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Experimental hypoglycemia is a human model of stress-induced hyperalgesia

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Keywords

Hypoglycemia; Hyperalgesia; Cytokines

1. Introduction

There is a complex relationship between stress and pain. Paradoxically, intense stress may both suppress and enhance pain sensitivity – phenomena known respectively as stress-induced analgesia and stress-induced hyperalgesia [39]. The physiological mechanisms and neural circuitry that underlie stress-induced analgesia are well-established [35;39], although these are not as well elucidated for stress-induced hyperalgesia [24;29].

Hypoglycemia is a physiological stress that leads to activation of the hypothalamic-pituitary adrenal axis and sympatho-adrenal system. The resulting increases in circulating levels of glucocorticoids and catecholamines serve as a counterregulatory defense against a decrease in blood glucose. Hypoglycemia also evokes increases in proinflammatory cytokines [14;28]. These are core features of the stress-response[26] and are implicated in the mediation and modulation of pain and in animal models of stress induced hyperalgesia [13;21;22].

An episode of hypoglycemia results in neurobiological changes that may persist for days. Antecedent hypoglycemia impairs the counterregulatory metabolic and autonomic responses to subsequent hypoglycemia thus increasing susceptibility to a vicious cycle of recurrent hypoglycemia [11]. We have shown that antecedent hypoglycemia also impairs the autonomic response to a non-hypoglycemic autonomic stress, suggesting that the effects of prior hypoglycemia on the response to provocations are not specific to a hypoglycemic stress but is more generalized [1]. The effects of antecedent hypoglycemia on pain processing are not known.

There are several animal models of stress-induced hyperalgesia [13;22;31;37]. There are no human models. We hypothesized that the stress of antecedent experimental hypoglycemia would result in a post-hypoglycemic state characterized by enhanced pain sensitivity consistent with stress-induced hyperalgesia.

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2. Research Design and Methods

2.1 Study population

Subjects were recruited through local advertisements and electronic postings. All participants were screened with a complete medical history, physical examination, complete blood count, electrolytes, BUN, creatinine, glucose, liver enzymes, hemoglobin A1c, and electrocardiogram. Subjects with abnormal blood or urine chemistries, diabetes, body mass index ≥ 30 kg/m², reported use of steroids within the last year, post menopausal status or current medical problems were excluded. All medications except for acetaminophen were stopped two weeks prior to and during all studies. Subjects with a family history of diabetes were not included in the study. Twenty healthy men and women, aged 18–50 years, participated in two 3-day visits separated in time by 1 to 3 months. On days 1 and 3 of each study visit, subjects underwent quantitative sensory testing of the left distal leg. On day 2, subjects were randomized to undergo either two 2-hour hypoglycemic-hyperinsulinemic clamp studies (target glucose 2.8 mmol/l) or two 2-hour euglycemic-hyperinsulinemic clamp studies (target glucose 5.0 mmol/l), as previously described [1]. Clamp studies began at 9am and 1pm. Those subjects that experienced hypoglycemia (50 mg/dl) during the initial visit were switched to euglycemia (90 mg/dl) during the second visit, and vice versa, as shown in Figure 1. All study procedures were approved by the Institutional Review Boards at Beth Israel Deaconess Hospital and Brigham and Women's Hospital and informed written consent was obtained from all subjects.

2.2 Quantitative sensory testing

Quantitative sensory testing to determine heat-detection, cold-detection, heat-pain and cold-pain thresholds was based on an average of 3 trials of each stimulus using the method of limits (Medoc CoVAS, Medoc Inc). Thermal temporal summation involved 10 consecutive cycles of pulsed heat (duration 0.5 seconds) with a baseline temperature of 40°C and maximum of 49°C with an inter-stimulus frequency of 0.33 Hz; subjects reported pain rated on a 0–100 scale (100 maximum pain) after the first and last stimuli, and reported the maximal perceived pain during the test. Subjects unable to complete all 10 thermal stimuli were assigned the maximum pain rating (100). Subject testing was performed on day 1 and 3 of each test admission by the same technician blinded to whether subject was participating in a euglycemic or hypoglycemic protocol.

2.3 Hormone and cytokine testing

Blood was drawn through an indwelling intravenous catheter and assayed for cortisol, adrenocorticotrophic hormone [ACTH], interleukin-6, growth hormone and insulin levels every 15–30 minutes during the euglycemic and hypoglycemic clamp studies [1]. Plasma catecholamines (epinephrine and norepinephrine) were measured every 15–30 minutes in 7 out of the 16 individuals. Baseline hormone and cytokine values were drawn 15 minutes before hypoglycemia or euglycemia. The peak value for hormone and cytokine testing was chosen as the highest of 3 samples drawn between 75 and 135 minutes of hypoglycemia or euglycemia which occurred from 10:30–11:30 am for morning clamps and 2:30–3:30 pm for afternoon clamps.

2.4 Laboratory Procedures

Cortisol was measured by Access Cortisol Immunoassay (Beckman Coulter, Chaska, MN) with a detection limit of 0.4 µg/dl. IL-6 was measured using ELISA (R&D Systems, Minneapolis, MN) with a detection limit of 0.039 pg/ml. Insulin was measured by AccessHigh Sensitive Insulin Immunoassay (Beckman Coulter, Chaska, MN) with a detection limit of 0.03 uIU/mL. ACTH was measured by immunoradiometric Assay

(DiaSorin, Stillwater, MN) with a detection limit of 1.5 pg/mL. Epinephrine was measured using RIA (Immuno Biological Laboratories, Minneapolis, MN) with a detection limit of 1 pg/mL. Norepinephrine was measured using RIA (Immuno Biological Laboratories, Minneapolis, MN) with a detection limit of 9 pg/mL. Growth hormone was measured by RIA (Beckman Access, Chaska, MN) with a detection limit of <0.04 ng/mL.

2.5 Statistical analysis

Data in text and figures are reported as mean $\pm$ standard error of the mean (SEM). The effects of treatment (euglycemia versus hypoglycemia) and time (Day 1 versus Day 3) were analyzed by repeated measures ANOVA, significance set at $P < 0.05$. For tests performed only once per testing visit, results were analyzed by paired t-tests. For temporal summation, the difference and the ratio between the initial and maximal pain score (wind-up difference and wind-up ratio) [32] were calculated for each test day, with change secondary to euglycemia/hypoglycemia reported. The number of subjects in each group that were unable to complete the temporal summation protocol was compared by Fisher's exact test. Pearson correlations between hormone/cytokine levels and sensory pain scores are reported.

3. Results

3.1 Demographics

Sixteen of 20 subjects participating in the euglycemic hyperinsulinemic and hypoglycemic hyperinsulinemic clamp protocol, had quantitative sensory testing performed before and after each clamp. Four subjects were unable to complete both testing protocols; two subjects withdrew after completing the euglycemia protocol, one after completing the hypoglycemia protocol and one withdrew after screening but before testing. Of the 16 participants included in the analysis, the average age was 29.3 years (range 19–46 years), six were female and ten male, BMI was 23.8 ± 0.7 kg/m², supine systolic blood pressure was 105.5 ± 2.4 mmHg, diastolic blood pressure was 67.1 ± 1.9 mmHg, and resting heart rate was 63.4 ± 1.6 beats per minute.

3.2 Quantitative Sensory Testing

No significant differences were noted in baseline test scores (day 1 of the hypoglycemia vs. day 1 of the euglycemia clamp protocol). There was no effect of treatment order on baseline measurements. Thresholds for cold detection assessed on day 3 were similar between treatment groups. Day 3 cold-pain detection threshold was increased (increased thermal pain sensitivity) after hypoglycemia compared with after euglycemia ($6.3 \pm 1.3^\circ\text{C}$ after hypoglycemia vs. $3.6 \pm 1.3^\circ\text{C}$ after euglycemia, $P < 0.01$) (Fig. 2). Similarly, the heat detection threshold assessed on day 3 after hypoglycemia was reduced (increased thermal sensitivity) compared with the day 3 threshold after euglycemia ($33.3 \pm 0.2^\circ\text{C}$ after hypoglycemia vs. $33.6 \pm 0.2^\circ\text{C}$ after euglycemia, $P < 0.005$) (Fig. 2). In addition, the day 3 heat-pain detection threshold after hypoglycemia was reduced (increased thermal pain sensitivity) compared with the threshold after euglycemia ($45.6 \pm 0.7^\circ\text{C}$ after hypoglycemia vs. $47.1 \pm 0.6^\circ\text{C}$ after euglycemia, $P < 0.005$) (Fig. 2).

3.3 Temporal Summation

There were no significant differences noted in pain perception during the temporal summation test protocol on day 1 prior to hypoglycemia as compared with testing obtained on day 1 prior to euglycemia. There was no effect of treatment order on these measurements. In contrast, the pain scores for the 10th stimulus and for maximal pain during the protocol were increased ($P < 0.005$) on day 3 after exposure to hypoglycemia as compared with the scores on day 3 after euglycemia (Fig. 3). Further, windup pain (the difference between 1st and 10th stimuli pain score) was increased by prior exposure to hypoglycemia as compared

with prior euglycemia (13.8 ± 8.4 points after hypoglycemia (day 3) versus 5.7 ± 1.9 points after euglycemia (day 3), $P < 0.001$); equivalent to a windup ratio of 2.05 ± 0.02 (hypoglycemia) versus 1.71 ± 0.02 (euglycemia), $P < 0.001$. Finally, one subject was unable to tolerate the temporal summation stimulation protocol after antecedent euglycemia, while four subjects were unable to tolerate the protocol after antecedent hypoglycemia.

3.4 Hormone and cytokine levels

There were significant differences in the maximum hormone and cytokine levels released during hypoglycemia compared to euglycemia, as shown in Table 1. Levels of ACTH, IL-6, cortisol, growth hormone, epinephrine and norepinephrine were significantly higher during hypoglycemia compared to euglycemia and to baseline conditions. Insulin levels were the same in both groups, and significantly higher than during baseline conditions. There was a positive correlation between the change in interleukin-6 levels during the hypoglycemic clamp and temporal summation pain on day 3 of testing ($r = 0.49$, $P < 0.01$, Fig. 4A). Changes in norepinephrine levels ($r = -0.53$, $P < 0.01$) and epinephrine levels ($r = -0.60$, $P < 0.01$, Fig. 4B) during the hypoglycemic clamp were inversely correlated with maximum pain during temporal summation on day 3 of testing. No other consistent, significant correlations between peak hormone levels and results of quantitative sensory testing or thermal temporal summation were noted.

4. Discussion

4.1 Major findings

The major findings of this study are that in comparison to a euglycemic, hyperinsulinemic clamp, experimental hypoglycemia evoked by a hyperinsulinemic clamp results in (1) enhanced pain sensitivity to a cold stimulus; (2) enhanced pain sensitivity to a warm stimulus; (3) enhanced temporal summation to repeated heat pain stimuli. These data suggest that antecedent hypoglycemia gives rise to a state of enhanced pain sensitivity that is consistent with stress-induced hyperalgesia.

4.2 Neurobiology of stress induced hyperalgesia

Stress may result in both pain suppression and pain enhancement. In contrast to stress induced hypoalgesia, the neurobiological basis for stress induced hyperalgesia is not well-established. Experimental studies in rodents suggest that stimulation of the dorsomedial nucleus of the hypothalamus activates pain facilitating neurons in the rostral ventromedial medulla. This neural circuitry plays a pivotal role in the stress response [24]. The neuroanatomical circuitry and molecular mechanisms of the counterregulatory response to hypoglycemia overlap with these rodent models of stress induced hyperalgesia.

4.3 Neurobiology of the counterregulatory response to hypoglycemia

Hypoglycemia activates the sympatho-adrenal, sympathetic and parasympathetic divisions of the autonomic nervous system and leads to increases in circulating levels of epinephrine, glucagon, norepinephrine, ACTH, cortisol, pancreatic polypeptide, and growth hormone. Hypoglycemia also evokes increases in proinflammatory cytokines including interleukin-6, interleukin-1 β , and tumor necrosis factor alpha [14;28]. The brain regions that play a critical role in the detection of hypoglycemia and activation of the stress response localize to the ventromedial hypothalamus – in particular the ventromedial and arcuate nuclei [6;7] – and brainstem [30].

4.4 Mechanisms of stress induced hyperalgesia

Chronic pain disorders such as migraine, fibromyalgia, and irritable bowel syndrome may be provoked or perpetuated by stress through unknown mechanisms. Animal models of stress-induced hyperalgesia have implicated cytokines [21–23], glucocorticoids [21;22] and catecholamines [21;22] and suggest that co-activation of immune-mediators, the hypothalamic-pituitary axis and the sympatho-adrenal axis [13] in varying combination may produce hyperalgesia. The features of and factors involved in these preclinical models of stress-induced hyperalgesia appear dependent upon the magnitude, duration and temporal features of the specific evocative stress paradigm [9]. The present data suggest that the biological features of experimental hypoglycemia in humans, which include transient elevations in catecholamines, glucocorticoids and cytokines, induce a hyperalgesic state consistent with stress-induced hyperalgesia.

In the present study, no hormone or cytokine was a consistent predictor of hyperalgesia or altered pain processing. While it is tempting to attribute the positive correlation of interleukin-6 and negative correlations of the catecholamines (epinephrine and norepinephrine) with temporal summation pain with mechanism, i.e., interleukin-6 promotes hyperalgesia while catecholamines inhibits hyperalgesia, this should be undertaken with caution; the study sample was small and most correlations were non-significant. The absence of consistent correlations with altered pain sensitivity or pain processing may be, in part, a consequence of the experimental paradigm in which many of the hormone and cytokine levels reach high physiologic levels during hypoglycemia and may not allow assessment of a “dose-response” to cytokine and/or catecholamine increases. A protocol with a range of hormone and cytokine responses may shed more light on the relative contributions of individual components of the stress response.

4.5 Hypoglycemia associated autonomic failure, stress and hyperalgesia

It is well-established from studies in healthy and diabetic subjects that recent exposure to hypoglycemia reduces the counterregulatory hormone and autonomic nervous system responses to subsequent hypoglycemia. The spectrum of reduced counterregulatory hormone responses and decreased symptom perception of hypoglycemia due to decreased autonomic nervous system activation following recent antecedent hypoglycemia has been termed “hypoglycemia induced autonomic failure” [11].

Hypoglycemia associated autonomic failure or dysfunction leads to decreased ability to sense hypoglycemia and to restore euglycemia, resulting in increased susceptibility to repeated episodes of hypoglycemia in insulin-treated diabetes [12]. Furthermore, we have recently shown that antecedent hypoglycemia impairs the autonomic response to *non-hypoglycemic stresses* such as simulated orthostatic stress and pharmacologically induced transient hypotension, thereby demonstrating that the effects of antecedent hypoglycemia on the autonomic nervous system are more generalized and not specific to subsequent hypoglycemic stimuli [1]. The present data suggest that the effects of antecedent hypoglycemia may be extended to alterations in pain processing.

The corticotropin-releasing factor receptors (CRFR 1 and CRFR 2) and their ligands, the corticotropin-releasing factor (CRF) family of neuropeptides (CRF and urocortin 1–3) are possible mediators of both the alterations in pain sensitivity and the attenuated counterregulatory response to hypoglycemia. The CRF/CRFR pathway mediates the hypothalamic-pituitary-adrenal axis and behavioral responses to multiple stressors [5]. Rodent studies have implicated this system in modulation of counterregulation and pain although the responses vary depending upon the experimental paradigm. For example, in vivo application of urocortin I, an endogenous CRFR2 agonist to the ventromedial

hypothalamus suppressed the counterregulatory response to hypoglycemia while application of CRF, a predominantly CRFR1 agonist, amplified the response [10;25]. Whereas, peripheral delivery of the CRFR1 ligand, CRF, (but not ACTH or corticosterone) attenuates the counterregulatory response to hypoglycemia [15]. Furthermore, there is evidence that CRFR and CRF also are involved in pain processing and the transmission of nociceptive information although the role of this factor and its receptors in nociception is not fully elucidated and appears dependent on the site, dose and receptor family activated or inhibited [18–20]. The experimental hypoglycemic model may prove useful in enhancing our understanding of the role of the CRF and the CRFR pathways in stress, pain, down-regulation of the counterregulatory response to subsequent hypoglycemia, and hypoglycemia associated autonomic failure.

4.6 Implications

The present data show greater temporal summation of repeated thermal stimuli in the post-hypoglycemic state compared to the euglycemic state. This finding, which is thought to represent the subjective equivalent of the progressive response increment in second order spinal neurons, suggest that central mechanisms, at least in part play a role in this response [27;38]. This observation suggests that hypoglycemia, possibly via the increases in stress hormones and cytokines provoked by hypoglycemia, disrupt inhibitory control mechanisms or enhanced descending facilitation leading to central sensitization. Further study is warranted to determine if repeated exposure to hypoglycemia leads to chronic changes in pain sensing in individuals with diabetes.

In addition, these findings may have implications for understanding the functional pain disorders such as fibromyalgia, interstitial cystitis and irritable bowel syndrome which have a well established association with stress and may, in some patients, represent, central sensitization [3;38]. Individuals with fibromyalgia also exhibit blunted ACTH and epinephrine responses to hypoglycemia induced by the hypoglycemic hyperinsulinemic clamp technique [2]. Thus, exposure to antecedent hypoglycemia in healthy subjects induces two characteristics observed in patients with fibromyalgia -- impaired counterregulatory response to hypoglycemia [2] and enhanced temporal summation [27;34].

Several studies have reported enhanced temporal summation of thermal and electrical stimuli in patients with fibromyalgia [27] providing the basis for the hypothesis that central sensitization underlies the wide-spread pain in this disorder [17]. The present data may thus provides a human model for examining underlying mechanisms and hypothesis-based testing of pharmacological interventions in these disorders associated with chronic widespread pain including fibromyalgia and related disorders.

These data may also have implications for treatment induced neuropathy [16] (also known as insulin neuritis [8]). A disorder characterized by the acute onset of severe, reversible distal and/or generalized severe neuropathic pain with hyperalgesia and allodynia that occurs following a rapid, substantial improvement of glycemic control. The pathophysiological mechanisms of this disorder are unknown, however, the present data raise the possibility that stress response systems, such as the catecholamines, glucocorticoids and cytokines, activated by hypoglycemia associated with aggressive glycemic control, may be implicated in this disorder. In this context, it is of interest that elevated cytokine levels, including interleukin-1 β , interleukin-6 and tumor necrosis factor- α have been associated with painful neuropathy [4;33;36]. These cytokines are all elevated by experimental hypoglycemia [14;28].

4.7 Future Directions

In summary, we report a human model of stress induced hyperalgesia provoked by experimental hypoglycemia. This model could provide a useful framework for hypothesis testing and targeted, mechanism-based pharmacological interventions to delineate the molecular basis of hyperalgesia and pain susceptibility.

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References

1. Adler GK, Bonyhay I, Failing H, Waring E, Dotson S, Freeman R. Antecedent hypoglycemia impairs autonomic cardiovascular function - implications for rigorous glycemic control. *Diabetes*. 2009; 58:360–366. [PubMed: 19056608]
2. Adler GK, Kinsley BT, Hurwitz S, Mossey CJ, Goldenberg DL. Reduced hypothalamic-pituitary and sympathoadrenal responses to hypoglycemia in women with fibromyalgia syndrome. *Am J Med*. 1999; 106:534–543. [PubMed: 10335725]
3. Arendt-Nielsen L, Graven-Nielsen T. Central sensitization in fibromyalgia and other musculoskeletal disorders. *Curr Pain Headache Rep*. 2003; 7:355–361. [PubMed: 12946288]
4. Backonja MM, Coe CL, Muller DA, Schell K. Altered cytokine levels in the blood and cerebrospinal fluid of chronic pain patients. *J Neuroimmunol*. 2008; 195:157–163. [PubMed: 18325600]
5. Bale TL, Vale WW. CRF and CRF receptors: role in stress responsivity and other behaviors. *Annu Rev Pharmacol Toxicol*. 2004; 44:525–557. [PubMed: 14744257]
6. Borg WP, During MJ, Sherwin RS, Borg MA, Brines ML, Shulman GI. Ventromedial hypothalamic lesions in rats suppress counterregulatory responses to hypoglycemia. *J Clin Invest*. 1994; 93:1677–1682. [PubMed: 8163668]
7. Borg WP, Sherwin RS, During MJ, Borg MA, Shulman GI. Local ventromedial hypothalamus glucopenia triggers counterregulatory hormone release. *Diabetes*. 1995; 44:180–184. [PubMed: 7859938]
8. Caravati CM. Insulin neuritis: a case report. *Va Med Monthly*. 1933; 59:745–746.
9. Chen X, Green PG, Levine JD. Stress enhances muscle nociceptor activity in the rat. *Neuroscience*. 2011; 185:166–173. [PubMed: 21513773]
10. Cheng H, Zhou L, Zhu W, Wang A, Tang C, Chan O, Sherwin RS, McCrimmon RJ. Type 1 corticotropin-releasing factor receptors in the ventromedial hypothalamus promote hypoglycemia-induced hormonal counterregulation. *Am J Physiol Endocrinol Metab*. 2007; 293:E705–E712. [PubMed: 17578887]
11. Cryer PE. Mechanisms of hypoglycemia-associated autonomic failure and its component syndromes in diabetes. *Diabetes*. 2005; 54:3592–3601. [PubMed: 16306382]
12. Cryer PE. The barrier of hypoglycemia in diabetes. *Diabetes*. 2008; 57:3169–3176. [PubMed: 19033403]
13. Donello JE, Guan Y, Tian M, Cheevers CV, Alcantara M, Cabrera S, Raja SN, Gil DW. A peripheral adrenoceptor-mediated sympathetic mechanism can transform stress-induced analgesia into hyperalgesia. *Anesthesiology*. 2011; 114:1403–1416. [PubMed: 21540738]
14. Dotson S, Freeman R, Failing HJ, Adler GK. Hypoglycemia increases serum interleukin-6 levels in healthy men and women. *Diabetes Care*. 2008; 31:1222–1223. [PubMed: 18332163]
15. Flanagan DE, Keshavarz T, Evans ML, Flanagan S, Fan X, Jacob RJ, Sherwin RS. Role of corticotrophin-releasing hormone in the impairment of counterregulatory responses to hypoglycemia. *Diabetes*. 2003; 52:605–613. [PubMed: 12606499]
16. Gibbons CH, Freeman R. Treatment-induced diabetic neuropathy: a reversible painful autonomic neuropathy. *Ann Neurol*. 2010; 67:534–541. [PubMed: 20437589]

17. Gracely RH, Petzke F, Wolf JM, Clauw DJ. Functional magnetic resonance imaging evidence of augmented pain processing in fibromyalgia. *Arthritis Rheum.* 2002; 46:1333–1343. [PubMed: 12115241]
18. Hummel M, Cummons T, Lu P, Mark L, Harrison JE, Kennedy JD, Whiteside GT. Pain is a salient “stressor” that is mediated by corticotropin-releasing factor-1 receptors. *Neuropharmacology.* 2010; 59:160–166. [PubMed: 20470804]
19. Ji G, Neugebauer V. Differential effects of CRF1 and CRF2 receptor antagonists on pain-related sensitization of neurons in the central nucleus of the amygdala. *J Neurophysiol.* 2007; 97:3893–3904. [PubMed: 17392412]
20. Ji G, Neugebauer V. Pro- and anti-nociceptive effects of corticotropin-releasing factor (CRF) in central amygdala neurons are mediated through different receptors. *J Neurophysiol.* 2008; 99:1201–1212. [PubMed: 18171711]
21. Khasar SG, Burkham J, Dina OA, Brown AS, Bogen O, Alessandri-Haber N, Green PG, Reichling DB, Levine JD. Stress induces a switch of intracellular signaling in sensory neurons in a model of generalized pain. *J Neurosci.* 2008; 28:5721–5730. [PubMed: 18509033]
22. Khasar SG, Dina OA, Green PG, Levine JD. Sound stress-induced long-term enhancement of mechanical hyperalgesia in rats is maintained by sympathoadrenal catecholamines. *J Pain.* 2009; 10:1073–1077. [PubMed: 19576859]
23. Khasar SG, Green PG, Levine JD. Repeated sound stress enhances inflammatory pain in the rat. *Pain.* 2005; 116:79–86. [PubMed: 15936144]
24. Martenson ME, Cetas JS, Heinricher MM. A possible neural basis for stress-induced hyperalgesia. *Pain.* 2009; 142:236–244. [PubMed: 19232470]
25. McCrimmon RJ, Song Z, Cheng H, McNay EC, Weikart-Yeckel C, Fan X, Routh VH, Sherwin RS. Corticotrophin-releasing factor receptors within the ventromedial hypothalamus regulate hypoglycemia-induced hormonal counterregulation. *J Clin Invest.* 2006; 116:1723–1730. [PubMed: 16741581]
26. O'Connor TM, O'Halloran DJ, Shanahan F. The stress response and the hypothalamic-pituitary-adrenal axis: from molecule to melancholia. *QJM.* 2000; 93:323–333. [PubMed: 10873181]
27. Price DD, Staud R, Robinson ME, Mauderli AP, Cannon R, Vierck CJ. Enhanced temporal summation of second pain and its central modulation in fibromyalgia patients. *Pain.* 2002; 99:49–59. [PubMed: 12237183]
28. Razavi NL, Kitabchi AE, Stentz FB, Wan JY, Larijani BA, Tehrani MM, Gozashti MH, Omidfar K, Taheri E. Proinflammatory cytokines in response to insulin-induced hypoglycemic stress in healthy subjects. *Metabol.* 2009; 58:443–448.
29. Richebe P, Rivat C, Cahana A. Stress-induced hyperalgesia: any clinical relevance for the anesthesiologist? *Anesthesiology.* 2011; 114:1280–1281. [PubMed: 21532462]
30. Ritter S, Llewellyn-Smith I, Dinh TT. Subgroups of hindbrain catecholamine neurons are selectively activated by 2-deoxy-D-glucose induced metabolic challenge. *Brain Res.* 1998; 805:41–54. [PubMed: 9733914]
31. Rivat C, Becker C, Blugeot A, Zeau B, Mauborgne A, Pohl M, Benoliel JJ. Chronic stress induces transient spinal neuroinflammation, triggering sensory hypersensitivity and long-lasting anxiety-induced hyperalgesia. *Pain.* 2010; 150:358–368. [PubMed: 20573451]
32. Rolke R, Baron R, Maier C, Tolle TR, Treede RD, Beyer A, Binder A, Birbaumer N, Birklein F, Botefur IC, Braune S, Flor H, Hoge V, Klug R, Landwehrmeyer GB, Magerl W, Maihofner C, Rolko C, Schaub C, Scherens A, Sprenger T, Valet M, Wasserka B. Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): standardized protocol and reference values. *Pain.* 2006; 123:231–243. [PubMed: 16697110]
33. Sommer C, Kress M. Recent findings on how proinflammatory cytokines cause pain: peripheral mechanisms in inflammatory and neuropathic hyperalgesia. *Neurosci Lett.* 2004; 361:184–187. [PubMed: 15135924]
34. Staud R, Price DD, Robinson ME, Mauderli AP, Vierck CJ. Maintenance of windup of second pain requires less frequent stimulation in fibromyalgia patients compared to normal controls. *Pain.* 2004; 110:689–696. [PubMed: 15288410]

35. Terman GW, Shavit Y, Lewis JW, Cannon JT, Liebeskind JC. Intrinsic mechanisms of pain inhibition: activation by stress. *Science*. 1984; 226:1270–1277. [PubMed: 6505691]
36. Uceyler N, Rogausch JP, Toyka KV, Sommer C. Differential expression of cytokines in painful and painless neuropathies. *Neurology*. 2007; 69:42–49. [PubMed: 17606879]
37. Vidal C, Jacob J. Hyperalgesia induced by non-noxious stress in the rat. *Neurosci Lett*. 1982; 32:75–80. [PubMed: 7145227]
38. Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. *Pain*. 2011; 152:S2–15. [PubMed: 20961685]
39. Yilmaz P, Diers M, Diener S, Rance M, Wessa M, Flor H. Brain correlates of stress-induced analgesia. *Pain*. 2010; 151:522–529. [PubMed: 20817354]

Figure 1. Study Design

Subjects participated in two, 3-day protocols separated in time by 1 to 3 months. Subjects were randomized by admission to participate in either euglycemic (target blood glucose 5.0 mmol/l) hyperinsulinemic clamp studies or hypoglycemic (target blood glucose 2.8 mmol/l) hyperinsulinemic clamp studies on day 2. Cytokine and hormone levels were obtained at baseline and at 45 minute intervals during clamp sessions. Sensory testing was conducted on days 1 and 3 by investigators unaware of whether a subject was participating in the euglycemia or hypoglycemia protocol. Sensory testing included thermal detection and thermal pain detection thresholds and thermal temporal summation.

Figure 2. Thermal sensitivity increases after hypoglycemia

Effect of exposure to antecedent euglycemia (black circles) and antecedent hypoglycemia (white circles) on thermal detection thresholds and thermal pain detection thresholds. Panel A: Day 3 cold detection thresholds were similar after antecedent euglycemia and antecedent hypoglycemia. Panel B: There is a higher cold-pain detection threshold (increased sensitivity) after antecedent hypoglycemia. Panel C: There is a lower heat detection threshold (increased sensitivity) after antecedent hypoglycemia. Panel D: There is a lower heat-pain detection threshold (increased sensitivity) after antecedent hypoglycemia.

Figure 3. Temporal summation increases after hypoglycemia

Thermal temporal summation after exposure to antecedent euglycemia (black circles) and antecedent hypoglycemia (white circles). The initial and 10th stimuli pain scores during thermal temporal summation are reported. There were no differences in initial pain response to temporal summation between treatments. There was an increase in windup pain after antecedent hypoglycemia.

Figure 4. IL-6 and epinephrine correlate with temporal summation pain

In (A), there was a positive correlation between temporal summation (windup) pain and the change in interleukin-6 levels during hypoglycemia ($r=0.49$, $P<0.01$; Figure 4A). In (B), the association between temporal summation (windup) pain and was inversely correlated with epinephrine levels during hypoglycemia ($r=-0.60$, $P<0.01$).

Table 1
Means and standard deviation of cytokine and hormone levels before and during hypoglycemia or euglycemia

Hormone or Cytokine	Baseline before euglycemia	Peak level during euglycemia	Baseline before hypoglycemia	Peak level during hypoglycemia
ACTH pg/ml	28.6±12.0	34.0±8.0	32.5±7.1	80.7±52.4 ^{**†}
Cortisol µg/dl	9.7±2.0	9.8±2.0	9.5±4.2	19.6±7.2 ^{**†}
Growth Hormone ng/ml	1.4±2.4	4.4±5.3	2.2±3.9	24.9±15.3 ^{**†}
Insulin µIU/ml	6.7±9.9	129.4±21.8 [‡]	4.8±3.6	126.8±73.5 [‡]
Epinephrine pg/ml	33.7±17.9	40.0±10.8	34.0±22.5	526.1±514.0 ^{**}
Norepinephrine pg/ml	195±140	233±91	113±75	268±221 ^{*‡}
IL-6 pg/ml	1.5±1.2	1.7±1.2	1.7±3.5	3.1±3.3 ^{*‡}

*=P<0.05,

**=P<0.001 peak hypoglycemia vs. peak euglycemia

[‡]=P<0.05,

[‡]=P<0.001 peak hypo/euglycemia vs. baseline hypo/euglycemia