a low dose of capsaicin (1 ug/10ul).

Results—We found that 0.01% capsaicin did not significantly increase Fos immunoreactivity in control mice fed a regular diet. In contrast, this low dose of capsaicin caused a 3.3-fold increase in Fos in the TNC in obese mice ($P < 0.001$).

Conclusions—These results support the hypothesis that diet-induced obesity in mice enhances nociceptive processing within the TNC. Diet induced obesity may be a useful model for mechanistic studies. Future studies will improve our understanding of how obesity may contribute to trigeminal pain by sensitizing the trigeminal nociceptive system.

INTRODUCTION

Obesity represents a serious public health problem of epidemic proportions (Flegal et al., 2012). Obesity is also well-recognized risk factor associated with several pain syndromes, including osteoarthritis, low back pain, fibromyalgia and headache (Hitt et al., 2007; D'Arcy, 2011; Marcus, 2004). It is unclear what mechanisms underlie this association.

We are particularly interested in the effects of obesity on trigeminal nociception, which is implicated in the pathophysiology of head pain (Goadsby et al., 2002). While excess weight could have a detrimental effect on weight-bearing structures, such as the back and lower

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Conflict of Interest Statement

The authors declare that there are not conflicts of interest.

Authors' contributions

H.R. performed the experiments and assisted with data analysis and manuscript preparation. A.L and J.D. assisted with all the experiments. A.R. planned and directed the experiments, analysed the data and wrote the paper. All authors discussed the results and commented on the manuscript.

body joints, this would not explain the connection between obesity and pain affecting sites that are not weight-bearing, such as head pain. The study of pain in the trigeminal region also provides an advantage over extra-cephalic regions because the trigeminal nerve is typically spared by diabetic peripheral neuropathy commonly associated with obesity (Arap et al., 2010).

We hypothesized that obesity facilitates the development of trigeminal sensitization. We used Fos immunoreactivity to quantify neuronal activation. Fos is the product of the immediate-early gene *c-fos* and is a well-accepted marker of pain-related neuronal activation in the trigeminal nucleus caudalis (TNC) and spinal cord dorsal horn (Hunt et al., 1987; Nozaki et al., 1992; Harris, 1998). Activation of meningeal nociceptors with capsaicin leads to Fos expression in the TNC and has been used to test the efficacy of analgesics in animal models of headache (Nozaki et al., 1992; Mitsikostas et al., 2002; Sixt et al., 2009).

To test our hypothesis we used a mouse model of diet-induced obesity (Hariri and Thibault, 2010) and quantified neuronal activation in the TNC in response to a facial injection of capsaicin (Pelissier et al., 2002). Chemical stimulation of the face is known to activate the TNC and evokes nocifensive behavior in rats and mice (Pelissier et al., 2002; Alvarez et al., 2003; Luccarini et al., 2006). Capsaicin was injected in an area of the face that is represented in the same brainstem region as dural blood vessels (Strassman et al., 1994), which are involved in head pain (Burstein et al., 1998). Capsaicin is known to induce afferent-driven central sensitization, in part by evoking the release of the calcitonin gene related peptide (CGRP) (Latremoliere and Woolf, 2009). Thus it is a reasonable tool to study the effects of obesity on sensitization of the trigeminal system.

In this study, we demonstrate that obese animals have increased neuronal activation in the TNC in response to a low dose of capsaicin. This suggests that sensitization of the trigeminal system could underlie the aggravation of trigeminal pain disorders by obesity.

METHODS

(online only)

RESULTS

High fat diet induces significant weight gain in mice

At the time of testing, males on high fat diet weighed 39.4 ± 1.8 g, 20% more than control males ($P < 0.005$) and females weighed 24.3 ± 0.8 g, 11% more than their regular diet counterparts ($P < 0.05$) (Fig S1 a). Males and females on high fat diet gained 12.9 and 4.3 g more, respectively, than those on regular diet ($P < 0.0001$ and $P < 0.0005$, respectively) (Fig S1 b).

Diet induced obesity in mice increases Fos expression in the TNC in response to capsaicin

To test our hypothesis that obesity promotes sensitization of the trigeminal system, we used capsaicin-induced Fos immunoreactivity as a marker for neuronal activation in the TNC

after noxious stimulation. We first determined that 0.1% capsaicin significantly increased Fos immunoreactivity in the TNC in a control group of mice (data not shown). Since our goal was to test whether a mild stimulus would cause higher neuronal activation in the TNC of obese animals, we decided to test a lower dose of 0.01% capsaicin.

We found that 0.01% capsaicin did not evoke a significant increase in Fos expression relative to vehicle treatment in control mice (Fig. S2 b,d, Fig. 1). In contrast, this dose of capsaicin caused a significant increase in Fos immunoreactivity ipsilateral to the injection in mice maintained on the high fat diet (Fig. S2 a,c, Fig. 1). Capsaicin-induced Fos expression was predominantly limited to the superficial laminae of the TNC (Fig. S2). There was no difference between male (Fig. 1 a) and female (Fig. 1 b) mice thus data from both sexes were pooled to reduce the number of animals used (Fig. 1 c,d). Two-way ANOVA revealed a significant treatment effect ($P = 0.0001$) and a significant interaction between diet and treatment ($P = 0.01$) (Fig. 1 c). Total number of Fos-IR cells in response to 0.01% capsaicin was significantly higher in the high fat diet group compared with the regular diet group ($P < 0.01$) (Fig. 1 c). Furthermore, 0.01% capsaicin did not have a significant effect on the regular diet group compared with vehicle, but it caused a 3.3-fold increase in Fos expression in the high fat diet group ($P < 0.001$) (Fig. 1 c).

There was no increased Fos immunoreactivity in the TNC contralateral to the injected side in any of the groups. The total number of Fos-IR neurons contralateral to the injection ranged between 50 and 110, below the level of activation seen ipsilaterally with vehicle injection.

DISCUSSION AND CONCLUSIONS

This is the first study to show that diet-induced obesity is associated with increased neuronal activation in the TNC following stimulation with capsaicin. Importantly, the low dose of capsaicin used increased Fos-IR in the TNC of obese mice but had no significant effect in lean mice. This suggests that obesity may contribute to trigeminal sensitization. Whether this represents a decrease in the threshold necessary to activate this system, an exaggerated response to stimuli, or both, remains to be determined. These findings provide early support for our hypothesis that diet-induced obesity is associated with enhanced activation of the trigeminal nociceptive pathway.

There are no well-established mechanisms by which diet-induced obesity could enhance trigeminal reactivity to capsaicin. However, capsaicin activates TRPV1 channels on peripheral nociceptors (Caterina et al., 1997), which are known to be sensitized by inflammatory mediators, such as tumor necrosis factor alpha (TNF alpha) (Khan et al., 2008; Hagenacker et al., 2010). TNF alpha is elevated in the plasma of obese humans (Dandona et al., 1998) and mice (Flores et al., 2012). Therefore, TNF alpha may be one of many possible contributors to the differences we found between diet groups. Enhanced nociceptor activity in obese animals could induce activity-dependent central sensitization in the TNC. On the other hand, diet-induced obesity has also been associated with increased reactivity of microglia and astrocytes in the hippocampus (Puig et al., 2012). If this also occurs in the TNC it could influence activation and Fos expression in second order neurons. Future

studies will examine the role of peripheral and central inflammation in diet-induced obesity, using molecular and electrophysiological techniques.

In this study we have combined the capsaicin nociception model (Pelissier et al., 2002) with the well-established mouse model of diet-induced obesity (Hariri and Thibault, 2010). We chose diet-induced obesity rather than a genetic model because diet-induced obesity is likely more representative of the most prevalent form of human obesity. However, some caveats need to be considered, such as the possibility that our effects are the result of the high fat diet itself rather than adiposity or an interaction between the two. Dietary composition influences inflammatory and cell signaling processes (Calder, 2011) which could interact with nociception. In the future we plan to study the effects of obesity on the trigeminal nociceptive pathway in other rodent models of obesity. This will determine if our findings are exclusive to high fat diet-induced obesity or an effect of the obese state regardless of the etiology.

One of the strengths of this model is that it allows us to investigate in a controlled manner the effect of diet-induced obesity on a specific population of neurons in the brainstem that have been implicated in trigeminal pain disorders. Future mechanistic studies will assess the role of different molecular mediators in the association between pain and obesity. We have focused on trigeminal pain, but similar studies in the dorsal horn of the spinal cord may provide useful information about the effects of obesity on other pain disorders.

In summary, this study demonstrates that diet-induced obesity results in increased nociceptive activation of the TNC. Whether this occurs through central and/or peripheral mechanisms remains unknown. We acknowledge that the association between pain and obesity is complex and multiple factors are likely involved. Nevertheless, these findings suggest that obesity may promote sensitization of the trigeminal nociceptive pathway to aggravate trigeminal pain disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank Dr Andrew F. Russo, Dr Donna L. Hammond, and the members of the University of Iowa Pain Research Group for their invaluable suggestions. We also thank Dr Alicia Olivier for her help with the microphotographs and Dr Andrew Ahn for his suggestions during the manuscript preparation.

Funding Sources

This work was supported by the National Institutes of Health (National Institute of Neurological Disorders and Stroke) [K08 – NS066087-01]; the University of Iowa Clinical and Translational Science Award [UL1 RR024979]; and the University of Iowa Pain Research Program Training Grant [T32NS045549]. The funding sources were not involved in study design, data collection/analysis, manuscript preparation, or publication decision.

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what's already known about this topic?

Obesity is associated with different pain syndromes. Specifically, it has been identified as a risk factor for migraine aggravation, leading to more frequent and severe migraine attacks. The mechanisms underlying this association remain unknown.

what does this study add?

We have identified obesity-induced changes in the trigeminal nucleus caudalis. This provides a potential anatomical substrate for the aggravation of trigeminal pain disorders by obesity and a model for future mechanistic studies.

Figure 1. Number of Fos-IR positive cells in the ipsilateral TNC in response to 0.01% capsaicin or vehicle in mice fed high fat or regular diet (a). Total number of Fos-IR cells in males. (b). Total number of Fos-IR cells in females. (c). Total number of Fos-IR cells in males and females (pooled data). (d). Distribution of Fos-IR cells throughout the TNC in males and females (pooled data). Data are mean $\pm$ S.E.M. * P < 0.01 ** P < 0.001.