Absence of glucocorticoids augments stress-induced *Mkp1* mRNA expression within the hypothalamic–pituitary–adrenal axis

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Abstract

Stress-induced activation of hypothalamic paraventricular nucleus (PVN) corticotropin-releasing hormone (CRH) neurons trigger CRH release and synthesis. Recent findings have suggested that this process depends on the intracellular activation (phosphorylation) of ERK1/2 within CRH neurons. We have recently shown that the presence of glucocorticoids constrains stress-stimulated phosphorylation of PVN ERK1/2. In some peripheral cell types, dephosphorylation of ERK has been shown to be promoted by direct glucocorticoid upregulation of the MAP kinase phosphatase 1 (*Mkp1*) gene. In this study, we tested the hypothesis that glucocorticoids regulate *Mkp1* mRNA expression in the neural forebrain (medial prefrontal cortex, mPFC, and PVN) and endocrine tissue (anterior pituitary) by subjecting young adult male Sprague–Dawley rats to various glucocorticoid manipulations with or without acute psychological stress (restraint). Restraint led to a rapid increase in *Mkp1* mRNA within the mPFC, PVN, and anterior pituitary, and this increase did not require glucocorticoid activity. In contrast to glucocorticoid upregulation of *Mkp1* gene expression in the peripheral tissues, we found that the absence of glucocorticoids (as a result of adrenalectomy) augmented basal mPFC and stress-induced PVN and anterior pituitary *Mkp1* gene expression. Taken together, this study indicates that the presence of glucocorticoids may constrain *Mkp1* gene expression in the neural forebrain and endocrine tissues. This possible constraint may be an indirect consequence of the inhibitory influence of glucocorticoids on stress-induced activation of ERK1/2, a known upstream positive regulator of *Mkp1* gene transcription.

Key Words

- *Mkp1*
- ACTH
- paraventricular nucleus of hypothalamus
- medial prefrontal cortex
- corticosterone
- ERK1/2

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Introduction

Corticotropin-releasing hormone (CRH) neurons of the hypothalamic–pituitary–adrenal (HPA) axis integrate stress-dependent changes in neural input and direct negative feedback effects of glucocorticoids (Dallman *et al*. 1987, Sawchenko *et al*. 1996, Bali *et al*. 2008). Excitation of CRH neurons is often coupled to not only CRH neurohormone secretion but also altered gene expression and neurohormone synthesis (Kovács & Sawchenko 1996, Watts 2005, Pace *et al*. 2009). Recent evidence indicates that a molecular element of this coupling process within CRH neurons is phosphorylation/activation of ERK1/2 (Khan & Watts 2004, Khan *et al*.

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2007, 2011). ERK1/2 are members of the MAP-kinase family. MAP-kinases are essential intracellular signaling proteins for virtually all cell types, including neurons and endocrine cells (Grewal *et al*. 1999). We recently have reported that acute exposure to psychological stress (restraint) increased the activated (phosphorylated) form of ERK1/2 in the hypothalamic CRH neurons, and this ERK1/2 activation was constrained by the tonic activity of glucocorticoid (Osterlund *et al*. 2011).

The active phosphorylation state of ERK1/2 is regulated by both the kinase MEK and by phosphatases, including MAP kinase phosphatase 1 (MKP1, also known as DUSP1) (Keyse 2000, Patterson *et al*. 2009). MKP1 is a dual specificity phosphatase that inactivates MAP-kinases by dephosphorylating tyrosine and threonine residues essential for catalytic activity (Camps *et al*. 2000, Theodosiou & Ashworth 2002). A key feature of MKP1 is that its encoding gene (*Mkp1*) is rapidly induced in a wide range of cell types by a large number of excitatory stimuli (Sun *et al*. 1993, Caunt & Keyse 2013). Interestingly, *in vitro* studies found that glucocorticoids increase *Mkp1* mRNA levels in a variety of peripheral cell types and cell lines (Clark 2003, Clark *et al*. 2008). Glucocorticoid treatment in mice has also been shown to rapidly increase *Mkp1* mRNA in the lung, spleen, and liver (Wang *et al*. 2008, Vandevyver *et al*. 2012). *In vitro* studies have also shown that glucocorticoid suppression of some MAP-kinase-dependent cellular processes depends on glucocorticoid-mediated upregulation of *Mkp1* gene expression (Kassel *et al*. 2001, Issa *et al*. 2007, Zhou *et al*. 2007, Komatsu *et al*. 2008, Nicoletti-Carvalho *et al*. 2010, Burke *et al*. 2012).

Glucocorticoids provide a protein-synthesis-dependent negative feedback inhibition of the HPA axis that is evident within 1–3 h after glucocorticoid treatment (Dallman *et al*. 1987, Shipston 1995, Osterlund & Spencer 2011). This short-term glucocorticoid negative feedback action is probably due to rapid glucocorticoid rapid induction of one or more genes that encode proteins important for the coupling of cellular excitation with (neuro)hormone secretion (Shipston 1995, Osterlund & Spencer 2011). We considered the possibility that glucocorticoid suppression of stress-induced ERK1/2 activation in hypothalamic paraventricular nucleus (PVN) CRH neurons depends, at least in part, on rapid glucocorticoid upregulation of *Mkp1* gene expression. To determine whether induction of the *Mkp1* gene could be a mechanism for short-term glucocorticoid regulation of the HPA axis, we examined *Mkp1* mRNA levels (*in situ* hybridization) in the hypothalamic PVN, medial prefrontal cortex (mPFC), and anterior pituitary of male rats subjected to acute stress and corticosterone (CORT) manipulations. The PVN contains the HPA axis-related CRH neuron cell bodies. For comparison purposes, we also examined *Mkp1* mRNA levels in another brain region important for the modulation of HPA axis activity, the mPFC (Radley *et al*. 2006, Weinberg *et al*. 2010), as well as in an additional anatomical element of the HPA axis, the anterior pituitary. For these studies, we challenged rats with the acute stressor restraint, which is considered a moderate-intensity psychological stressor (Herman & Cullinan 1997, Dayas *et al*. 2001, Pace *et al*. 2005). We manipulated CORT levels by either removing endogenous CORT (adrenalectomy) or by treating rats with an acute injection of CORT. Although to date there has been very limited study of *Mkp1* gene expression in mammalian brain, there is evidence for it being regulated in an activity-dependent manner, contributing to neuronal axonal plasticity, and being associated with major depressive disorder (Sgambato *et al*. 1998, Kodama *et al*. 2005, Doi *et al*. 2007, Jeanneteau *et al*. 2012).

Materials and methods

Animals

Young adult male Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IA, USA) weighed between 280 and 305 g at time of experimentation were housed two per-cage. The colony room lights were maintained on a 12 h light:12 h darkness cycle and rats were given rat chow (Purina Rat Chow, Ralston Purina, St Louis, MO, USA) and tap water and allowed to feed and drink *ad libitum*. Rats were given at least a 2-week acclimation period to the colony room before initiation of experimental procedures. All experiments were performed during the first half of the rats' inactive period, when basal CORT secretion is at its circadian trough. Handling and testing of all rats were approved by the University of Colorado Institutional Animal Care and Use Committee.

Surgery

Rats were adrenalectomized bilaterally (ADX) or were sham-ADX under halothane anesthesia. Adrenal glands were excised and removed through bilateral incisions that were made through the dorsal-lateral skin and peritoneal wall in close proximity to each kidney. Sham-ADX rats experienced the same surgical procedure as ADX rats, except that adrenal glands were left in place after their localization. All ADX and sham-ADX rats were given

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4 days to recover from surgery before the experimental test day. ADX rats were given 0.9% saline drinking water and allowed to drink *ad libitum*.

Restraint stress

Acute stress challenge consisted of placing rats in clear plexiglass tubes (23.5 cm in length and 7 cm in diameter; with multiple air holes). The size of the tube restricted lateral, forward, and backward movement but did not interfere with breathing. Restraint is widely accepted as a psychological stressor within the stress neurobiology field, which has not only a conceptual basis, but is also supported by a study on neurocircuit activity (Dayas *et al*. 2001).

Experimental procedures

Experiment 1 Effect of acute stress on PVN *Mkp1* mRNA expression. Rats were exposed to 15 or 30 min of stress or no-stress (n=3–4). Immediately after restraint, or at the same time of day (no-stress group), rats were killed by guillotine decapitation. Brains were flash frozen in isopentane (chilled between −30 and −40 °C) and then stored at −80 °C until subsequent analysis.

Experiment 2 Effect of long-term absence of endogenous glucocorticoid (ADX) and acute stress challenge (restraint) on ACTH secretion and *Mkp1* mRNA expression in the PVN, anterior pituitary, and mPFC. On the test day, ADX and sham-ADX rats were either subjected to 30 min of restraint or left in their respective home cages (2×2 between-subjects factorial design; n=6, N=24). Immediately after decapitation, brains were removed, flash frozen in isopentane (chilled between −30 and −40 °C) and then stored at −80 °C until subsequent analysis. In addition, trunk blood was collected for subsequent plasma ACTH hormone measurement.

Experiment 3 Effect of a phasic 1 or 3 h CORT pretreatment on mPFC and PVN *Mkp1* mRNA expression of non-stressed adrenal-intact rats. On the test day, rats were given an injection of CORT (2.5 mg/kg, i.p.) or vehicle (1 ml/kg, i.p.) 1 or 3 h before decapitation (2×2 between-subjects factorial design; n=6; N=24). This exogenous CORT treatment procedure produces plasma CORT levels in rats that closely match the endogenous CORT levels and time course associated with a moderate intensity stressor, such as restraint (Pace *et al*. 2001, Barnum *et al*. 2008). The period of 1 to 3 h post CORT treatment is within the time range that another study detected a significant increase in mast cell *Mkp1* gene expression after glucocorticoid treatment (Kassel *et al*. 2001). After each injection, rats were returned to their home cage and home room. In order to minimize stress-induced CORT increase in the vehicle-treated rats, we habituated rats to the injection procedure by poking rats with the blunt end of a 1 ml syringe (no needle attached) for 2 min over a 3-day period before testing. CORT was purchased from Steraloids, Inc. (Newport, RI, USA) and dissolved in vehicle (10% ethanol, 30% propylene glycol, and 60% sterile saline).

ACTH RIA

Trunk blood was collected into EDTA-containing vacutainer tubes (Becton-Dickinson, Franklin Lakes, NJ, USA), placed on wet ice, and then centrifuged for 15 min (4 °C). Plasma was then aliquoted into microfuge tubes and snap-frozen on dry ice. The blood-processing procedure was completed within 45 min after decapitation. ACTH (pg/ml) was determined in duplicate (100 μl of plasma) by using a competitive RIA protocol as described previously (Osterlund & Spencer 2011). Radiolabeled 125-I ACTH-Tracer was obtained from DiaSorin (Stillwater, MN, USA) and primary ACTH anti-serum Rb 7 (diluted to a final concentration of 1:30 000) was provided courtesy of Dr W Engeland (University of Minnesota, twin cities campus, Department of Neuroscience). The detection limit for this assay was 15 pg/ml; the intra-assay coefficient of variability was 4%.

CORT ELISA

Plasma CORT samples were measured using 20 μl of plasma with an enzyme immunoassay kit (Assay Design, Ann Arbor, MI, USA). All samples were diluted in a ratio of 1:50 in assay buffer and incubated in a 70 °C water bath for 1 h to denature corticosteroid-binding globulin. Heat-inactivated samples were then processed as per the instructions from the assay kit. Sensitivity of the CORT assay, as reported by the manufacturer, is 27 pg/ml. The intra-assay coefficient of variability was 6.3%.

In situ hybridization assay

Coronal brain sections and horizontal pituitary sections (12 μm thick) were obtained with a cryostat (model 1850; Leica Microsystems, Nussloch, Germany) and thaw mounted onto Superfrost Plus slides (Fisher Scientific,

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Pittsburgh, PA, USA). The 35S-UTP-labeled cRNA probes for *Mkp1* mRNA were generated using a cDNA template from a portion of the *Rattus norvegicus* gene *Dusp1* (accession number AF357203) corresponding to a 206 nucleotide mRNA sequence (positions 817–1022 from the origin) that was cloned within the Spencer lab into a transcription vector (pSCA, Agilent Technologies, Santa Clara, CA, USA). The *Mkp1* cDNA-containing plasmids were subsequently linearized with the restriction endonuclease *Hin*DIII and transcribed using T7 RNA polymerase. The identity of *Mkp1* cloned DNA was verified by DNA sequencing (University of Colorado Molecular, Cellular and Developmental Biology sequencing facility). After the hybridization assay procedure (Girotti *et al*. 2006), slides were exposed to X-ray film (Kodak Biomax MR film) for 14 days. Semiquantitative analyses were performed on digitized images from X-ray films using the linear range of the gray values obtained from an acquisition system (Northern Lights lightbox, model B 95, Ontario, Canada; CCD camera, model XC-77, Sony, Tokyo, Japan; image capture with National Institutes of Health scion Image v1.59 software, Frederick, MD, USA), as previously described (Campeau *et al*. 2002). Brain regions of interest (PVN and prelimbic (PrL) and infralimbic (IL) subregions of mPFC) were identified by matching digitized rat hypothalamic structures to rat brain atlas diagrams (Paxinos *et al*. 1980). Quantification and analysis of images were performed by individuals that were blind to treatment condition assignments. For the PVN, bilateral measurements were taken from four tissue sections (ca. 1.8 mm posterior to bregma) for each rat (4–8 measurements for each rat brain). Measurements were also taken from 4 to 6 sections of the anterior pituitary. For the PrL and IL subregions of the mPFC, bilateral measurements were taken from 2 to 4 tissue sections (ca. 3.2 mm anterior to bregma) for each rat (4–8 measurements for each rat brain). Average uncalibrated optical densities for each region of interest were measured using the program NIH ImageJ (version 1.42q).

Statistical analysis

One-way (Experiment 1) or two-way (Experiments 2 and 3) between groups ANOVA was used to analyze the dependent measures (Statistical Package for the Social Sciences, SPSS, Macintosh computer version). Significant *F*-test results were followed with a Fisher's least significant difference (FLSD) *post-hoc* test in order to assess the statistical significance of differences between pairs of groups. Additionally, a Pearson's *r* correlational analysis was performed (SPSS) for Experiment 2. An alpha-level of $P \leq 0.05$ was used to determine statistical significance. All graphical presentations of data represent group means ± S.E.M.

Results

Experiment 1: PVN *Mkp1* mRNA increased after 15 and 30 min restraint challenge

In this first experiment, we examined whether acute psychological stress (restraint) would lead to a rapid increase in *Mkp1* mRNA levels within the PVN. Under no-stress conditions, there was very little *Mkp1* mRNA detected within the PVN. Restraint substantially increased PVN *Mkp1* mRNA; $F_{(3,11)}=4.2$, $P<0.05$. *Post-hoc* tests indicate that there was a significant increase in PVN *Mkp1* mRNA within 15 min of restraint and a progressively greater increase after 30 min of restraint (Fig. 1).

Experiment 2: restraint increased *Mkp1* mRNA in the PVN, anterior pituitary, and PFC, and this increase in the PVN and anterior pituitary was augmented by the long-term absence of endogenous glucocorticoids (ADX)

In this second experiment, we examined whether acute stress (30 min restraint) would also lead to a rapid increase

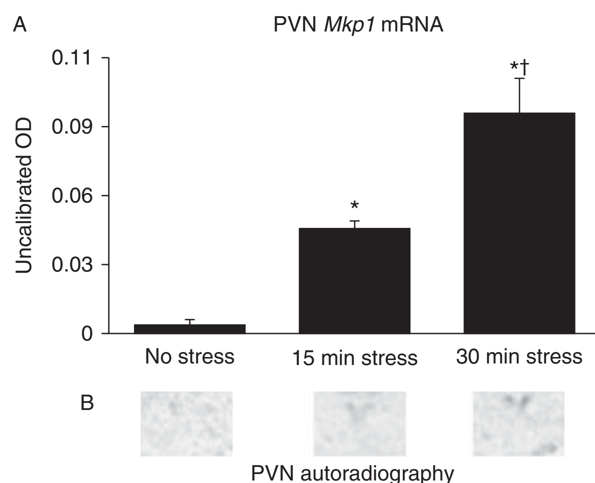

Figure 1
Stress rapidly increased *Mkp1* mRNA in the PVN. (A) *Mkp1* mRNA (optical density of autoradiographs) was significantly increased after 15 min of restraint and was increased to a greater extent after 30 min of restraint. *Significant stress effect compared with the no-stress group; †significant stress effect compared with 15 min stress group, $P<0.05$, FLSD. (B) Representative portions of autoradiographs surrounding the PVN taken from a rat within each treatment group as denoted in the aligned (A) bar graph. *Mkp1* mRNA in the PVN was not detectable on basal condition autoradiographs.

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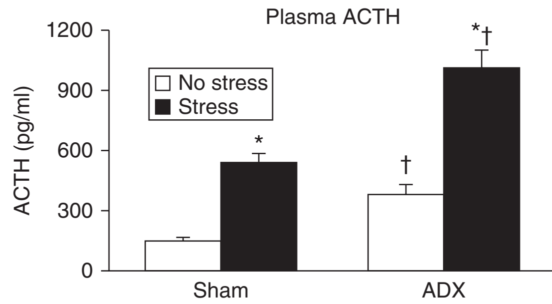

Figure 2
Both basal and stress-stimulated ACTH plasma levels were augmented in ADX rats. Four days after ADX or sham-ADX surgery, rats were challenged with 30 min restraint. *Significant stress effect compared with the no-stress group for the same surgical condition; †significant tonic CORT condition (ADX) effect compared with the sham group for the same stress condition; $P<0.05$, FLSD.

in *Mkp1* mRNA in an additional anatomical element of the HPA axis (the anterior pituitary) as well as in a separate brain region (mPFC). We also examined whether a stress-induced increase in *Mkp1* mRNA depended on stress-induced increases in endogenous CORT. As expected, restraint-challenged rats displayed an increase in ACTH secretion (stress effect: $F_{(1,19)}=78.3$, $P<0.05$), and the absence of glucocorticoids resulted in a substantial increase in basal and stress-stimulated ACTH secretion (ADX effect: $F_{(1,19)}=33.8$, $P<0.05$; Fig. 2). Plasma CORT analysis indicated that sham-ADX non-stressed rats displayed normal low-basal levels of CORT ($M=48.8$, $\pm$s.e.m. 16.0 ng/ml) and stressed sham-ADX rats had a significant increase in plasma CORT levels ($M=464.4$, $\pm$s.e.m. 21.8 ng/ml). All ADX rats had CORT levels near or below the detection threshold of the assay.

Under non-stressed conditions, *Mkp1* mRNA levels were very low in the PVN and anterior pituitary (Fig. 3). Within the PrL and IL subregions of the mPFC, *Mkp1* mRNA levels were also low, but were clearly visible on autoradiograms (Fig. 4). Sham-operated rats challenged with 30 min of restraint displayed a significant increase in *Mkp1* mRNA expression within PVN (stress effect: $F_{(1,19)}=37.4$, $P<0.05$), anterior pituitary (stress effect: $F_{(1,19)}=29.8$, $P<0.05$), PrL cortex (stress effect: $F_{(1,19)}=14.7$, $P<0.05$), and IL cortex (stress effect: $F_{(1,19)}=42.8$, $P<0.05$). Stress-stimulated CORT levels of sham-operated rats were not significantly correlated with the stress-induced increase in *Mkp1* mRNA levels in the PVN ($r=0.27$; $P>0.05$), PrL cortex ($r=0.34$; $P>0.05$), or IL cortex ($r=-0.063$; $P>0.05$). Interestingly, there was a trend for a negative correlation between CORT levels and stress-induced *Mkp1* mRNA levels in the anterior pituitary

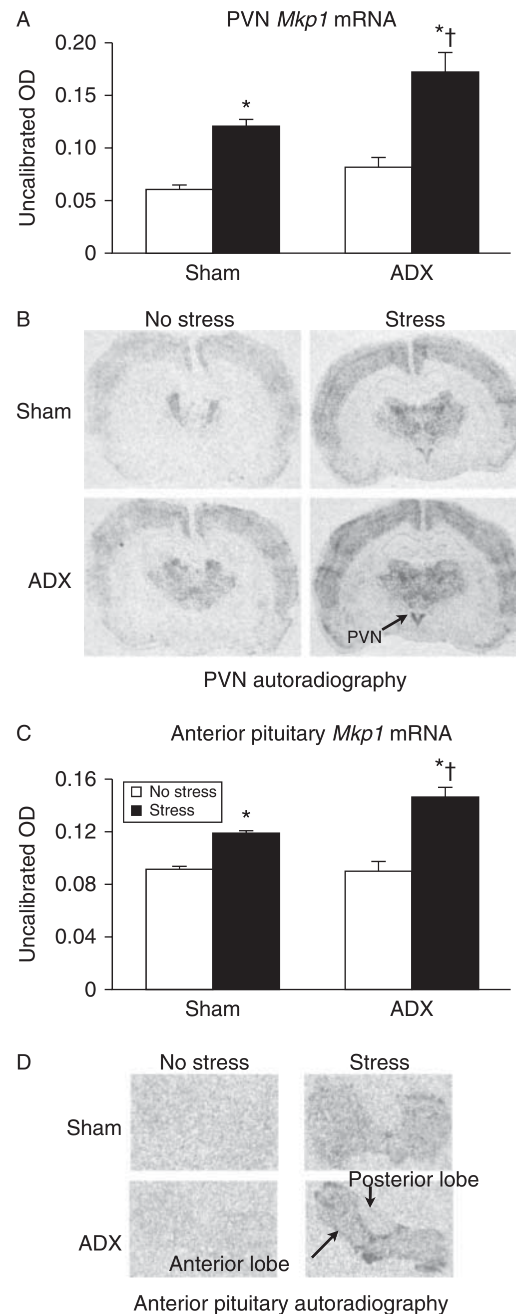

Figure 3
Stress-stimulated *Mkp1* mRNA levels were augmented in PVN and anterior pituitary of ADX rats. (A) PVN and (C) anterior pituitary relative *Mkp1* mRNA levels for sham-operated or ADX rats 30 min restraint challenge 4 days after surgery. *Significant stress effect compared with the no-stress group for the same surgical conditions; †significant tonic CORT condition (ADX) effect compared with the sham group, for the same stress conditions; $P<0.05$, FLSD. Representative autoradiographs for *Mkp1* mRNA expression in (B) PVN (coronal brain section) and (D) anterior pituitary (horizontal section); regions of interest are denoted on a representative autoradiograph taken from a rat from the ADX-Stress group.

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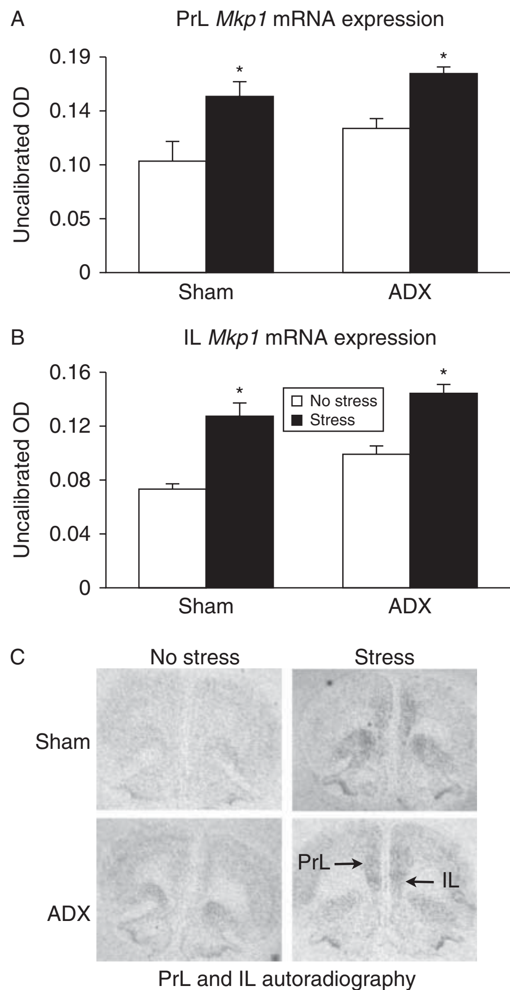

Figure 4
Stress increased *Mkp1* mRNA levels to a similar degree in the mPFC of sham-operated and ADX rats. Four days after ADX or sham-ADX surgery, rats were challenged with 30 min restraint. Restraint increased *Mkp1* mRNA levels in the prelimbic (PrL, A) and infralimbic (IL, B) subregions of the mPFC, and ADX increased basal *Mkp1* mRNA levels in the IL. *Significant stress effect compared with the no-stress group for the same surgical condition; †significant tonic CORT condition (ADX) effect compared with the sham group for the same stress condition; $P<0.05$, FLSD. (C) Representative autoradiographs for *Mkp1* mRNA expression in mPFC (coronal brain section); regions of interest are denoted on a representative autoradiograph taken from a rat from the ADX-stress group. Note that there were low but visible *Mkp1* mRNA levels in the mPFC visible on basal-condition autoradiographs.

($r=-0.64$, $P=0.244$), suggesting that acute CORT may have had an inhibitory influence on stress-induced *Mkp1* mRNA levels. Rather than attenuating stress-induced *Mkp1* mRNA levels, as predicted, ADX augmented stress-stimulated *Mkp1* mRNA levels within the PVN (stress×ADX interaction $F_{(1,19)}=11.0$, $P<0.05$) and anterior pituitary (stress×ADX $F_{(1,19)}=5.1$, $P<0.05$). There was also a trend towards higher stress-induced *Mkp1* mRNA levels in the PrL and IL cortex of ADX rats; however, there was not a significant stress×ADX interaction in either brain region. On the other hand, within the IL cortex there was a significant increase in *Mkp1* mRNA levels of non-stressed ADX rats compared with sham-operated rats (FLSD, $P<0.05$).

Experiment 3: acute CORT treatment was not sufficient to upregulate PVN or mPFC *Mkp1* mRNA levels

Although Experiment 2 demonstrated that an increase in endogenous CORT was not necessary for a stress-induced increase in *Mkp1* mRNA, there is still the possibility that an acute increase in CORT is sufficient to produce an increase in *Mkp1* mRNA in PVN and mPFC, which may be masked by the effect of restraint stress. Thus, this experiment examined the effect of vehicle or CORT injection in the absence of restraint stress on subsequent *Mkp1* mRNA expression. As expected, plasma CORT measures indicated that there was a greater level of plasma CORT present 1 h after CORT injection ($M=149.1$, ±S.E.M. 51.3 ng/ml) compared with vehicle injection ($M=33.4$, ±S.E.M. 13.0 ng/ml). By 3 h after CORT injection, the exogenous CORT had cleared such that plasma CORT levels were low in both CORT-injected rats ($M=7.5$, ±S.E.M. 1.5 ng/ml) and vehicle-injected rats ($M=22.0$, ±S.E.M. 11.2 ng/ml).

We observed no difference in *Mkp1* mRNA levels of CORT vs vehicle-injected rats in either brain region (Fig. 5). Similar to non-stressed conditions in Experiments 1 and 2, we observed almost undetectable levels of *Mkp1* mRNA within the PVN. Within the PrL, there was a moderate level of *Mkp1* mRNA expression present 1 h after injection, but it did not differ between CORT and vehicle treatments. Interestingly, for both CORT and vehicle treatment groups, there was a lower level of *Mkp1* mRNA expression in PrL 3 h after injection compared with 1 h after injection (post injection time: $F_{(1,10)}=2.4$, $P<0.05$), perhaps indicating that the stress of injection produced a transient increase in *Mkp1* mRNA levels in PrL that was evident 1 h, but less so by 3 h after injection. A similar pattern of *Mkp1* mRNA was observed in IL (data not shown).

Discussion

In this study, we found that *Mkp1* mRNA was rapidly increased by acute psychological stress within anatomical elements of the HPA axis (PVN and anterior pituitary) and in a stress-responsive brain region that provides regulatory

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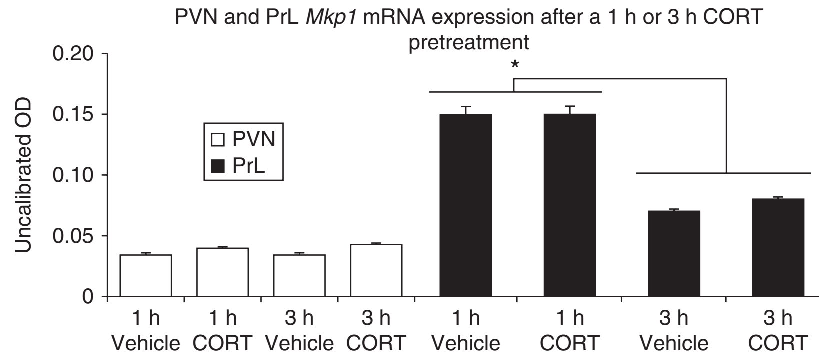

Figure 5
Acute CORT treatment did not increase PVN or prelimbic (PrL) cortex *Mkp1* mRNA levels. Adrenal-intact rats received injections of CORT (2.5 mg/kg, i.p.) or vehicle 1 3 h before they were killed. There was very low *Mkp1* mRNA expression in the PVN for the four treatment groups. There was a moderately high level of *Mkp1* mRNA expression in the PrL subregion of the mPFC 1 h after injection and lower levels 3 h after injection ($P<0.05$), but the levels did not differ between CORT or vehicle-injected rats. *Significant pretreatment-time difference effect in the PrL.

modulation over the HPA axis (mPFC) (Diorio *et al.* 1993, Radley *et al.* 2006, Weinberg *et al.* 2010). Contrary to the predictions based on studies of glucocorticoid regulation of *Mkp1* gene expression in peripheral tissues and cell lines (Clark *et al.* 2008), we found that acute CORT treatment was not sufficient to increase *Mkp1* mRNA within the brain and endocrine tissues examined. Moreover, stress-induced CORT secretion was not necessary for the rapid increase in *Mkp1* mRNA observed after acute stress. Instead, we found that stress-induced *Mkp1* gene expression was augmented within the PVN and anterior pituitary of the rats that lacked endogenous adrenal glucocorticoids. These results suggest that Mkp1 expression is dynamically regulated in brain and neuroendocrine tissue, and that endogenous glucocorticoids may have a tonic suppressive role in regulating *Mkp1* gene expression in these tissues, perhaps by indirectly constraining activity-dependent regulation of MAP-kinase (see discussion below).

A number of studies have found that the *Mkp1* gene behaves as an activity-dependent immediate early gene in response to a wide variety of stimuli within various peripheral cell types and transformed cell lines (Clark 2003, Patterson *et al.* 2009, Caunt & Keyse 2013). Initial indication that the *Mkp1* gene may be regulated in a similar fashion within mammalian neural tissue was provided by studies that observed a rapid increase in *Mkp1* mRNA in striatal and hippocampal subregions of the rodent brain after direct electrical stimulation or electroconvulsive seizure (Sgambato *et al.* 1998, Davis *et al.* 2000, Kodama *et al.* 2005). Subsequently, activity-dependent *Mkp1* gene induction in the mammalian brain has also been observed in the suprachiasmatic nucleus in response to a light pulse during the subjective night (Doi *et al.* 2007). Our first experiment showed that PVN *Mkp1* gene expression was rapidly increased within 15 min of restraint, an acute stress challenge that is predominantly psychological in nature (Herman & Cullinan 1997, Dayas *et al.* 2001). Our second experiment showed that this same stressor produced a rapid increase in *Mkp1* mRNA within the anterior pituitary and mPFC (PrL and IL). Despite the contrasting influence that the PrL and IL cortex have over the PVN (inhibitory and excitatory respectively) (Radley *et al.* 2006), both subregions of the medial-PFC display stress-dependent rapid induction of other experience-dependent genes such as *c-Fos* and *Fra2* (*FOSL2*) (Weinberg *et al.* 2007). We observed nearly undetectable levels of *Mkp1* mRNA within the PVN and the anterior pituitary under no-stress conditions, whereas there was a greater degree of constitutive *Mkp1* mRNA expression within the mPFC. Moreover, our third experiment indicates that there may be a greater sensitivity of *Mkp1* mRNA induction to the mild stress of vehicle injection in the mPFC than in the PVN. Overall, our study extends the extent of known activity-dependent operation of the *Mkp1* gene to neural and endocrine elements of the HPA axis and to the mPFC. In addition, our results indicate that a moderate-intensity stressor is an effective experience for producing a rapid and substantial increase in *Mkp1* mRNA in the adult rat brain.

In this study, we explored the possibility that CORT serves as both an intercellular and intracellular stress-dependent signal for *Mkp1* gene induction (Clark 2003, Clark *et al.* 2008). We found, however, that the presence of endogenous CORT was not necessary for the stress-induced increase in *Mkp1* mRNA within the tissue examined. In addition, acute CORT treatment was not

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sufficient to increase *Mkp1* mRNA levels. An *in vitro* study has also indicated that glucocorticoid treatment alone does not increase *Mkp1* gene expression, but that study revealed that glucocorticoid treatment augmented *Mkp1* gene induction in response to other cellular stimuli (Zhou *et al.* 2007). In contrast to the finding of that study, we observed an opposite effect. In the anatomical elements of the HPA axis there was a greater stress-induced increase in *Mkp1* mRNA in rats lacking endogenous CORT. It should be noted that the mPFC, PVN, and anterior pituitary all express high levels of GR (Gustafsson *et al.* 1987, Herman 1993, Francis *et al.* 2006). Furthermore the levels of CORT typically secreted in response to restraint are sufficient to activate GR in those tissues (Reul & de Kloet 1985, Spencer 1993). Thus, we conclude that CORT-activated GR does not contribute to a rapid induction of *Mkp1* mRNA in the PVN, mPFC, and anterior pituitary.

Several functional glucocorticoid response elements (GREs) have recently been identified in the distal promoter region of the human *Mkp1* gene (Shipp *et al.* 2010, Tchen *et al.* 2010). Although some of those putative GREs exhibit high homologous nucleotide sequences at approximately the same upstream locations as for the rat *Mkp1* gene, they fail to exhibit similar functional responsiveness to glucocorticoids when assessed in gene transcription reporter assays (Tchen *et al.* 2010). Whether glucocorticoids influence transcriptional processes is often dependent on cellular function and location. For example, glucocorticoids provide suppressive transcriptional regulation of the *Crh* gene in the PVN CRH neuroendocrine neurons (Swanson & Simmons 1989, Ginsberg *et al.* 2003, Bali *et al.* 2008, Sharma *et al.* 2013), but in other forebrain regions, such as the amygdala and bed nucleus of the stria terminalis, glucocorticoids either upregulate *Crh* gene expression or have no effect (Makino *et al.* 1995, Schulkin *et al.* 1998). Glucocorticoid regulation of the *Mkp1* gene may also vary with cell phenotype and cell state, and these factors may differ across species (Tchen *et al.* 2010).

The profile of glucocorticoid regulation of *Mkp1* gene expression in the PVN that we observed in this study is similar to what we have seen in previous studies for the immediate early genes *c-Fos*, *Zif268* and *Ngfi-b* (Girotti *et al.* 2007, Pace *et al.* 2009). Whereas the absence of endogenous glucocorticoids produced a large increase in both basal and stimulated ACTH hormone levels, for each of these immediate early genes only stimulated levels were augmented in the PVN of ADX rats. This expression profile may indicate that glucocorticoids indirectly regulate the expression of Mkp1 and other immediate early genes within the PVN. One possible mechanism for this indirect effect could be alteration of CRH neuron responses to acute stress by altering CRH receptor (CRH-R1) expression within PVN CRH neurons (Luo *et al.* 1995, Imaki *et al.* 2001). For example, ADX has been shown to increase CRH-R1 expression in the rat PVN (Luo *et al.* 1995). Thus, the long-term absence of CORT may lead to an augmented positive CRH feedback loop onto PVN CRH neurons, resulting in increased signaling activity (e.g., MAP-kinase activation), transcriptional processes (e.g., *Mkp1* gene expression), and neuropeptide release (e.g., CRH) within these neuroendocrine cells. Regardless of the mechanism, there appears to be some process by which the tonic presence of glucocorticoids constrains the general reactivity of these cells to excitation (Pace *et al.* 2009, Osterlund & Spencer 2011, Osterlund *et al.* 2011, Weiser *et al.* 2011).

Although the specific process remains undetermined, the augmented *Mkp1* gene expression in the absence of glucocorticoids may be secondary to an augmentation of ERK1/2 activation in CRH neurons. We have previously observed an increased number of stress-induced phospho-ERK1/2-positive cells in the PVN of ADX rats, which was normalized by giving ADX rats CORT in their drinking water (Osterlund *et al.* 2011). MAP-kinase pathway activation has been found to converge on *Mkp1* gene expression in a number of cell types (Brondello *et al.* 1997, Camps *et al.* 2000, Kassel *et al.* 2001). This *Mkp1* gene induction may serve as a form of intracellular negative feedback control over the pluripotent MAP-kinase intracellular signaling network. There is some evidence that in the neural tissue the activity-dependent *Mkp1* gene induction depends specifically on ERK1/2 (Sgambato *et al.* 1998, Jeanneteau *et al.* 2012). Interestingly, in cultured cortical neurons, ERK1/2 activation was necessary for activity-dependent *Mkp1* gene induction, with the subsequent upregulated MKP1 protein responsible for negative regulation of the phosphorylation state and function of a different MAP-kinase (c-jun N-terminal kinase) (Jeanneteau *et al.* 2012). Basal CORT levels may not only constrain *Mkp1* expression within the HPA axis, they may also have an important modulatory influence on *Mkp1* gene expression in a variety of extrinsic hypothalamic forebrain regions. In this study, we saw a trend towards greater stress-induced *Mkp1* mRNA in the mPFC of ADX rats. In addition, there was a significant increase in basal *Mkp1* mRNA in the IL subregion of the mPFC of ADX rats.

Our results, taken together with others, indicate that the *Mkp1* gene may be induced in a wide-range of brain regions by an extensive set of experiential conditions.

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Thus, the activity dependence of *Mkp1* gene expression may resemble that of some other activity-dependent immediate early genes, such as *c-Fos*, *Zif268*, and *Arc*, which are critical for experience-dependent neuroplasticity (Guzowski *et al*. 2001, Loebrich & Nedivi 2009, Okuno 2011). Given the importance of the MAP-kinase intracellular signaling network for neuroplasticity in the developing and adult brain, it would not be surprising that tight regulation of this molecular network is critical for optimal function (Sweatt 2001, Waltereit & Weller 2003, Davis & Laroche 2006, Ayroldi *et al*. 2012). Mice lacking the *Mkp1* gene exhibit a range of heightened inflammatory processes, presumably due to the over-activity of MAP-kinases (Chi *et al*. 2006, Maier *et al*. 2007, Wang *et al*. 2008, Patterson *et al*. 2009, Vandevyver *et al*. 2012). This anti-inflammatory role of MKP1 may extend to brain microglia (Zhou *et al*. 2007). Cortical neurons cultured from *Mkp1* gene-knockout mice are deficient in their axonal branching response to brain-derived neurotrophic factor (Jeanneteau *et al*. 2012). Support for the importance of *MKP1* gene expression levels in human brain function is provided by a study that found increased *Mkp1* mRNA levels in the postmortem brains of individuals with major depressive disorder (Duric *et al*. 2010). The dynamic interaction between MAP-kinases and MKP1 appears to be an important element of neural function, and may be an effective pharmacotherapeutic target. Further study of how that interaction may be modulated by the tonic changes in glucocorticoids associated with chronic stress and various mental and physical disorders is warranted.

Declaration of interest

The authors declared no conflict of interest that could be perceived as prejudicial to the impartiality of the research reported.

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