An initial exploration of *in vivo* hair cortisol responses to a brief pain stressor: Latency, localisation and independence effects.

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Running title: Hair cortisol responses to a brief pain stressor

Summary

Cortisol is secreted by the central HPA-axis and affects many target organs and tissues, particularly in response to stressor demands and infection. Recent data reporting cortisol synthesis in hair follicles have shown the existence of a parallel "peripheral" HPA-axis. However, although there is evidence from *in vitro* studies and single-observation comparisons between groups that cortisol from hair follicles reflects endocrine changes associated with stressor demands, there are no reports to date of repeated measurements of *in vivo* cortisol responsivity in hair to transitory stressors. This issue was investigated with three males who underwent 1 min Cold Pressor test. Cortisol response in hair to stressor demand appears to be (a) swift but transitory, (b) localized to the site of the demand and (c) independent of central HPA-axis activity.

Key words: cortisol, hair, stress, peripheral HPA axis

Introduction

Glucocorticoids are an important class of steroid hormones that modulate a diverse range of physiological effects. They are produced primarily by the adrenal cortex in response to the pituitary hormone ACTH which is in turn regulated by the hypothalamic peptide CRH (Guyton & Hall, 2006). Together, these processes form the hypothalamic-pituitary-adrenal axis (HPA). While cortisol is the major glucocorticoid in humans and was first recognized for its role in glucose homeostasis, it is now known to have important anti-inflammatory and immunosuppressive effects on all tissues including the skin (Klaitman & Almog, 2003; Weissman & Thomas, 1963).

A basal concentration of cortisol is required at all times (Guyton & Hall, 2006). Although the onset of physical or mental stress produces elevated concentrations (Dickerson & Kemenyi, 2004), increased secretion of cortisol is also stimulated by perceived (i.e, before the actual threat occurs: Gaab, et al, 2005) as well as actual threat. Cortisol has traditionally been assayed from serum, urine and saliva in various studies as a measure of stress (Umeda et al, 1981). An elevated plasma cortisol response to a stressor takes about 8 minutes to occur after the onset of a stressor (Buono, et al, 1986), while salivary cortisol rises after about 15 to 20 minutes after a physical (Filaire et al, 1996) or psychological (Sharpley & McLean, 1992) stressor. Salivary cortisol provides a reliable measure of the free cortisol concentration in blood (Umeda, et al, 1981), is not affected by salivary flow or composition, indicates physiologically relevant changes in the HPA axis (Khan et al, 1988) and is relatively easy to sample (Walker, et al, 1978).

The skin is continuously exposed to a range of environmental stressors throughout life. It responds to acute stress in the classically recognized manner such as flushing and

sweating but also with increased numbers of immunocompetant cells (Dhabhar, 2000). As well as recent reports of cortisol synthesis within skin cells (Arck et al, 2006), there have been a number of studies showing that steroid hormones including cortisol can be identified in hair samples from humans (Raul et al, 2004; Sauve et al, 2007; Van Uum et al, 2008; Yamada et al, 2007; Yang et al, 1998; Wheeler et al, 1998;) and other species (Davenport et al, 2006; Klein et al, 2004; Koren et al, 2002). Although it has been assumed that the hair shaft above skin level is dead (Harkey, 1993), some studies have challenged this view by showing variability in hair shaft steroid levels during the female menstrual cycle in parallel with serum steroid concentrations (Yang et al, 1998). In addition, the lack of difference in steroid concentration across the length of the hair shaft (Yang et al, 1998) implies that hair steroid levels are actively altered along the "dead" hair shaft in tandem with serum concentrations. Several studies have shown that washing of the hair removes less than 7% of the total hormone extracted (Cirimele et al, 2000; Raul et al, 2004), suggesting that washing is an unnecessary step in the assay process.

These and other data indicate that, as well as being *targets* for HPA axis activity, skin and hair are also *producers* of cortisol via their own neuroendocrine systems that have been called the "peripheral" HPA axis (e.g., Arck et al, 2006; Paus et al, 2006; Slominski et al, 2005). Given that CRH, POMC, ACTH (Aron, et al, 2007) and cortisol itself have been shown to be secreted by human hair follicles (Ito, et al, 2005; Klein, et al, 2004; Kalra, et al, 2005), it is reasonable to expect that a localized cortisol response to a stressor applied to a specific area of the body may occur. However, previous studies of hair cortisol responsivity were performed *in vitro* using expurgated human hair follicles and the secretion of cortisol in response to ACTH stimulation was delayed by at least 48

hours (Ito et al, 2005). In addition, those reports linking hair cortisol with stress gathered data from comparison groups (e.g., depressed vs non-depressed pregnant women: Kalra,et al, 2005) or at a single point in time (babies vs adults: Klein, et al, 2004).Thus, direct comparisons between the immediate responses of the intact central and peripheral HPA-axes have not yet been performed, leading Arck et al (2006) to note that one of the "major, as-yet unmet challenges in cutaneous stress research …(is)…the study of the cross-talk between peripheral and systemic responses to psychological stress" (p. 1697). Therefore, the aims of the current study were to collect some initial *in vivo* exploratory data on hair cortisol in response to acute stress, to determine whether those responses were localized, and to assess the presence of any links between the peripheral and central HPA axes.

Methods

Participants

Data were collected from three healthy male subjects who were taking no medication. S1 was about 50 years at the time of experimentation, S2 was about 20 years and S3 was about 30years.

Sample collection and Assay

Saliva was collected via Salivette (Sarstelt), and then centrifuged and stored frozen at -20C until assayed for cortisol. Hair was collected by shaving about $3cm^2$ of the wrist area of the hand which was immersed into the CPT and the lower opposite leg near the ankle with a disposable razor that was washed in methanol between shaves, with a new razor being used for each subject. Hair was brushed into separate paper envelopes for each sample and transferred into scintillation vials and chopped with scissors before

being extracted with 3ml of methanol for 24 hours. The methanol was then decanted into polyethylene tubes (4mm) and evaporated under vacuum. Gel buffer (100μL), phosphate buffer (0.05M), saline (0.15M), pH 7.5 containing 0.1% gelatin were added and allowed to stand at room temperature for 60 minutes prior to assay. Cortisol concentrations in both saliva and hair were determined by radioimmunoassay as previously described (Yuen et al, 2004).

Procedure

Subjects underwent the experimental procedure individually in a small room with no distracting features. After being greeted and answering some background questions, each subject sat reading for 6 mins, underwent the CPT (0 to 4C) for 1 min on his right (preferred) hand and then remained sitting and reading for a further 29 min. Eight hair samples were collected from each subject's CPT arm and opposite leg, plus eight saliva samples at regular intervals (see Fig 1). None of the subjects had previously experienced the CPT. Immediately after CPT, the subject's hand was dried and remained stationary for the rest of the experimental period. Hair was shaved from the wrist immediately adjacent to his immersed hand and also from his opposite lower leg at intervals prior to and following the administration of the CPT as shown in Figure 1. Salivary cortisol was also collected at the same intervals.

Results

Figure 1 shows the hair and saliva cortisol values for each S. Hair cortisol values in ng/g for the CPT-immersed arm may be seen to rise for each S from pre-CPT rest to a maximum during the CPT and then to reduce to values which approach resting for two Ss and to about 50% of CPT for S3. By contrast, cortisol values for the opposite leg (which

did not undergo CPT) were relatively unchanged during the entire experimental period. Figure 2 shows these individual arm and opposite leg hair cortisol data collapsed into group means and recalculated as percentage change from pre-CPT rest. Mean salivary cortisol values are shown in Figure 3.

Insert Figures 1, 2 and 3 here

Discussion

Although they represent only initial explorations of the *in vivo* hair cortisol responses to a standard (CPT) pain stressor, these data show consistency across the three Ss in several aspects. First, the application of pain showed dramatic individual and group mean increases in hair cortisol in the arm which underwent CPT, followed by a similar decrease almost immediately afterwards. These data suggest the presence of a dramatic and short-term hair cortisol production following the application of pain. Second, each S's hair cortisol responses were restricted to the arm which received the CPT pain and did not occur in the opposite leg. These site-specific responses support a "localization" hypothesis that might argue for specific hair cortisol synthesis according to the body area under threat. Third, the time lag observed for Ss' salivary cortisol is similar to that reported in the previous literature and argues that the hair cortisol response was independent of the central HPA-axis response, and that hair and salivary cortisol responses to the CPT stressor were independent of each other in terms of the organic source of their respective responses (i.e., hair cortisol was synthesised locally and salivary cortisol was synthesised centrally) to the same demand (i.e., the CPT).

These results require replication and must be considered as initial explorations at this stage. However, the data shown here suggest the presence of an *in vivo* cortisol response in hair that is (a) swift but transitory to pain, (b) localized to the site of the pain and (c) independent of the central HPA-axis as represented by salivary cortisol.

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Figure captions

Figure 1: Hair and salivary extract cortisol concentrations prior to and following CPT of Ss' arm, opposite leg and saliva.

Figure 2: Mean (n = 3) hair extract cortisol concentrations prior to and following CPT of Ss' arm and opposite leg.

Figure 3: Mean (n = 3) salivary extract cortisol concentrations during experimental conditions.

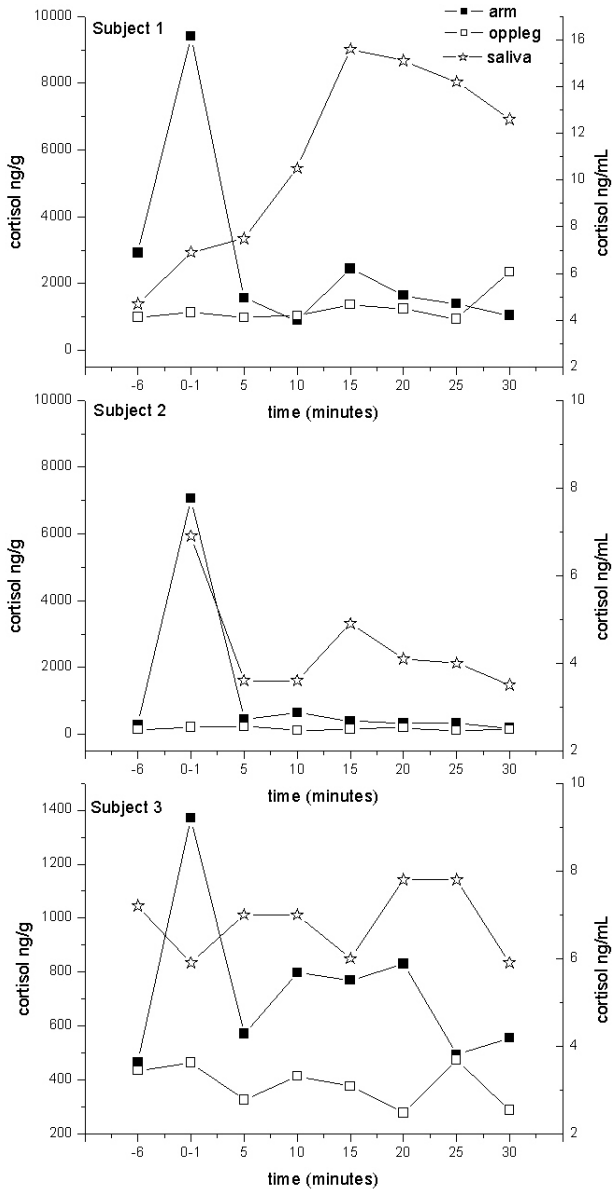

Subject 1
arm
oppleg
saliva
cortisol ng/g
cortisol ng/mL
time (minutes)
Subject 2
cortisol ng/g
cortisol ng/mL
time (minutes)
Subject 3
cortisol ng/g
cortisol ng/mL
time (minutes)

Fig. 1.

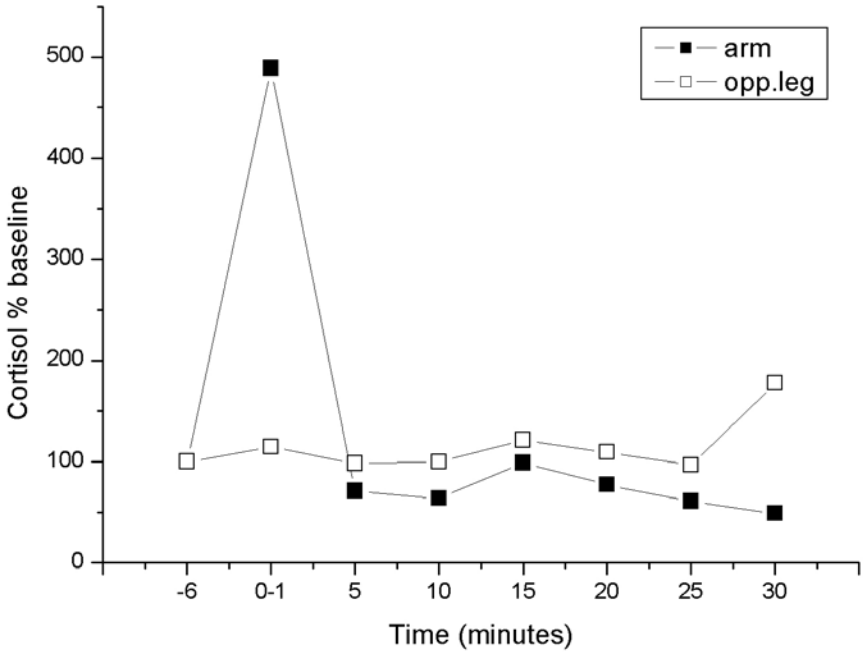
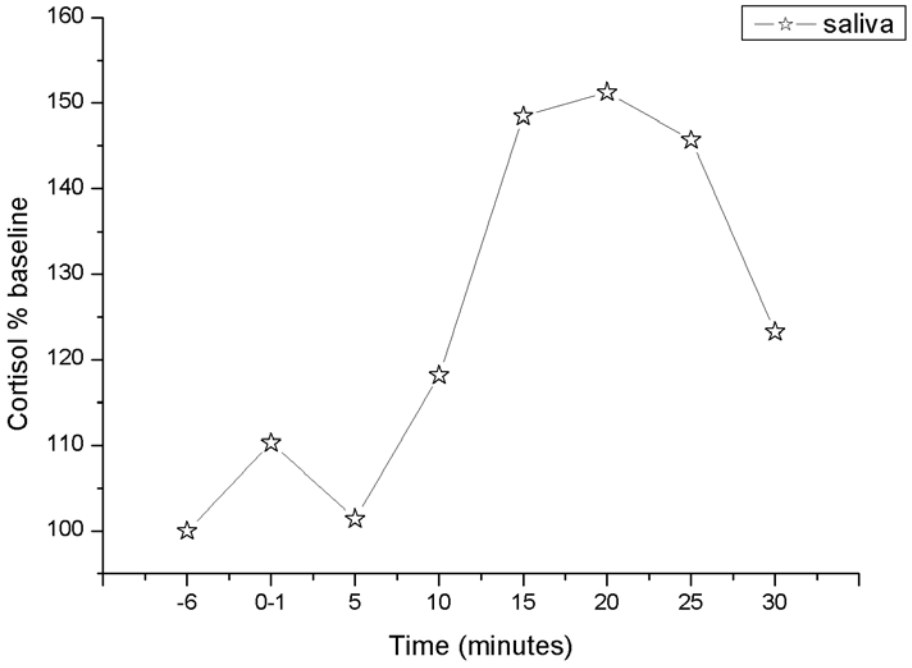

arm
opp.leg
Cortisol % baseline
0
100
200
300
400
500
-6
0-1
5
10
15
20
25
30
Time (minutes)

Fig. 2.


saliva
Cortisol % baseline
100
110
120
130
140
150
160
-6
0-1
5
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15
20
25
30
Time (minutes)

Fig. 3.