

A Study of Three Biomarkers in Human Saliva After the Cold-Pressor Task

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Introduction

Ineffective acute pain management is associated with the development of chronic pain. Prolonged pain is physically debilitating and psychologically distressing. Current pain assessment methods rely on self-reporting and are not suited to patients who are unable to provide their input, such as people with intellectual and developmental disabilities or unconscious patients.¹

Reliable, objective and non-invasive methods of detecting acute pain would be very desirable. Salivary cortisol has been shown to rise after exposure to acute pain.^{2,3} Glutamate and substance P (SP) are biomolecules associated with nociceptive pathways and their salivary concentrations have been found to be higher in people with chronic pain.^{4,5}

Methods

We have explored the variation in salivary concentrations of three biomolecules -cortisol, glutamate, and SP- after acute pain was induced by the cold-pressor task (CPT) in healthy pain-free volunteers.

Pain induction



Figure 1. Participants submerged their forearm and hand into an ice bath (0-5°C) for a maximum of 5 minutes with full control over when to start and end the immersion. After the CPT, participants recorded their maximum pain intensity score on a 0-10 Numeric Rating Scale (NRS).

Saliva sampling



Figure 2. Whole saliva samples were collected by passive drool at different time points before and after the CPT. Biomolecule concentrations were quantified using enzyme-linked immunospecific assay (ELISA) for SP and cortisol, and a colorimetric assay for glutamate.

Timeline for saliva sampling with respect to the painful task

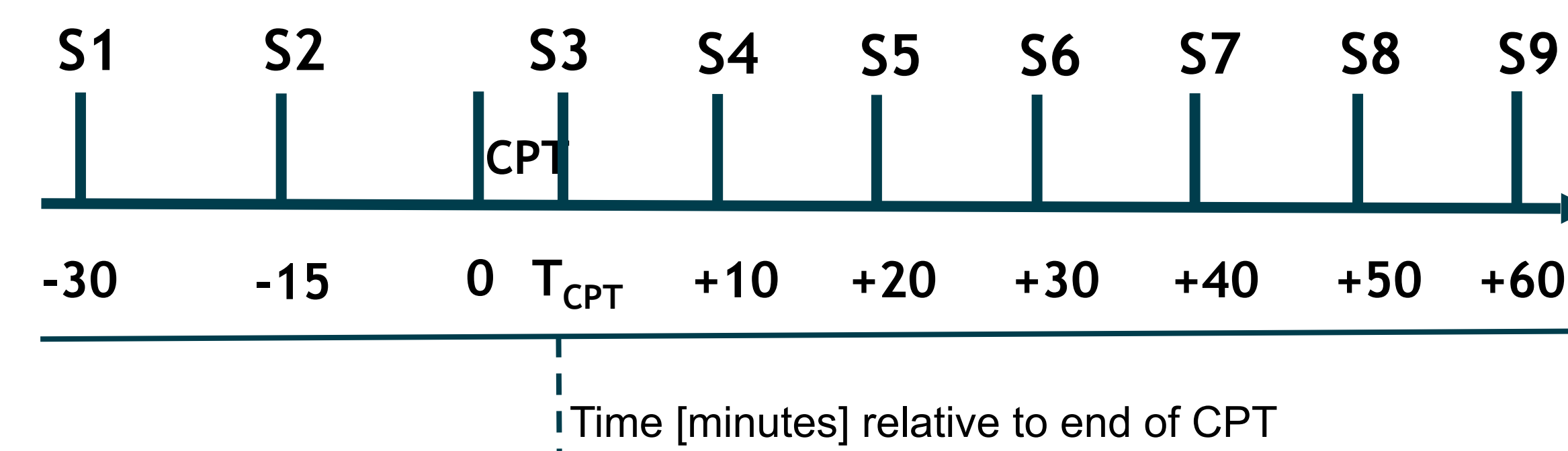


Figure 3. Two baseline samples (S1,S2) were collected at t=-30' and -15' before the CPT, and another 7 samples (S3-S9) were collected after the CPT by the participants. CPT, cold-pressor task.

Results

Eighteen participants (sex ratio 1:1) were recruited with a median age of 25 years (range of 21-40 years). The median CPT duration was 5 minutes and the median of the NRS scores reported was 6.25.

Cortisol: Median salivary cortisol concentration rose significantly between baseline (0.14 µg/dL, IQR 0.1) and t=+10' (0.34 µg/dL, IQR 0.4) (P=0.007) (Fig. 4A). Cortisol levels at t=+10' were significantly higher than at t=T_{CPT} and t=+60' (P=0.03 and P=0.02 respectively), but not significantly higher compared with t=+20' and +30' (Fig. 4A). In male participants, rise in cortisol concentrations was greater at t=+10', +20' and +30' compared to females (P=0.01, P=0.02, and P=0.04, respectively) (Fig. 4D).

Glutamate: Salivary glutamate increased immediately after the CPT from a median of 4.90 ng/µL (IQR=4.7) to 5.66 ng/µL (IQR=4.6) and fluctuated thereafter. These changes were not significant except at t=+50' when the levels dropped below baseline to 2.08 ng/µL (IQR=3.3) (P=0.014) (Fig. 4B and 4E).

SP: SP analysis was only possible for 10 participants (median age 25 years, sex ratio 1:1) and no significant differences were found between the SP concentrations at different time points (Fig. 4C and 4F).

Salivary cortisol, glutamate and SP response to the CPT

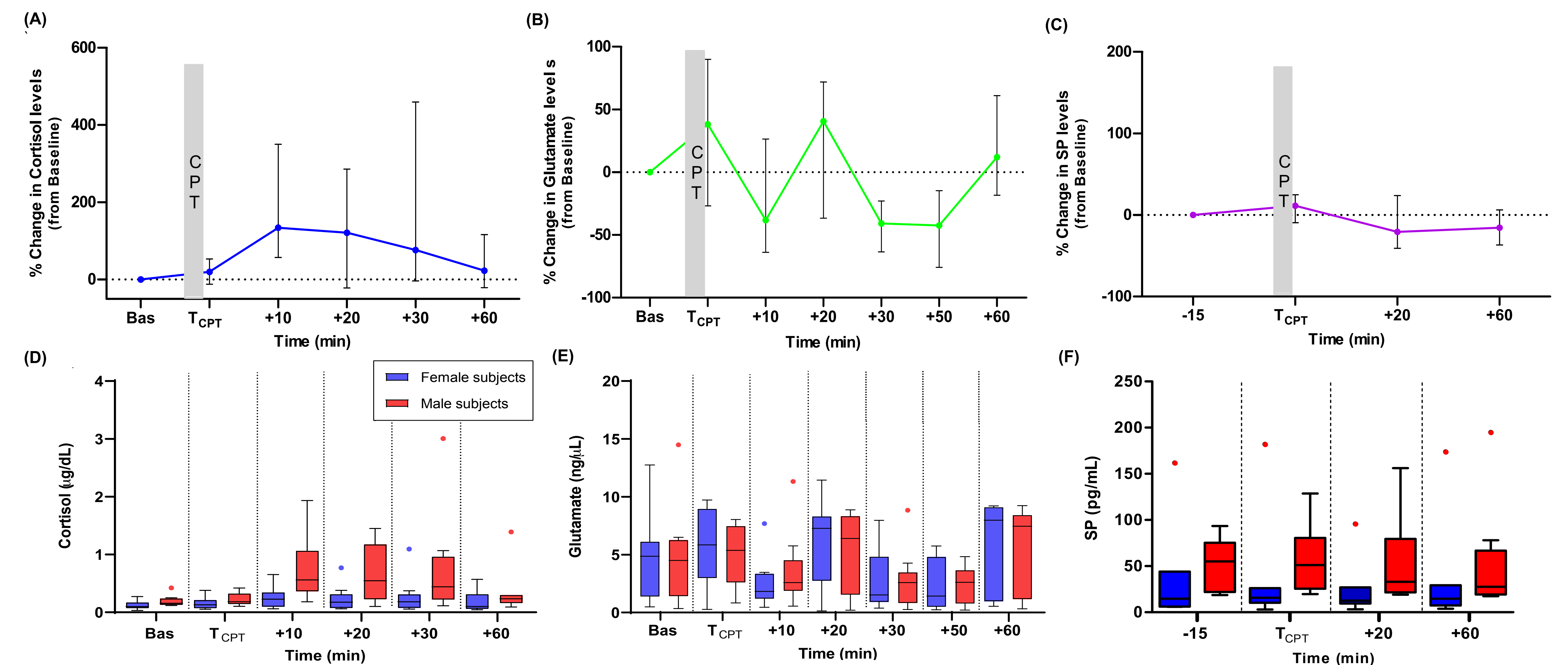


Figure 4. Medians of normalised (to the baseline (Bas)) levels percentile changes in cortisol (A), glutamate (B), and SP (C) levels, and the medians of absolute values of cortisol (D), glutamate (E), and SP (F) levels in the female and male groups are presented. Cortisol levels increased at 10-30 minutes (min) after the cold pressor task (CPT) and no significant differences were found in glutamate and SP (Friedman's test with Dunn's adjusted post hoc test). The interquartile ranges (IQRs) are presented with vertical lines. The average of the baseline values was used in the analyses for glutamate and cortisol. Sex differences were investigated using the unpaired t-test or the Mann-Whitney U test depending on data normality.

Conclusion

We found a peak in salivary cortisol levels 10-30 minutes after the CPT, which is in line with previous studies.^{2,3} This is the first study of salivary glutamate and SP reported in the literature after acute pain induction. No significant changes were found in either of these, but it is possible that we missed a peak in glutamate or SP levels right after the CPT due to the sampling time points in our protocol being aligned to the known time scale for change in salivary cortisol.

Limitations of this study include a) uncertainties about the applicability of data on healthy volunteers to real-world scenarios, b) biomolecule response to acute pain influenced by factors such as circadian rhythm, chronic pain or illness, and c) changes in biomolecule levels may be a stress response. Due to loss of some samples, the SP study may also be underpowered to detect change.

Further work on pain-related biomarkers is warranted to build a clear picture of how salivary biomolecules could reliably be used to detect nociception. The development of such technology can provide a non-invasive, easy-to-use means of monitoring acute pain and treating it.

References

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