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Non-Genomic Glucocorticoid Suppression of a Postsynaptic Potassium Current via Emergent Autocrine Endocannabinoid Signaling in Hypothalamic Neuroendocrine Cells following Chronic Dehydration

Postsynaptic glucocorticoid-cannabinoid signaling

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ABSTRACT

Glucocorticoids rapidly stimulate endocannabinoid synthesis and modulation of synaptic transmission in hypothalamic neuroendocrine cells via a nongenomic signaling mechanism. The endocannabinoid actions are synapse-constrained by astrocyte restriction of extracellular spatial domains. Exogenous cannabinoids have been shown to modulate postsynaptic potassium currents, including the A-type potassium current (I_A), in different cell types. The activity of magnocellular neuroendocrine cells is shaped by a prominent I_A. We tested for a rapid glucocorticoid modulation of the postsynaptic I_A in magnocellular neuroendocrine cells of the hypothalamic paraventricular nucleus (PVN) using whole-cell recordings in rat brain slices. Application of the synthetic glucocorticoid dexamethasone (Dex) had no rapid effect on the I_A amplitude, voltage dependence, or kinetics in magnocellular neurons in slices from untreated rats. In magnocellular neurons from salt-loaded rats, however, Dex application caused a rapid suppression of the I_A and a depolarizing shift in I_A voltage dependence. Exogenously applied endocannabinoids mimicked the rapid Dex modulation of the I_A and CB1 receptor antagonists and agonists blocked and occluded the Dex-induced changes in the I_A, respectively, suggesting an endocannabinoid dependence of the rapid glucocorticoid effect. Preincubation of control slices in a gliotoxin resulted in the partial recapitulation of the glucocorticoid-induced rapid suppression of the I_A. These findings demonstrate a glucocorticoid suppression of the postsynaptic I_A in PVN magnocellular neurons via an autocrine endocannabinoid-dependent mechanism, and suggest a possible role for astrocytes in the control of the autocrine endocannabinoid actions.

SIGNIFICANCE STATEMENT

Stress causes elevated levels of glucocorticoid hormones and rapid and delayed glucocorticoid feedback effects in the brain. Glucocorticoids regulate synaptic inputs to hypothalamic neuroendocrine cells via a non-genomic release of endocannabinoid. We report a non-genomic glucocorticoid modulation of a postsynaptic A-type potassium current in magnocellular neurons via a novel autocrine endocannabinoid mechanism. The A-current modulation by glucocorticoids occurred in neurons from rats subjected to chronic dehydration via salt loading, but not in neurons from normally hydrated rats. Our findings suggest that chronic dehydration leads to an glucocorticoid-induced endocannabinoid autocrine signaling in magnocellular neuroendocrine cells. The neuroplastic mechanisms for this emergent signaling may be related to neuronal-glial structural plasticity or to changes in rapid postsynaptic glucocorticoid and/or endocannabinoid actions.

Osmotic challenge elicits a neuroendocrine stress response that results in an increase in the circulating level of glucocorticoids (Roberts et al., 2011). Stress levels of glucocorticoids cause endocannabinoid synthesis in PVN magnocellular and parvocellular neuroendocrine cells (Di et al., 2003; Di et al., 2005a; Malcher-Lopes et al., 2006; Di et al., 2009). Endocannabinoids are synthesized from lipid precursors in neuronal membranes and are released canonically as retrograde signals to regulate the presynaptic release of glutamate and GABA (Di Marzo, 2011). Endocannabinoid synthesis is elicited in magnocellular neuroendocrine cells of the hypothalamus by depolarization (Hirasawa et al., 2004; Di et al., 2005b) and in response to oxytocin (Oliet et al., 2007), as well as in response to rapid glucocorticoid actions (Di et al., 2003; Di et al., 2005a). Glucocorticoid- and depolarization-induced retrograde endocannabinoid release from MNCs of the hypothalamic paraventricular nucleus (PVN) and supraoptic nucleus causes a synapse-specific suppression of glutamate release at excitatory synapses (Tasker, 2006; Tasker et al., 2006). Restriction of the retrograde endocannabinoid actions exclusively to glutamate synapses is controlled by astrocytes, since salt-loading-induced neuronal-glial plasticity and pharmacologic inhibition of glial cell metabolism lead to spillover actions of endocannabinoids at neighboring GABA synapses (Di and Tasker, 2013).

While endocannabinoids have been identified predominantly as retrograde messengers that regulate presynaptic neurotransmitter release, they also have been reported to modulate postsynaptic potassium channels (Deadwyler et al., 1995; Mackie et al., 1995; Schweitzer, 2000). Glucocorticoids also have been shown to rapidly regulate postsynaptic properties by inhibiting potassium currents in PVN neurons (Zaki and Barrett-Jolley, 2002) and hippocampal neurons (ffrench-Mullen, 1995; Olijslagers et al., 2008) and by modulating calcium-dependent potassium

channels in pituitary GH3 and AtT-20 cells (Huang et al., 2006). The mechanisms of these postsynaptic actions of glucocorticoids have not been characterized.

The A-type potassium current (I_A) is a prominent voltage-gated conductance in PVN magnocellular neurons that influences magnocellular neuron firing properties (Luther and Tasker, 2000; Ellis et al., 2007). Water deprivation or chronic salt loading induces dramatic structural and functional plasticity among magnocellular neuroendocrine cells and astrocytes of the hypothalamic PVN and supraoptic nucleus (Tasker et al., 2017). This plasticity results in functional changes in magnocellular neuron electrical activity that leads generally to an increase in excitability (Tasker et al., 2002). Here, we investigated whether glucocorticoids rapidly modulate the I_A of magnocellular neurons in a transcription-independent fashion via putative autocrine endocannabinoid signaling, and whether the rapid glucocorticoid modulation of the I_A is altered with chronic osmotic stress via salt loading.

Methods

Animals

Male Sprague Dawley rats (5-6 weeks, Harlan, Indianapolis, IN) were used with the approval of the Tulane University Animal Care and Use Committee and in accordance with United States Public Health Service guidelines. Rats were dehydrated via chronic salt loading by restricting them to drinking water with 2% NaCl for 6-8 days. Untreated control rats were age-matched and were provided with regular tap drinking water.

Hypothalamic slice preparation

Rats were decapitated with a rodent guillotine under deep halothane anesthesia. The brain was quickly removed and placed into ice-cold artificial cerebrospinal fluid (aCSF) containing (in mM): 140 NaCl, 3 KCl, 1.3 $MgSO_4$, 1.4 NaH_2PO_4, 2.4 $CaCl_2$, 11 glucose, and 5 HEPES; the pH was adjusted to 7.2–7.3 with NaOH, the osmolarity was 290-300 mOsm, and the aCSF was bubbled with 100% oxygen. The hypothalamus was blocked and the caudal surface of the tissue block was glued to the chuck of a vibrating tissue slicer. Two coronal hypothalamic slices 300 µm in thickness and containing the PVN were sectioned, bisected at the midline, and submerged in a holding chamber in oxygenated aCSF at room temperature, where they were allowed to equilibrate for >1.5 hours before being transferred to a recording chamber.

Electrophysiology

Patch pipettes were pulled from borosilicate glass with a Flaming/Brown P-97 micropipette puller (Sutter Instruments, Novato, CA) to a tip resistance of 3-4 MΩ. They were filled with a solution containing (in mM): 120 K-gluconate, 10 KCl, 1 NaCl, 1 $MgCl_2$, 1 EGTA, 2 Mg-ATP, 0.3 Na-GTP, and 10 HEPES; the pH was adjusted to 7.2 with KOH and the osmolarity was adjusted to 300 mOsm with 20 mM D-sorbitol. For recordings, single hemi-slices were transferred from the holding chamber to a submersion recording chamber, where they were perfused with oxygenated aCSF at a rate of 2 ml/min and allowed to equilibrate for at least 15 min before starting the recordings. PVN neurons were visualized on a video monitor with a cooled CCD camera using infrared illumination and differential interference contrast optics, and were patch-clamped under visual control. After achieving the whole-cell configuration, the series resistance was compensated by ≥ 60% and the series resistance and whole-cell capacitance were

continuously monitored during experiments. Magnocellular neurons in the PVN were identified based on their location, size, morphology and electrophysiological properties (i.e., prominent I_A in voltage clamp and transient outward rectification in current clamp) (Tasker and Dudek, 1991; Luther and Tasker, 2000). Recordings were performed using a Multiclamp 700A amplifier (Molecular Devices, Sunnyvale, CA). Data were low-pass filtered at 2 kHz with the amplifier and sampled at 10 kHz using the pClamp 9 data acquisition and analysis software package (Molecular Devices, Sunnyvale, CA).

Prior to experiments, cells were tested in current-clamp mode and excluded from analyses if they did not meet the following criteria: action potential amplitudes ≥50 mV from the threshold to peak, an input resistance at resting potential of at least 500 MΩ, a resting membrane potential negative to -50 mV, and a characteristic transient outward rectification (Tasker and Dudek, 1991; Luther and Tasker, 2000). Slices were then bathed in aCSF with tetrodotoxin (TTX, 1 μM) and $CdCl_2$ (200 μM) to block voltage-gated sodium channels and calcium channels, respectively, and the recording configuration was switched to voltage clamp. The liquid junction potential (calculated at 15 mV) was corrected *post hoc* during data analysis. All voltage-clamp recordings were leak subtracted using a P/4 protocol. Series resistance compensation of ≥ 60% was routinely applied and changes in series resistance were monitored and compensated for throughout the experiments. The series resistance at the beginning of recordings was less than 20 MΩ and recordings were discarded if changes in series resistance greater than 20% occurred during the recordings.

Voltage-dependence of activation and inactivation of the I_A and I_K

We used two separate voltage protocols to isolate the I_A in PVN magnocellular neurons (Fig. 1A). The first protocol included a 200-ms hyperpolarizing conditioning step to -115 mV, which removed the inactivation of the I_A, followed by sequential 200-ms depolarizing test pulses to between -75 mV and +35 mV in 10-mV increments, which resulted in the activation of both the I_A and I_K. The second protocol consisted of the same series of test pulses, but from a 200-ms depolarizing conditioning step to -45 mV. The I_K was isolated from the I_A in the second protocol because the I_A in magnocellular neurons is inactivated at -45 mV (Luther and Tasker, 2000). The I_A was isolated by digitally subtracting the current responses generated by the second protocol from those generated by the first protocol, which removed the I_K, leak current and capacitive currents. The inactivation of the I_A was then studied with a third voltage protocol, consisting of sequential 200-ms conditioning steps to between -135 mV and -25 mV in 10-mV increments, which removed a variable amount of inactivation of I_A, followed by a command step to -15 mV. We introduced a small step, to -75 mV for 5 ms, between the conditioning steps and the test steps in both protocols, which allowed all test steps to be delivered from the same initial voltage level. This way the voltage-clamp artifact was the same size and pulses were all more conveniently subtracted from each other or compared. We tested the effect of this short pretest step on I_A and did not observe it to change I_A significantly, since very little I_A was activated at -75 mV and 5 ms was not sufficient to inactivate I_A (Luther and Tasker, 2000).

Drug application

Water-soluble forms of the steroids dexamethasone (dexamethasone-cyclodextrin complex, Dex) (1 μM**)** and corticosterone (corticosterone, 2-hydroxypropyl-β-cyclodextrin, 1 μM) (Sigma-

Aldrich, St. Louis, MO) were directly dissolved in aCSF to their final concentrations and applied in the bath perfusion. The Dex-bovine serum albumin (BSA) conjugate (10 μM) was dissolved in aCSF with 25% β-cyclodextrin (Sigma-Aldrich) as a carrier to increase its solubility. The concentration of Dex-BSA (10 μM) was selected to obtain an effective concentration of Dex of ~ 1 μM, as the BSA conjugate had a steroid-to-BSA ratio of 8:1. Tetrodotoxin (TTX, 1 μM) (Sigma-Aldrich) and $CdCl_2$ (200 μM) (Sigma-Aldrich) were dissolved in sterile water and stored in 10 mM stock solutions at -20°C, and were dissolved to their final concentrations in aCSF immediately prior to bath application. The endogenous cannabinoids, anandamide (AEA) and 2-arachidonoylglycerol (2-AG) (Tocris), were dissolved in DMSO and stored in 10 mM stock solutions at -20°C, and were dissolved to their final concentrations in aCSF just before their application in the bath. The cannabinoid receptor inverse agonists *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251) (1 μM) (Tocris) and rimonobant (SR141716, 1 μM) (kindly provided by the NIMH Chemical Synthesis and Drug Supply Program) were stored as 10 mM stock solutions in DMSO at -20°C and dissolved to their final concentrations in aCSF before bath application. The vanilloid receptor agonist, capsaicin (Tocris), and antagonist, capsazepine (Tocris), were stored as 10 mM stock solutions in DMSO at -20°C and dissolved to their final concentrations in aCSF before bath application. The protein synthesis inhibitor, cycloheximide (CHX) (Tocris) was dissolved in sterile water and stored in a 10 mM stock solution at -20°C. Fluorocitrate (D,L-fluorocitric acid, barium salt, 100 μM) (Sigma) was dissolved in aCSF with 15 min of sonication. The DMSO and β-cyclodextrin vehicles without the cannabinoids or glucocorticoids had no effect on the waveform of the I_A.

192 **Statistical methods and curve fitting**

193 Values are expressed as means ± standard errors of the mean. Statistical analyses were
194 performed using a two-way ANOVA with the Bonferroni multiple comparisons test for between-
195 group comparisons and the Student's paired *t* test for within-cell comparisons. Differences were
196 considered significant at $p < 0.05$. Boltzmann and exponential fits were used to fit data plots and
197 current traces using the fitting methods provided in the Graphpad Prism software (Graphpad
198 Software, La Jolla, CA) and Clampfit 9 (pCLAMP 9, Molecular Devices, Sunnyvale, CA),
199 respectively.

200

201 ## Results

202 **Rapid glucocorticoid modulation of I_A**

203 Corticosteroids rapidly modulate postsynaptic voltage-gated potassium currents in
204 hippocampal pyramidal neurons (Olijslagers et al., 2008). Magnocellular neuroendocrine cells of
205 the hypothalamic paraventricular nucleus (PVN) generate a prominent A-type potassium current
206 (I_A) that shapes their pattern of action potential firing (Luther and Tasker, 2000; Ellis et al.,
207 2007). We first tested for a rapid effect of glucocorticoids on the activation and inactivation of
208 the I_A in magnocellular neurons. The passive properties of the recorded magnocellular neurons
209 are presented in Table 1. The synthetic glucocorticoid dexamethasone (Dex), applied at a
210 concentration determined in previous studies to be near-saturating for rapid actions on synaptic
211 transmission (1 μM) (Di et al., 2003; Di et al., 2016), had no effect on the I_A current. Based on a
212 two-way ANOVA ($p > 0.05$, $n = 9$), there was no change at the end of a 10-min bath application
213 of Dex in the I_A mean current amplitude, the I_A current density, or the voltage dependence of I_A
214 activation (Fig. 1B-E). Dex also had no effect on the activation kinetics of the I_A (10-90% rise

time measured with voltage steps from -115 mV to -25 mV; $p > 0.05$, Student's paired *t* test) (Fig. 1F) or on the voltage dependence of I_A inactivation ($p > 0.05$, two-way ANOVA) (Fig. 1G and H). The rate of inactivation was examined by fitting the I_A decay phase with a single exponential function to determine the inactivation time constant, which was also unchanged by Dex treatment (data not shown; $p > 0.05$, Student's paired *t* test). Dex had no effect on the amplitude, current density or voltage dependence of activation of I_K ($p > 0.05$, ANOVA; data not shown).

Chronic dehydration causes loss of glial coverage, enhanced glutamatergic, GABAergic and noradrenergic synaptic regulation of magnocellular neurons, and altered voltage-gated currents (Tasker et al., 2017). We next tested the possibility that glucocorticoid modulation of the I_A in PVN magnocellular neurons is altered under conditions of chronic dehydration caused by salt loading. Five to 7 days of salt loading caused an increase in the membrane capacitance and a decrease in the input resistance of magnocellular neurons (Table 1), which are characteristic of dehydration-induced hypertrophy in the magnocellular neurons (Miyata and Hatton, 2002; Di and Tasker, 2004; Shah et al., 2014). Unlike neurons from untreated rats, bath application of Dex (1 μM) to PVN magnocellular neurons ($n = 15$) in slices from salt-loaded rats caused decreases in both the I_A current amplitude and the I_A current density ($p < 0.05$, 2-way ANOVA followed by the Bonferroni multiple comparisons test for each test step) (Fig. 2A-C) and shifted the activation curve of I_A positive by 4.9 mV ($p < 0.01$) (Fig. 4D) within 10 min. The I_A 10-90% rise time (Fig. 4E) and the voltage dependence of I_A inactivation (Fig. 4F, G) were unchanged. Like magnocellular neurons from untreated rats, Dex had no effect on the amplitude, current density, or voltage dependence of activation of I_K in magnocellular neurons recorded in slices from salt-loaded rats (data not shown).

The endogenous glucocorticoid corticosterone had a similar effect on the I_A activation in MNCs from dehydrated rats. A 10-min bath application of corticosterone (1 μM) reduced the I_A mean current amplitude ($p < 0.01$, 2-way ANOVA, Bonferroni's test) (Fig. 3A) and induced a 4.3-mV positive shift in the I_A half-activation potential in MNCs from dehydrated rats ($p < 0.05$, $n = 10$; Student's unpaired *t* test) (Fig. 3B). Corticosterone had no effect on the voltage dependence of inactivation or on the inactivation time constant of I_A (data not shown).

Rapid glucocorticoid modulation of synaptic inputs to hypothalamic magnocellular and parvocellular neuroendocrine cells are mediated by a membrane-associated receptor (Di et al., 2003; Di et al., 2005a). Here, we investigated whether the rapid glucocorticoid modulation of the I_A in PVN magnocellular neurons from dehydrated rats is also mediated by a membrane-associated glucocorticoid receptor. Bath application of the membrane-impermeant Dex-bovine serum albumin (Dex-BSA) conjugate (10 μM), like Dex, reduced the mean current amplitude ($p < 0.01$, two-way ANOVA followed by Bonferroni multiple comparisons test for each test step, $n = 7$) (Fig. 3C) and caused a 3.4-mV positive shift in the I_A half-activation potential ($p < 0.01$, $n = 7$; Student's unpaired *t* test) (Fig. 3D). This implicated a membrane site of steroid action. We used a 10-fold higher concentration of Dex-BSA because the Dex-to-BSA ratio of the Dex-BSA compound was 8 to 1, and we assumed that a single Dex molecule per Dex-BSA was available to bind to the receptor. The weaker effect of the Dex-BSA compared to Dex could be due to a lower effective concentration of available Dex to bind to the receptor or to a steric hindrance of Dex binding by the Dex-BSA three-dimensional structure. We have shown recently that free molecules of Dex do not dissociate from the Dex-BSA conjugate to penetrate into the cell (Weiss et al., in press).

To determine whether the rapid glucocorticoid effect on I_A in MNCs from dehydrated rats is independent of *de novo* protein synthesis, we tested the sensitivity of the Dex effect to the protein synthesis inhibitor cycloheximide. Following a 30-min pre-incubation of slices in cycloheximide (10 μM), the Dex-induced suppression of I_A amplitude ($p < 0.05$, two-way ANOVA, Bonferroni's *post-hoc* test, n = 7) (Fig. 3E) and positive shift in I_A half-activation potential (by 3.2 mV) ($p < 0.01$, n = 7; Student's unpaired *t* test) (Fig. 3F) within 10 min were maintained. This indicated that the Dex effect on I_A does not require protein synthesis and is mediated by a non-transcriptional mechanism.

Endocannabinoid dependence of the glucocorticoid modulation of the I_A

Rapid glucocorticoid modulation of glutamatergic synaptic inputs to magnocellular and parvocellular neuroendocrine cells of the hypothalamus are mediated by the retrograde release of an endocannabinoid (Di et al., 2003; Di et al., 2005a). Here, we tested for the endocannabinoid dependence of the rapid glucocorticoid modulation of the postsynaptic I_A in PVN magnocellular neuroendocrine cells from untreated rats. Bath application of the endocannabinoid 2-AG (1 μM) caused a decrease in the mean I_A peak amplitude ($p < 0.05$, 2-way ANOVA, Bonferroni multiple comparisons test, n = 16) (Fig. 4A) and a 5-mV positive shift in the I_A half-activation potential ($p < 0.01$, Student's paired *t* test, n = 16) (Fig. 4B) in PVN magnocellular neurons. This effect of 2-AG on the I_A was blocked by the CB1 receptor inverse agonist SR141716 (1 μM, n = 6) (Fig. 4C and D).

The other main endocannabinoid, anandamide (AEA), activates both CB1 receptors and transient receptor potential-vanilloid 1 (TRPV1) receptors (Marinelli et al., 2003; Derbenev et al., 2006), so we blocked TRPV1 receptors with the TRPV1 receptor antagonist capsazepine during

AEA application to test AEA modulation of I_A via activation of the CB1 receptor. Preapplication of capsazepine (1 μM) 10 min prior to AEA application had no effect on the I_A in magnocellular neurons. AEA (1 μM) added to the capsazepine in the bath solution caused a decrease in the I_A peak amplitude ($p < 0.01$, 2-way ANOVA, Bonferroni multiple comparisons test, $n = 7$) (Fig. 4F) and induced a 10-mV positive shift in the I_A half-activation potential ($p < 0.01$, Student's paired *t* test, $n = 7$) (Fig. 4G). The TRPV1 agonist capsaicin (1 μM) had no effect on the voltage dependence of I_A activation (Fig. 4H). These data indicate that CB1 receptor activation with endocannabinoids modulates I_A activation in a manner similar to glucocorticoids.

Unlike glucocorticoids, which had no effect on the I_A in magnocellular neurons from normally hydrated rats, exogenously-applied endocannabinoids induced similar effects on the I_A in magnocellular neurons from both normally hydrated and salt-loaded rats. Similar to magnocellular neurons from untreated rats, exogenous application of 2-AG (1 μM) to magnocellular neurons from salt-loaded rats caused a significant decrease in the mean peak current amplitude ($p < 0.05$, 2-way ANOVA, Bonferroni multiple comparisons test) and shifted the activation curve of I_A to the right by 3.9 mV ($p < 0.05$, Student's paired *t* test) (compared to 5 mV in untreated rats). Exogenous application of the other main endocannabinoid, AEA (0.5 μM), also decreased the I_A peak amplitude ($p < 0.05$, 2-way ANOVA, Bonferroni multiple comparisons test, $n = 11$), and shifted the I_A half-activation potential positive by 4.1 mV ($p < 0.01$, Student's paired *t* test, $n = 11$) in PVN magnocellular neurons from salt-loaded rats.

We next tested whether the postsynaptic modulation of the I_A by glucocorticoids in magnocellular neuroendocrine cells from salt-loaded rats is dependent on CB1 receptor activation. The CB1 receptor inverse agonists AM251 (1 μM) and SR141716 (1 μM) by themselves had no effect on I_A amplitude or activation voltage dependence (data not shown), but

both AM251 and SR141716 blocked the Dex-induced decrease in I_A peak current amplitude (Fig. 5A, C) and the rightward shift in the I_A activation curve (Fig. 5B, D) in MNCs in slices from salt-loaded rats, suggesting the involvement of endocannabinoid in the rapid glucocorticoid modulation of I_A.

We then tested whether glucocorticoids and endocannabinoids act through the same signaling pathway or through separate, parallel pathways, by testing for occlusion of the glucocorticoid-induced modulation of I_A by previous endocannabinoid exposure. In slices from salt-loaded rats, 2-AG applied alone caused a decrease in the I_A peak current amplitude ($p < 0.05$, 2-way ANOVA, Bonferroni multiple comparisons test, $n = 4$) and a 5.5-mV positive shift in the I_A half-activation potential ($p < 0.05$, Student's paired *t* test, $n = 4$). The subsequent application of Dex (1 μM), in the presence of 2-AG (1 μM), failed to further decrease the peak I_A amplitude ($p > 0.05$; ANOVA) or to further shift the I_A half-activation potential to the right ($p > 0.05$, Student's paired *t* test, $n = 4$) (Fig. 5E). Similarly, AEA decreased the I_A peak current amplitude ($p < 0.05$, 2-way ANOVA, Bonferroni multiple comparisons test, $n = 4$) and shifted the I_A half-activation potential positive by 5-mV ($p < 0.05$, Student's paired *t* test, $n = 4$). Dex (1 μM) failed to decrease the peak current amplitude further ($p > 0.05$; ANOVA) or elicit any further rightward shift in the I_A half-activation potential in the presence of AEA ($p > 0.05$, Student's paired *t* test, $n = 4$) (Fig. 5F).

Possible glial regulation of the glucocorticoid modulation of I_A

Chronic dehydration causes a structural change in astrocytic morphology that results in a reduction in the coverage of neurons and synapses by astrocytic processes (Miyata et al., 1994; Theodosis et al., 1995; Hatton, 1997; Tasker et al., 2017) and leads to spillover of synaptic

329 signals to extrasynaptic sites (Fleming et al.; Boudaba et al., 2003). We have found that salt
330 loading causes spillover of endocannabinoid from excitatory synapses to inhibitory synapses,
331 which was mimicked by suppressing glial activity with a gliotoxin (Di et al., 2013). The
332 emergence of rapid glucocorticoid modulation of the I_A with dehydration suggests a possible role
333 for glial coverage in the control of autocrine actions of endocannabinoids. To test for a role of
334 astrocyte buffering in the glucocorticoid-endocannabinoid modulation of I_A channels, we used
335 the gliotoxin fluorocitrate to impair astrocyte buffering capability. Fluorocitrate is preferentially
336 taken up by glia and blocks glial metabolic activity, including membrane transport, by inhibiting
337 the citric acid cycle (Clarke, 1991; Gordon et al., 2005). Slices from normally hydrated rats were
338 preincubated for approximately two hours in fluorocitrate (100 μM). Dexamethasone (1 μM) had
339 a small but significant effect on the I_A in PVN magnocellular neurons in slices from untreated
340 rats exposed to fluorocitrate. Whereas Dex had no effect on the I_A peak current amplitude
341 following fluorocitrate treatment ($p > 0.05$, ANOVA, $n = 8$) (Fig. 6A), it caused a small, but
342 significant, shift to the right in the I_A half-activation potential, by 3.2 mV ($p < 0.05$, Student's
343 paired *t* test, $n = 8$) (Fig. 6B). Thus, blocking glial metabolism in slices from untreated rats
344 partially reproduced the effect of *in-vivo* salt loading on the glucocorticoid modulation of the I_A.
345

346 **Discussion**

347 Glucocorticoids rapidly stimulate the synthesis and dendritic release of endocannabinoids,
348 which act retrogradely to modulate glutamate release from excitatory synaptic terminals onto
349 PVN neuroendocrine cells (Di et al., 2005b; Di et al., 2009). In addition to their well-
350 characterized presynaptic actions, cannabinoids have also been shown to modulate postsynaptic
351 potassium currents (Deadwyler et al., 1995; Tang et al., 2005), although, to our knowledge, the

352 regulation of postsynaptic conductances by endogenously released cannabinoids has not been
353 reported. Here, we demonstrate a glucocorticoid-induced suppression of the A-type potassium
354 current in hypothalamic magnocellular neuroendocrine cells that is CB1 receptor-dependent,
355 suggesting a postsynaptic autocrine action of endogenously-released cannabinoid. The
356 glucocorticoid modulation of I_A occurred rapidly, within minutes of its introduction, and reached
357 saturation within 10 minutes. The glucocorticoid effect was maintained with the membrane-
358 impermeant Dex-BSA conjugate, indicating that it was mediated by a membrane-associated
359 receptor, and it was not blocked by inhibiting protein synthesis, suggesting a nongenomic
360 mechanism. Therefore, like the rapid glucocorticoid effects on glutamate and GABA
361 neurotransmission in the PVN (Di et al., 2003; Di et al., 2009), these findings implicate a
362 membrane glucocorticoid receptor in the postsynaptic modulation of I_A.

363 We have performed concentration-response analyses on the rapid Dex- and
364 corticosterone-induced endocannabinoid production and retrograde suppression of excitatory
365 synaptic transmission in magnocellular neurons (Di et al.,). In those studies, we found that the
366 glucocorticoid-induced endocannabinoid synthesis in magnocellular neurons has a half-effective
367 concentration of approximately 350 nM and a saturating concentration near 1 μM. We used the
368 near-saturating concentration of 1 μM for Dex and corticosterone in this study to approximate
369 the high circulating glucocorticoid levels encountered following stress activation of the
370 hypothalamic-pituitary-adrenal (HPA) axis. The 1-μM concentration of Dex and corticosterone
371 we used is equivalent approximately to 350-400 ng/mL of circulating glucocorticoid, which is
372 well within physiological limits. This concentration was ineffective in the magnocellular neurons
373 from untreated rats, which suggested that the effect seen in the magnocellular neurons from salt-
374 loaded rats was not the result of a non-specific action of the steroid.

375 We showed previously that glucocorticoids stimulate the synthesis of endocannabinoids
376 in the PVN (Malcher-Lopes et al., 2006), and that they suppress excitatory synaptic inputs to
377 PVN and SON neurons via the dendritic release and retrograde actions of endocannabinoids (Di
378 et al., 2003; Di et al., 2005a). Here we demonstrate the endocannabinoid dependence of the
379 glucocorticoid modulation of the I_A, which suggests that the glucocorticoid-induced release of
380 endocannabinoids also regulates postsynaptic conductances in these cells and is consistent with
381 cannabinoid modulation of I_A in other brain areas (Deadwyler et al., 1995; Tang et al., 2005). In
382 addition to their retrograde actions, therefore, glucocorticoid-induced endocannabinoids are
383 capable of acting in either an autocrine or a paracrine fashion to regulate postsynaptic potassium
384 currents in the PVN.

385 One possible mechanism of the negative modulation of the I_A by endocannabinoids is
386 that the activation of postsynaptic CB1 receptors triggers a change in the phosphorylation state of
387 the A-type potassium channels. There is a large body of evidence that supports modulation of I_A
388 by phosphorylation (Covarrubias et al., 1994; Birnbaum et al., 2004; Cai et al., 2005), and CB1
389 receptors are known to negatively couple via Gi to adenylyl cyclase activity, cAMP production
390 and PKA-dependent protein phosphorylation (Howlett et al., 2004). A decrease in the PKA-
391 dependent phosphorylation of Kv channel subunits may result in a decrease in the ability of the
392 voltage sensor of the I_A channel to respond to changes in membrane voltage (Park et al., 2006;
393 Yang et al., 2007). Another possibility is that CB1 receptor activation leads to the modulation of
394 one or more of the Kv channel auxiliary proteins, which are well known to regulate the I_A
395 (Covarrubias et al., 1994; Schrader et al., 2009).

396 The rapid glucocorticoid modulation of the I_A was seen only in magnocellular neurons
397 from rats subjected to chronic salt loading, a treatment known to induce the retraction of

astrocytic processes from around magnocellular neurons (Theodosis et al., 1995; Tasker et al., 2017) and result in synaptic spillover of endocannabinoid from excitatory to inhibitory synapses (Di et al., 2013). The emergent sensitivity to rapid glucocorticoid-induced endocannabinoid modulation of the I_A could be caused either by dehydration-induced plasticity of postsynaptic endocannabinoid or glucocorticoid signaling mechanisms or by the loss of glial endocannabinoid buffering due to astrocytic retraction. The neuronal-glial structural plasticity in magnocellular neurons under stimulated conditions (Theodosis et al., 1986; Miyata et al., 1994; Hatton, 1997) is accompanied by increasing levels of ambient neurotransmitters, such as glutamate (Oliet et al., 2001; Boudaba et al., 2003) and increasing heterosynaptic and extrasynaptic spillover of neurotransmitters (Piet et al., 2004). We have found that the glutamate synapse-specific effects of glucocorticoid-induced retrograde endocannabinoid actions (Di et al., 2009) are controlled by glial coverage and that, following dehydration-induced glial retraction, endocannabinoid also accesses and activates CB1 receptors on GABA synapses (Di et al., 2013). Our findings here are consistent with autocrine glucocorticoid-induced endocannabinoid actions that are constrained by glial restriction of extracellular endocannabinoid diffusion. That the I_A modulation by exogenously applied cannabinoids was similar in slices from both untreated and salt-loaded rats suggests that the lack of effect of endogenously released endocannabinoid in slices from untreated rats was not due to a change in the sensitivity of endocannabinoid signaling with dehydration. While these findings do not exclude the possibility of a change in the sensitivity to rapid glucocorticoid signaling with salt loading, they may be explained by dehydration-induced glial retraction that expands the extracellular diffusional reach of the endocannabinoid to allow access to postsynaptic CB1 receptors that are normally inaccessible due to astrocytic coverage. However, a role for astrocytes in limiting the glucocorticoid-induced autocrine endocannabinoid

421 actions is still speculative, as it was only partially supported by our recordings in slices in which
422 glial metabolism, and thus endocannabinoid reuptake, was inhibited by the gliotoxin fluorocitrate
423 (Clarke, 1991; Gordon et al., 2005). Thus, fluorocitrate treatment partially recapitulated the
424 dehydration-induced facilitation of glucocorticoid modulation of the I_A, as Dex shifted the I_A
425 activation curve but did not suppress the peak I_A following fluorocitrate preincubation. Therefore,
426 further experiments are required to conclusively rule out possible plastic changes in
427 glucocorticoid or endocannabinoid signaling in favor of the loss of astrocytic buffering to
428 explain the emergence of rapid glucocorticoid modulation of the I_A with salt loading.

429 In magnocellular neurons from chronically dehydrated rats, we observed a
430 glucocorticoid-induced decrease in the peak amplitude and rightward shift in the voltage
431 dependence of activation of the I_A. The rightward shift in the activation curve of the I_A, with no
432 change in the inactivation voltage dependence, means that a greater depolarizing stimulus is
433 required to activate the transient potassium current and that fewer A-type potassium channels are
434 activated with depolarization in the range of the action potential threshold. The lower peak
435 amplitude of the I_A indicates that significantly less current will be activated with depolarization.
436 The glucocorticoid modulation of the I_A, therefore, should increase the excitability of
437 magnocellular neurons following chronic dehydration. Contrary to our expectations, Dex had no
438 effect on spiking in neurons from salt-loaded rats. This may be because dehydration itself causes
439 a reduction in the I_A current density (Wu and Tasker, unpublished observation), as well as
440 changes in other voltage-gated conductances (Tanaka et al., 1999; Zhang et al., 2007), which
441 makes it difficult to isolate the impact of glucocorticoid modulation of I_A on firing properties.

442 Glucocorticoid suppression of the I_A in magnocellular neurons during chronic
443 dehydration should enhance the effect of synaptic excitation. Glucocorticoid-induced retrograde

endocannabinoid release suppresses presynaptic glutamate release and enhances presynaptic GABA release onto magnocellular neurons from normally hydrated rats (Di et al., 2005a; Di et al., 2009). This should negate any excitatory effect of suppressing the I_A; however, the glucocorticoid facilitation of GABA release is lost during dehydration due to the spillover inhibitory actions of endocannabinoid at GABA synapses (Di et al., 2013). Therefore, the excitation-inhibition balance established by glutamate and GABA release and I_A activation in the dendrites would be tilted toward excitation. This combined with the increased sensitivity to noradrenergic inputs (Di and Tasker, 2004) should enhance or maintain magnocellular neuron activity and neurohypophysial hormone release under conditions of osmotic stress (Hatton, 1997). Collectively, the different effects of glucocorticoids on magnocellular neurons in untreated and chronically dehydrated rats reveal a mechanism for the neuroendocrine system to respond to stress and maintain homeostasis by means of integration of afferent neural circuit information and circulating hormonal signals.

List of References

Birnbaum SG, Varga AW, Yuan LL, Anderson AE, Sweatt JD, Schrader LA (2004) Structure and function of Kv4-family transient potassium channels. Physiol Rev 84:803-833.

Boudaba C, Linn DM, Halmos KC, Tasker JG (2003) Increased tonic activation of presynaptic metabotropic glutamate receptors in the rat supraoptic nucleus following chronic dehydration. J Physiol 551:815-823.

Cai SQ, Hernandez L, Wang Y, Park KH, Sesti F (2005) MPS-1 is a K+ channel beta-subunit and a serine/threonine kinase. Nature neuroscience 8:1503-1509.

Clarke DD (1991) Fluoroacetate and fluorocitrate: mechanism of action. Neurochem Res 16:1055-1058.

Covarrubias M, Wei A, Salkoff L, Vyas TB (1994) Elimination of rapid potassium channel inactivation by phosphorylation of the inactivation gate. Neuron 13:1403-1412.

Deadwyler SA, Hampson RE, Mu J, Whyte A, Childers S (1995) Cannabinoids modulate voltage sensitive potassium A-current in hippocampal neurons via a cAMP-dependent process. J Pharmacol Exp Ther 273:734-743.

Derbenev AV, Monroe MJ, Glatzer NR, Smith BN (2006) Vanilloid-mediated heterosynaptic facilitation of inhibitory synaptic input to neurons of the rat dorsal motor nucleus of the vagus. J Neurosci 26:9666-9672.

Di Marzo V (2011) Endocannabinoid signaling in the brain: biosynthetic mechanisms in the limelight. Nature neuroscience 14:9-15.

Di S, Tasker JG (2004) Dehydration-induced synaptic plasticity in magnocellular neurons of the hypothalamic supraoptic nucleus. Endocrinology 145:5141-5149.

Di S, Popescu IR, Tasker JG (2013) Glial control of endocannabinoid heterosynaptic modulation in hypothalamic magnocellular neuroendocrine cells. J Neurosci 33:18331-18342.

Di S, Malcher-Lopes R, Halmos KC, Tasker JG (2003) Nongenomic glucocorticoid inhibition via endocannabinoid release in the hypothalamus: a fast feedback mechanism. J Neurosci 23:4850-4857.

Di S, Maxson MM, Franco A, Tasker JG (2009) Glucocorticoids regulate glutamate and GABA synapse-specific retrograde transmission via divergent nongenomic signaling pathways. J Neurosci 29:393-401.

Di S, Malcher-Lopes R, Marcheselli VL, Bazan NG, Tasker JG (2005a) Rapid glucocorticoid-mediated endocannabinoid release and opposing regulation of glutamate and gamma-aminobutyric acid inputs to hypothalamic magnocellular neurons. Endocrinology 146:4292-4301.

Di S, Boudaba C, Popescu IR, Weng FJ, Harris C, Marcheselli VL, Bazan NG, Tasker JG (2005b) Activity-dependent release and actions of endocannabinoids in the rat hypothalamic supraoptic nucleus. J Physiol 569:751-760.

Ellis LD, Krahe R, Bourque CW, Dunn RJ, Chacron MJ (2007) Muscarinic receptors control frequency tuning through the downregulation of an A-type potassium current. J Neurophysiol 98:1526-1537.

ffrench-Mullen JM (1995) Cortisol inhibition of calcium currents in guinea pig hippocampal CA1 neurons via G-protein-coupled activation of protein kinase C. J Neurosci 15:903-911.

503 Fleming TM, Scott V, Naskar K, Joe N, Brown CH, Stern JE State-dependent changes in
504 astrocyte regulation of extrasynaptic NMDA receptor signalling in neurosecretory
505 neurons. J Physiol 589:3929-3941.
506 Gordon GR, Baimoukhametova DV, Hewitt SA, Rajapaksha WR, Fisher TE, Bains JS (2005)
507 Norepinephrine triggers release of glial ATP to increase postsynaptic efficacy. Nature
508 neuroscience 8:1078-1086.
509 Hatton GI (1997) Function-related plasticity in hypothalamus. Annu Rev Neurosci 20:375-397.
510 Hirasawa M, Schwab Y, Natah S, Hillard CJ, Mackie K, Sharkey KA, Pittman QJ (2004)
511 Dendritically released transmitters cooperate via autocrine and retrograde actions to
512 inhibit afferent excitation in rat brain. J Physiol 559:611-624.
513 Howlett AC, Breivogel CS, Childers SR, Deadwyler SA, Hampson RE, Porrino LJ (2004)
514 Cannabinoid physiology and pharmacology: 30 years of progress. Neuropharmacology
515 47 Suppl 1:345-358.
516 Huang MH, So EC, Liu YC, Wu SN (2006) Glucocorticoids stimulate the activity of large-
517 conductance Ca2+ -activated K+ channels in pituitary GH3 and AtT-20 cells via a non-
518 genomic mechanism. Steroids 71:129-140.
519 Luther JA, Tasker JG (2000) Voltage-gated currents distinguish parvocellular from
520 magnocellular neurones in the rat hypothalamic paraventricular nucleus. J Physiol 523 Pt
521 1:193-209.
522 Mackie K, Lai Y, Westenbroek R, Mitchell R (1995) Cannabinoids activate an inwardly
523 rectifying potassium conductance and inhibit Q-type calcium currents in AtT20 cells
524 transfected with rat brain cannabinoid receptor. J Neurosci 15:6552-6561.
525 Malcher-Lopes R, Di S, Marcheselli VS, Weng FJ, Stuart CT, Bazan NG, Tasker JG (2006)
526 Opposing crosstalk between leptin and glucocorticoids rapidly modulates synaptic
527 excitation via endocannabinoid release. J Neurosci 26:6643-6650.
528 Marinelli S, Di Marzo V, Berretta N, Matias I, Maccarrone M, Bernardi G, Mercuri NB (2003)
529 Presynaptic facilitation of glutamatergic synapses to dopaminergic neurons of the rat
530 substantia nigra by endogenous stimulation of vanilloid receptors. J Neurosci 23:3136-
531 3144.
532 Miyata S, Hatton GI (2002) Activity-related, dynamic neuron-glial interactions in the
533 hypothalamo-neurohypophysial system. Microscopy research and technique 56:143-157.
534 Miyata S, Nakashima T, Kiyohara T (1994) Structural dynamics of neural plasticity in the
535 supraoptic nucleus of the rat hypothalamus during dehydration and rehydration. Brain
536 research bulletin 34:169-175.
537 Oliet SH, Piet R, Poulain DA (2001) Control of glutamate clearance and synaptic efficacy by
538 glial coverage of neurons. Science 292:923-926.
539 Oliet SH, Baimoukhametova DV, Piet R, Bains JS (2007) Retrograde regulation of GABA
540 transmission by the tonic release of oxytocin and endocannabinoids governs postsynaptic
541 firing. J Neurosci 27:1325-1333.
542 Olijslagers JE, de Kloet ER, Elgersma Y, van Woerden GM, Joels M, Karst H (2008) Rapid
543 changes in hippocampal CA1 pyramidal cell function via pre- as well as postsynaptic
544 membrane mineralocorticoid receptors. Eur J Neurosci 27:2542-2550.
545 Park KS, Mohapatra DP, Misonou H, Trimmer JS (2006) Graded regulation of the Kv2.1
546 potassium channel by variable phosphorylation. Science 313:976-979.

Piet R, Vargova L, Sykova E, Poulain DA, Oliet SH (2004) Physiological contribution of the astrocytic environment of neurons to intersynaptic crosstalk. Proc Natl Acad Sci U S A 101:2151-2155.

Roberts EM, Pope GR, Newson MJ, Lolait SJ, O'Carroll AM (2011) The vasopressin V1b receptor modulates plasma corticosterone responses to dehydration-induced stress. J Neuroendocrinol 23:12-19.

Schrader LA, Ren Y, Cheng F, Bui D, Sweatt JD, Anderson AE (2009) Kv4.2 is a locus for PKC and ERK/MAPK cross-talk. Biochem J 417:705-715.

Schweitzer P (2000) Cannabinoids decrease the K(+) M-current in hippocampal CA1 neurons. J Neurosci 20:51-58.

Shah L, Bansal V, Rye PL, Mumtaz N, Taherian A, Fisher TE (2014) Osmotic activation of phospholipase C triggers structural adaptation in osmosensitive rat supraoptic neurons. J Physiol 592:4165-4175.

Tanaka M, Cummins TR, Ishikawa K, Black JA, Ibata Y, Waxman SG (1999) Molecular and functional remodeling of electrogenic membrane of hypothalamic neurons in response to changes in their input. Proc Natl Acad Sci U S A 96:1088-1093.

Tang SL, Tran V, Wagner EJ (2005) Sex differences in the cannabinoid modulation of an A-type K+ current in neurons of the mammalian hypothalamus. J Neurophysiol 94:2983-2986.

Tasker JG (2006) Rapid glucocorticoid actions in the hypothalamus as a mechanism of homeostatic integration. Obesity (Silver Spring) 14 Suppl 5:259S-265S.

Tasker JG, Dudek FE (1991) Electrophysiological properties of neurones in the region of the paraventricular nucleus in slices of rat hypothalamus. J Physiol 434:271-293.

Tasker JG, Di S, Boudaba C (2002) Functional synaptic plasticity in hypothalamic magnocellular neurons. Progress in brain research 139:113-119.

Tasker JG, Di S, Malcher-Lopes R (2006) Minireview: rapid glucocorticoid signaling via membrane-associated receptors. Endocrinology 147:5549-5556.

Tasker JG, Voisin DL, Armstrong WE (2017) 3.15 - The Cell Biology of Oxytocin and Vasopressin Cells A2 - Pfaff, Donald W. In: Hormones, Brain and Behavior (Third Edition) (Joëls M, ed), pp 305-336. Oxford: Academic Press.

Theodosis DT, el Majdoubi M, Gies U, Poulain DA (1995) Physiologically-linked structural plasticity of inhibitory and excitatory synaptic inputs to oxytocin neurons. Advances in experimental medicine and biology 395:155-171.

Theodosis DT, Chapman DB, Montagnese C, Poulain DA, Morris JF (1986) Structural plasticity in the hypothalamic supraoptic nucleus at lactation affects oxytocin-, but not vasopressin-secreting neurones. Neuroscience 17:661-678.

Yang JW, Vacher H, Park KS, Clark E, Trimmer JS (2007) Trafficking-dependent phosphorylation of Kv1.2 regulates voltage-gated potassium channel cell surface expression. Proc Natl Acad Sci U S A 104:20055-20060.

Zaki A, Barrett-Jolley R (2002) Rapid neuromodulation by cortisol in the rat paraventricular nucleus: an in vitro study. Br J Pharmacol 137:87-97.

Zhang W, Star B, Rajapaksha WR, Fisher TE (2007) Dehydration increases L-type Ca(2+) current in rat supraoptic neurons. J Physiol 580:181-193.

Figure Legends

Figure 1. Dexamethasone had no rapid effect on I_A activation and inactivation in magnocellular neurons from untreated rats. A. Voltage-clamp protocol for isolating I_K and I_A. Combined high-voltage-activated (I_K) and low-voltage-activated (I_A) currents were evoked by stepping from a holding potential (V_h) of -115 mV to test steps of -75 mV to 35 mV in 10-mV increments (left: voltage steps below, current responses above). High-voltage-activated potassium current (I_K) was evoked by the same stepping protocol, except from a V_h of -45 mV (middle: voltage steps below, current responses above). Subtraction of the currents activated from a Vh of -45 mV from those activated from a Vh of -115 mV yielded the isolated low-voltage-activated I_A (right current traces). B. Representative traces showing voltage-dependent activation of I_A in PVN magnocellular neurons from untreated rats before and at the end of a 10-min Dex application (1 μM). Inset: activation voltage protocol. C. Plots of the mean current amplitude of the I_A versus the test step potential, showing no effect of Dex on the I_A current amplitude. D. Plots of the mean current density of the I_A (peak I_A/capacitance) versus the test step potential, showing no effect of Dex on the I_A current density. E. Plots of the normalized chord conductance of the I_A versus the test step potential, showing no effect of Dex on the voltage dependence of activation of the I_A. F. Plots of the mean I_A 10-90% rise time vs. test step potential; the Dex effect on the activation rate of I_A was not significant. G. Representative traces showing voltage-dependent inactivation of I_A in response to voltage steps to -15 mV from 200-ms conditioning steps between -135 mV and -25 mV before and after 10 min of DEX application (1 μM). Dexamethasone had no effect on the inactivation voltage dependence of the I_A. Inset: inactivation voltage protocol. H. Plots of the mean normalized current amplitude versus the

615 conditioning step potential, showing no effect of DEX on the voltage dependence of I_A
616 inactivation. All recordings were in PVN magnocellular neurons in slices from untreated rats.
617
618 **Figure 2. Rapid glucocorticoid modulation of I_A in magnocellular neurons from salt-loaded**
619 **rats.** A. Representative traces showing voltage-dependent activation of I_A in PVN magnocellular
620 neurons from salt-loaded rats before and at the end of a 10-min DEX application (1 μM). Inset:
621 activation voltage-clamp protocol. B. Plots of the mean peak amplitude of the I_A versus the test
622 step potential; Dex caused a significant reduction in the I_A current amplitude. C. Plots of the
623 mean current density (peak current/capacitance) of the I_A versus the test step potential; Dex
624 caused a significant reduction in the I_A current density. D. Plots of the normalized chord
625 conductance of the I_A versus the test step potential; Dex caused a significant shift to the right in
626 the voltage dependence of activation of the I_A. E. Plots of the mean 10-90% I_A rise time versus
627 the test step potential; Dex did not change the I_A activation kinetics. F. Representative traces
628 showing voltage-dependent inactivation of I_A in response to voltage steps to -15 mV from 200-
629 ms conditioning steps between -135 mV and -25 mV before and at the end of a 10-min Dex
630 application (1 μM). Inset: inactivation voltage-clamp protocol. G. Plots of the mean normalized
631 current amplitude versus the conditioning potential. Dexamethasone had no effect on the voltage
632 dependence of I_A inactivation. All recordings were in PVN magnocellular neurons in slices from
633 salt-loaded rats. *, $p < 0.05$; **, $p < 0.01$ with ANOVA.
634
635 **Figure 3: The rapid glucocorticoid effect on I_A in magnocellular neurons from salt-loaded**
636 **rats was mediated by a membrane-associated receptor.** Plots of the mean current amplitude
637 of the I_A (A, C, E) and the I_A voltage dependence of activation (Normalized Chord Conductance)
638 (B, D, F) versus the test step potential before and at the end of a 10-min application of the

endogenous glucocorticoid corticosterone (Cort), the membrane-impermeant glucocorticoid Dex-BSA, and Dex in the presence of the protein translation inhibitor cycloheximide (CHX). The endogenous glucocorticoid, Cort (A, B), and the membrane-impermeant glucocorticoid, Dex-BSA (C, D, reduced the I_A peak amplitude and shifted the I_A voltage dependence to the right. Blocking protein synthesis (E, F) failed to block the glucocorticoid modulation of the I_A. *, $p < 0.05$; **, $p < 0.01$ with ANOVA.

Figure 4. CB1 receptor agonists mimicked the glucocorticoid modulation of I_A. A, B. Bath application of the endocannabinoid 2-AG (1 μM) elicited a significant decrease in the I_A mean peak amplitude (A) and a positive shift in the I_A activation voltage dependence (Normalized Chord Conductance) (B) in magnocellular neurons from dehydrated rats. C, D. The CB1 receptor inverse agonist SR141716 (rimonabant, 1 μM) blocked the decrease in mean I_A peak amplitude (C) and the rightward shift in the chord conductance curve (D). E, F. Anandamide (AEA) elicited a decrease in the I_A mean peak amplitude (E) and a positive shift in the I_A activation voltage dependence (chord conductance) (F) in the presence of the TRPV1 receptor antagonist capsazepine (1 μM). G. The TRPV1 agonist capsaicin (1 μM) had no effect on the I_A voltage dependence of activation (chord conductance). *, $p < 0.05$; **, $p < 0.01$ with ANOVA.

Figure 5. CB1 receptor analogs blocked the glucocorticoid modulation of I_A. A, B. The CB1 receptor inverse agonist SR141716 (1 μM) blocked the decrease in I_A peak amplitude (A) and the rightward shift in the voltage dependence of activation (Normalized Chord Conductance) (B) induced by Dex (1 μM) in PVN magnocellular neurons in slices from salt-loaded rats. C, D. The CB1 receptor inverse agonist AM251 also blocked the Dex-induced decrease in I_A peak

662 amplitude (C) and the positive shift in activation voltage dependence (Normalized Chord
663 Conductance) (D) in PVN magnocellular neurons from salt-loaded rats. E, F. 2-AG (1 μM) (E)
664 and AEA (1 μM) (F) caused a positive shift in the I_A voltage-dependence curve (Normalized
665 Chord Conductance) from the control value, but subsequent application of Dex (1 μM) failed to
666 further shift the curve, suggesting occlusion of the Dex effect by prior CB1 receptor activation. *,
667 $p < 0.05$ with ANOVA
668
669 **Figure 6. Glucocorticoid modulation of I_A following gliotoxin treatment**. Brain slices from
670 untreated rats were pre-incubated in the gliotoxin fluorocitrate for approximately two hours prior
671 to recordings. A. Following fluorocitrate treatment, Dex had no effect on the I_A mean peak
672 amplitude. B. Dex caused a positive shift in the voltage dependence of I_A activation (normalized
673 chord conductance) in fluorocitrate-treated slices. *, $p < 0.05$, ANOVA.
674

Table 1. Effects of chronic dehydration on passive electrical properties of magnocellular neurons.

	Untreated (n = 9)	**Dehydrated (n = 15)**
Membrane capacitance (pF)	24.3 ± 2.5	40.0 ± 4.4*
Membrane resistance (MΩ)	794.3 ± 48.4	667.5 ± 51.0*
Holding current (pA)	17.7 ± 3.8	13.4 ± 5.0*

*, $p > 0.05$ vs. neurons from untreated rats; Student's unpaired *t* test.

Fig. 1

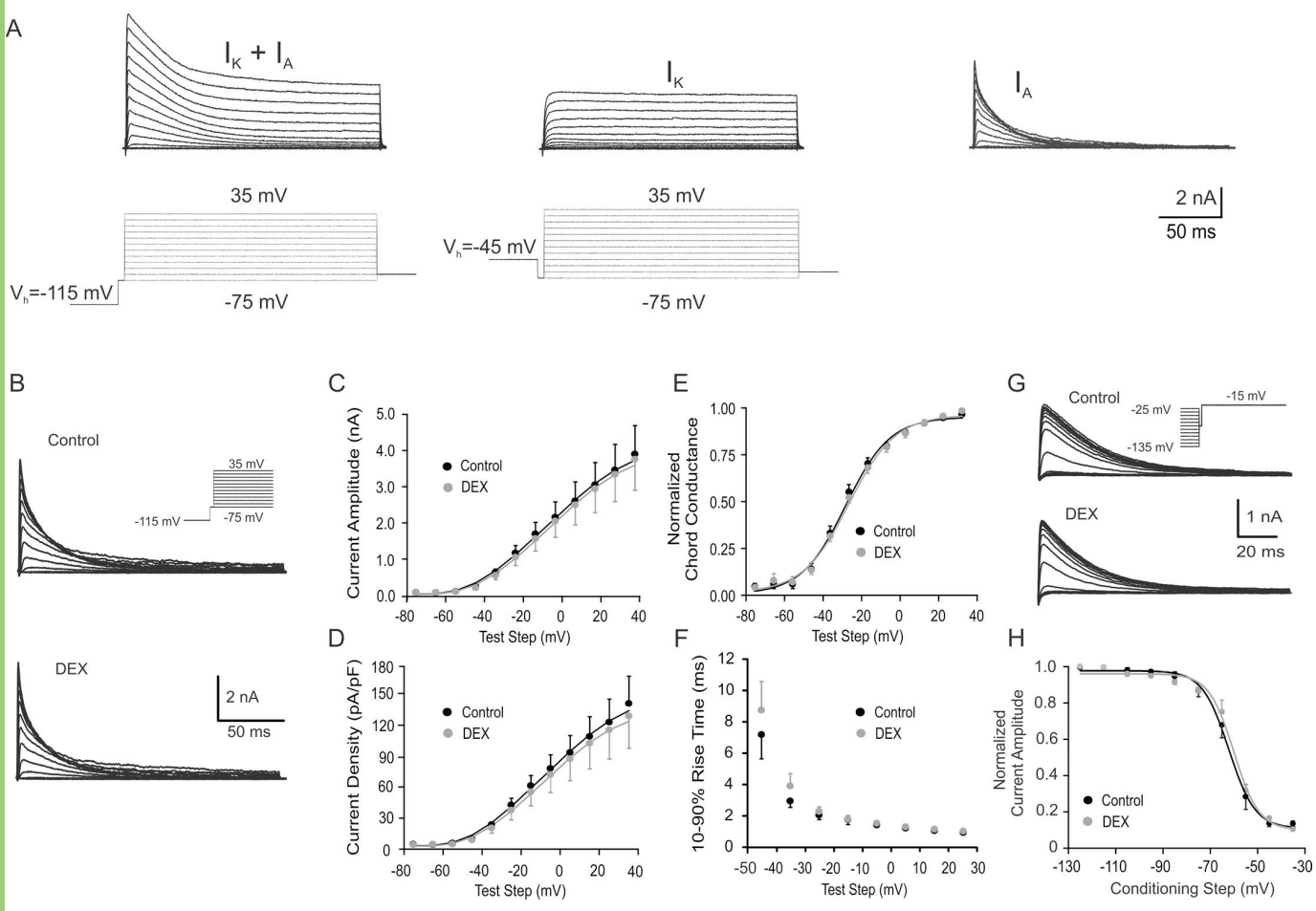

Fig. 2

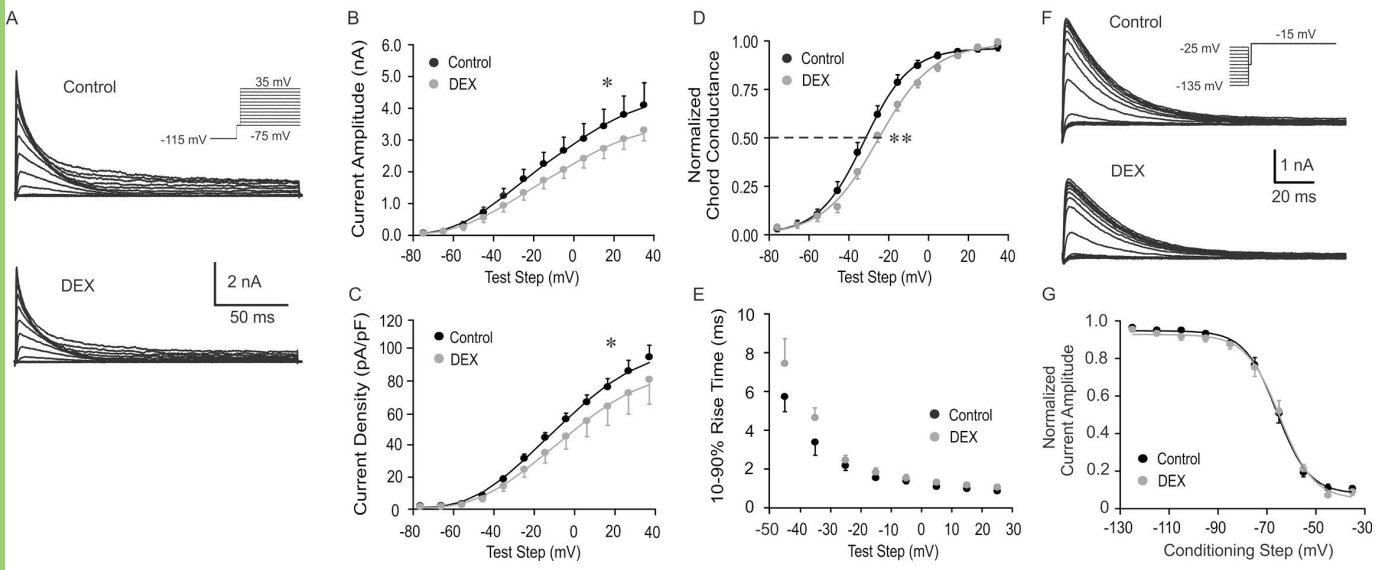

Fig. 3

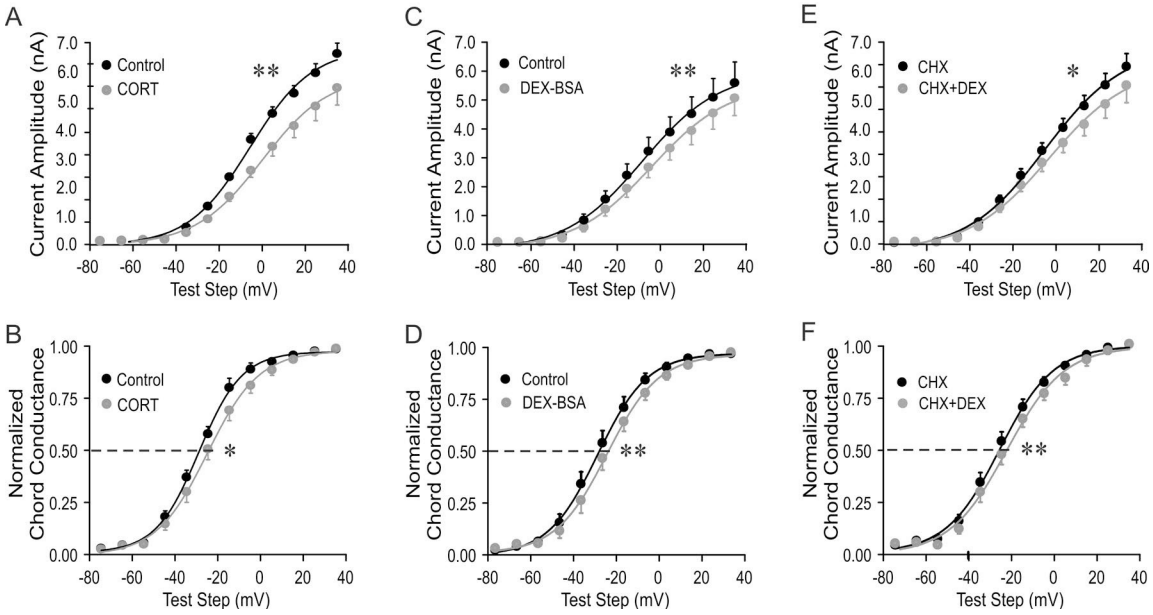

Fig. 4

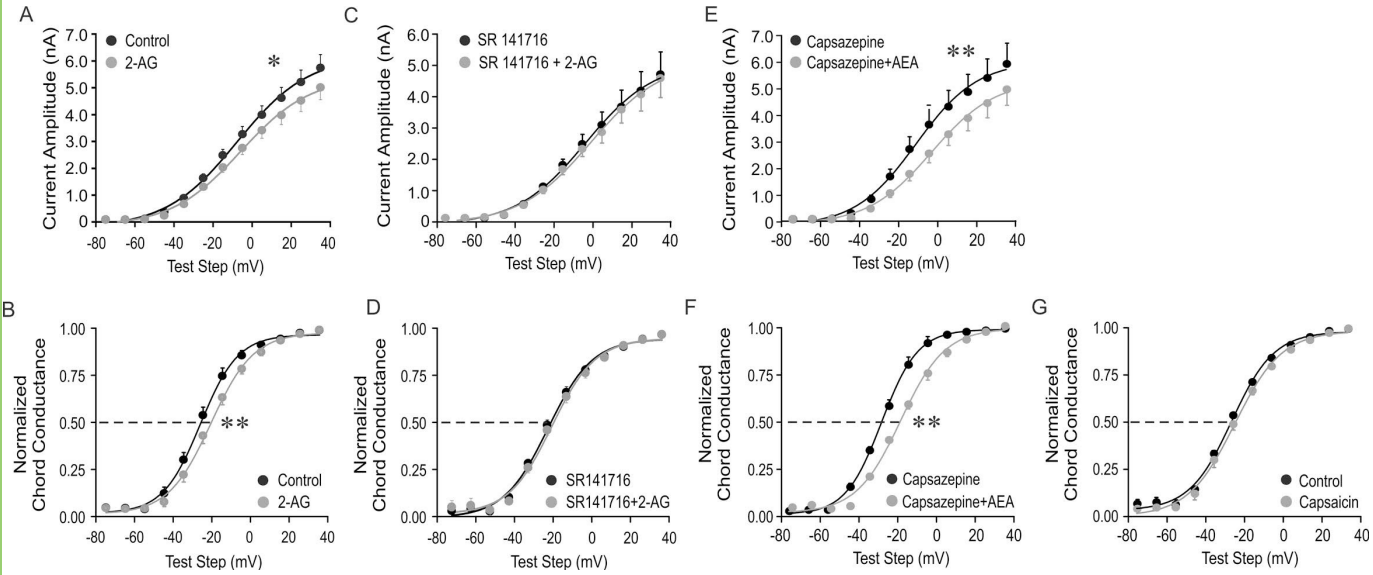

Fig. 5

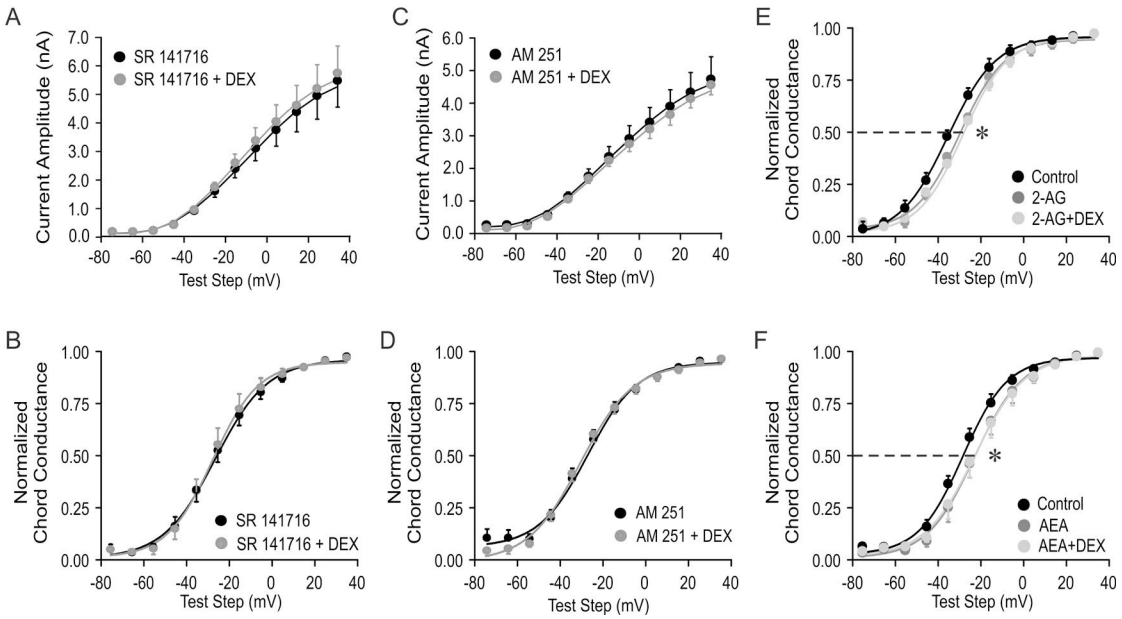

Fig. 6