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SHORT COMMUNICATION

An Initial Exploration of *in vivo* Hair Cortisol Responses to a Brief Pain Stressor: Latency, Localization and Independence Effects

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Summary

Cortisol is secreted by the central hypothalamo-pituitary-adrenal 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 (CP). 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

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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 and 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 and Almog 2003, Weissman and Thomas 1963).

A basal concentration of cortisol is required at all times (Guyton and Hall 2006). Although the onset of physical or mental stress produces elevated concentrations (Dickerson and 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 min to occur after the onset of a stressor (Buono *et al.* 1986), while salivary cortisol rises after about 15-20 min after a physical (Filaire *et al.* 1996) or psychological (Sharpley and 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 immunocompetent cells (Dhabhar 2000). As well as recent reports of cortisol synthesis within skin cells (Arck

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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 (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". 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.

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 30 years.

Saliva was collected *via* Salivette (Sarstelt), and then centrifuged and stored frozen at –20 °C until assayed for cortisol. Hair was collected by shaving about 3 cm^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 3 ml of methanol for 24 h. The methanol was then decanted into polyethylene tubes (4 mm) and evaporated under vacuum. Gel buffer (100 µl), phosphate buffer (0.05 M), saline (0.15 M), pH 7.5 containing 0.1 % gelatin were added and allowed to stand at room temperature for 60 min prior to assay. Cortisol concentrations in both saliva and hair were determined by radioimmunoassay as previously described (Yuen *et al.* 2004).

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 min, underwent the CPT (0 to 4 °C) 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 (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.

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 subject from pre-CPT rest to a maximum during the CPT and then to reduce to values which approach resting for two subjects 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

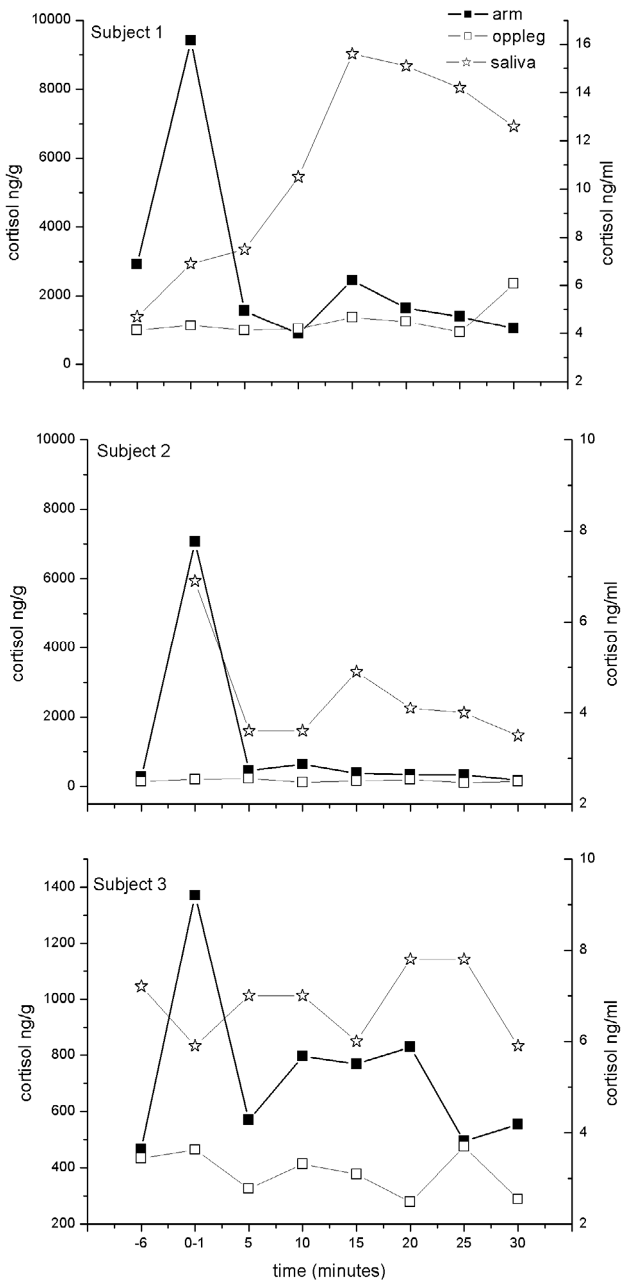

Fig. 1. Hair and salivary extract cortisol concentrations prior to and following CPT of subjects' arm, opposite leg and saliva.

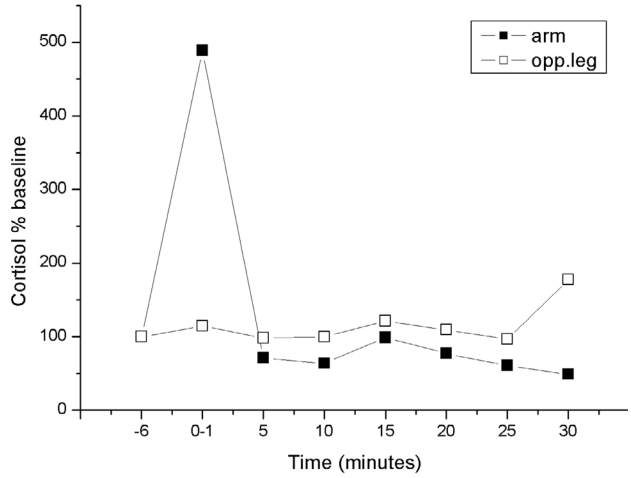

Fig. 2. Mean (n=3) hair extract cortisol concentrations prior to and following CPT of subjects' arm and opposite leg.

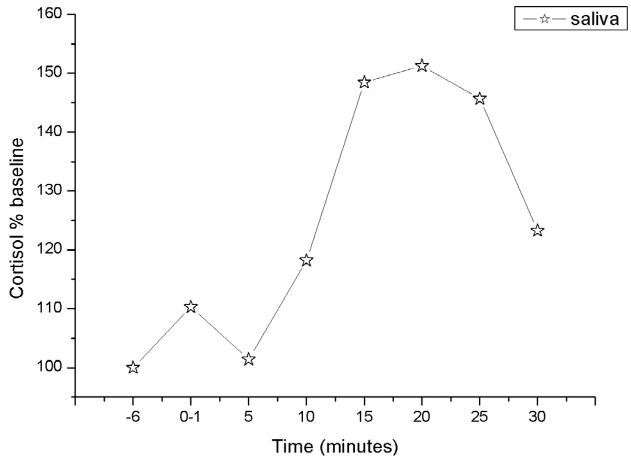

Fig. 3. Mean (n=3) salivary extract cortisol concentrations during experimental conditions.

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.

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 subjects 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 subject'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 subjects' 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 synthesized locally and salivary cortisol was synthesized 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.

Conflict of Interest

There is no conflict of interest.

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