Short communication

3α,5β-Reduced cortisol exhibits antagonist properties on cerebral cortical GABA$_A$ receptors

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Abstract

The tetrahydro-reduced derivatives of progesterone and deoxycorticosterone, allopregnanolone, and tetrahydrodeoxycorticosterone are potent positive modulators of GABA$_A$ receptors that are elevated by hypothalamic–pituitary–adrenal axis activation in rodents. In humans, 11-deoxycortisol and cortisol are important hypothalamic–pituitary–adrenal axis steroids. We hypothesized that C$_{3,5}$ reduction of 11-deoxycortisol and cortisol generates steroids with GABA$_A$ receptor activity. 3α,5β-Reduced cortisol dose-dependently inhibited muscimol-stimulated chloride flux and tetrahydrodeoxycorticosterone potentiation of muscimol responses. Cortisol, 11-deoxycortisol, 5α-dihydrocortisol, 3α,5α-reduced cortisol, 3α,5α-reduced 11-deoxycortisol, and 3α,5β-reduced 11-deoxycortisol had no activity at 1 μM and weaker negative modulatory activity at 10 μM. We conclude that cortisol metabolism may produce antagonistic GABAergic activity.
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1. Introduction

GABA$_A$ receptors are ubiquitously expressed throughout the brain and modulate overall synaptic activity by regulating chloride influx. Deficits in GABA neurotransmission are associated with a variety of disorders including epilepsy, anxiety, and alcoholism. Neuroactive steroids including 3α-hydroxy-5α-pregnane-20-one (allopregnanolone) and 3α,21-dihydroxy-5α-pregnan-20-one (tetrahydrodeoxycorticosterone) are endogenous modulators of GABA$_A$ receptors. These steroids increase chloride conductance (Harrison et al., 1987) and potentiate GABA responses at nanomolar concentrations (Morrow et al., 1987). Alterations in the normal plasma levels of these steroids have been demonstrated in several psychiatric conditions including alcoholism withdrawal (Romeo et al., 1996), major depression (Uzunova et al., 1998), panic

disorder (Strohle et al., 2002), and premenstrual dysphoric disorder (Girdler et al., 2001).

The major steroid mediators of the stress response in humans are cortisol and 11-deoxycortisol. The structures of cortisol and 11-deoxycortisol are very similar to progesterone and corticosterone, and both are substrates for 5α/β-reductases and 3α-hydroxysteroid dehydrogenase. Their tetrahydro-reduced derivatives (3α,5α/β-cortisol and 3α,5α/β-11-deoxycortisol) are found in the human central nervous system (Wilson, 2001) and meet the critical structural requirement of A ring reduction for activity at GABA$_A$ receptors (Harrison et al., 1987; Purdy et al., 1990). However, GABA receptor activity could be prevented by C$_{11}$ substituents on these molecules. 3α,5α-Reduced and 3α,5β-reduced cortisol and 11-deoxycortisol metabolites are major components of total urinary cortisol metabolites in humans (Sarkola et al., 2001). Moreover, there is an overall increase in 5α-reduced cortisol metabolites in several endocrine disorders. Polycystic ovarian syndrome is characterized by an increase in urinary 5α-reduced cortisol metabolites (Chin et al., 2000). Cortisol metabolism is also

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shifted from 5β to 5α reduction in hypothyroidism (Vantyghem et al., 1998) and obesity (Andrew et al., 1998). Patients with cirrhosis of the liver show an overall reduction of all urinary cortisol metabolites (Vogeser et al., 1998). Acute ethanol consumption increases plasma cortisol levels in humans. In heavy-drinking alcoholics, elevated cortisol levels are found during withdrawal and detoxification (Sarkola et al., 2001), as well as a shift in total urinary cortisol derivatives from 5α-reduced to 5β-reduced metabolites (Cronholm et al., 1994). We investigated the hypothesis that C_3- and C_5-reduced metabolites of cortisol and 11-deoxycortisol have modulatory activity at GABA$_A$ receptors.

2. Materials and methods

2.1. Subjects and materials

Adult male Sprague–Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) (200–250 g) were group housed with food and water access ad libitum. Animals were maintained on a 12:12 h light:dark cycle. Animals were sacrificed by decapitation during the early morning period of the light cycle. Protocols were approved by the University of North Carolina at Chapel Hill Institutional Animal Care and Use Committee. All steroids were purchased from Research Plus (Manasquan, NJ) except for 3α,5α-11-deoxycortisol and 3α,5β-11-deoxycortisol, which were purchased from Steraloids (Newport, RI).

2.2. Chloride uptake assay

Following decapitation, brains were immediately removed and placed in cold saline from which cerebral cortices were isolated. Synaptoneurosomes were prepared and chloride uptake was conducted as previously described (Morrow et al., 1990). The synaptoneurosome pellet was resuspended in 6.6 vol of ice-cold assay buffer (0.02 M Hepes, 0.118 M NaCl, 0.0047 M KCl, 0.0012 M MgSO$_4$, 0.0025 M CaCl$_2$, pH 7.4) for a final protein concentration of approximately 5 mg/ml. The homogenate (200 μl) was aliquoted per assay tube and preincubated at 30 °C for 12 min. Muscimol-stimulated chloride uptake was initiated by addition of 0.2 μCi of [^{36}Cl] (NEN, Boston, MA) in the presence of an EC$_{50}$ concentration of muscimol (3 μM) alone or in conjunction with tetrahydrodeoxycorticosterone (1 μM), cortisol, 11-deoxycortisol, or their derivatives (1 or 10 μM). The solution was vortexed and uptake terminated after 5 s by addition of 4 ml of ice-cold assay buffer containing 100 μM picrotoxin with rapid vacuum filtration over S&S no. 32 filters using a single vacuum manifold filter. The synaptoneurosomes were washed twice with 4 ml of buffer; the filter was allowed to dry, and radioactive counts were determined by liquid scintillation spectroscopy. Chloride uptake was measured in the absence of muscimol and subtracted from all tubes to determine muscimol-stimulated chloride uptake. Concentration–response curves were evaluated using nonlinear regression by Prism 3.0 (GraphPad, San Diego, CA). Each chloride flux assay was performed in quadruplicate in three to four experiments. The IC$_{50}$ value was determined by nonlinear regression using SigmaStat 2.0 (Chicago, IL). Statistics were performed using an ANOVA with a Fisher's PLSD post-hoc test. Data are expressed as the mean ± S.E.M.

3. Results

Rat cerebral cortical synaptoneurosome preparations were incubated with [^{36}Cl], the GABA analog muscimol, and various steroids for 5 s at 30 °C to determine basal and muscimol-stimulated chloride flux. Muscimol (3 μM) was also tested with the neuroactive steroid tetrahydrodeoxy-
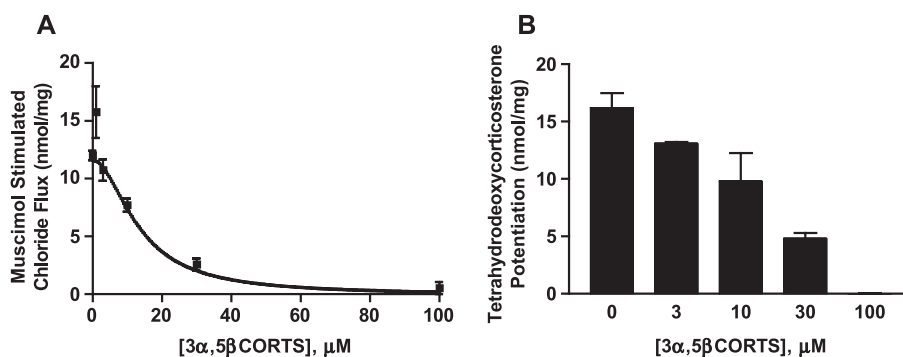

Fig. 1. (A) 3α,5β-Cortisol (1–100 μM) significantly reduces muscimol (3 μM)-stimulated chloride uptake in rat cerebral cortical synaptoneurosomes with an IC$_{50}$ concentration of 13.4 ± 9.9 μM. 3α,5β-Cortisol negatively modulates the GABA recognition sites on GABA$_A$ receptors. (B) 3α,5β-Cortisol (1–100 μM) significantly reduces tetrahydrodeoxycorticosterone (1 μM) potentiation of muscimol (3 μM)-stimulated chloride uptake in rat cerebral cortical synaptoneurosomes. The agonist tetrahydrodeoxycorticosterone potentiates muscimol-stimulated chloride flux by 16.2 ± 1.3 nmol/mg. 3α,5β-Cortisol also negatively modulates the neurosteroid recognition site(s) on GABA$_A$ receptors. The effect of 3α,5β-cortisol (100 μM) on tetrahydrodeoxycorticosterone potentiation of muscimol-stimulated chloride flux is −0.5 ± 0.5 nmol/mg. Data are from quadruplicate measures from three independent experiments and expressed as the mean ± S.E.M.

Table 1
Inhibition of GABA$_A$ receptor-mediated chloride flux by A-ring reduced metabolites of cortisol and 11-deoxycortisol in rat cerebral cortical synaptoneurosomes (percent inhibition versus basal; mean±S.E.M.)

Steroid name	IUPAC name	Inhibition of basal flux (1 μM)	Inhibition of GABA flux (1 μM)	Inhibition of neurosteroid flux (1μM)	Inhibition of GABA flux (10 μM)	Inhibition of neurosteroid flux (10 μM)
Cortisol	11β,17,21-Trihydroxypregn-4-ene-3,20-dione	−1.0±1.1	−2.8±1.0	−5.0±3.1	39.9±10.6*	27.6±5.3*
3α,5α-Cortisol	3α,11β,17,21-Tetrahydroxy-5α-pregnan-20-one	−1.2±1.0	6.0±5.4	−0.4±2.2	36.4±3.2*	29.2±2.6*
3α,5β-Cortisol	3α,11β,17,21-Tetrahydroxy-5β-pregnan-20-one	−0.4±1.0	7.7±2.5	9.2±1.7	36.0±2.7*	63.0±16.0*
5α-Dihydrocortisol	11β,17,21-Triol-5α-pregnane-3,20-dione	−0.4±1.6	16.2±9.4	3.0±4.6	43.9±4.9*	29.2±2.0*
11-Deoxycortisol	17,21-Dihydroxypregn-4-ene-3,20-dione	−0.9±1.4	4.9±1.7	−0.8±1.5	42.5±4.0*	31.9±1.3*
3α,5α-11-Deoxycortisol	3α,17,21-Trihydroxy-5α-pregnan-20-one	−1.1±1.0	−11.2±17.9	2.3±8.4	13.4±4.3	16.7±4.0
3α,5β-11-Deoxycortisol	3α,17,21-Trihydroxy-5β-pregnan-20-one	0.8±1.3	15.5±11.3	−2.2±8.5	38.9±3.5*	30.9±3.4*

The inhibition of GABA flux represents the effects of the steroids on GABA-mediated chloride flux. The inhibition of neurosteroid flux represents the steroids activity on tetrahydrodeoxycorticosterone-potentiated muscimol-stimulated chloride flux. Data were collected as quadruplicates of three independent experiments.

* $P<0.05$ versus basal levels. Data are expressed as percent inhibition versus basal values, but statistics were performed on original data expressed as total chloride flux (nmol/mg).

corticosterone to assess full positive modulatory activity of GABA$_A$ receptors. The cortisol and 11-deoxycortisol derivatives were tested either alone, in combination with muscimol, or with muscimol and tetrahydrodeoxycorticosterone. 3α,5β-Cortisol (1–100 μM) inhibited muscimol-stimulated chloride uptake with an IC$_{50}$ of 13.4±9.9 μM (Fig. 1A). In addition, 3α,5β-cortisol inhibited tetrahydrodeoxycorticosterone potentiation of muscimol (Fig. 1B).

Cortisol, 11-deoxycortisol, and 5α-dihydrocortisol (1 μM) did not alter muscimol-stimulated chloride flux or affect the ability of tetrahydrodeoxycorticosterone (1 μM) to potentiate muscimol-stimulated chloride flux in rat synaptoneurosomes (Table 1). C$_{3,5}$ reductions of 11-deoxycortisol and the 3α,5α–reduced derivative of cortisol had no effect on GABA-mediated chloride flux at 1 μM (Table 1). However, cortisol, 11-deoxycortisol, 5α-dihydrocortisol, 3α,5α-cortisol, and 3α,5β-11-deoxycortisol exhibited weak negative modulatory activity at 10 μM on muscimol-stimulated chloride flux as well as on tetrahydrodeoxycorticosterone-potentiated chloride flux (Table 1). Interestingly, 10 μM 3α,5α-11-deoxycortisol did not alter chloride flux (Table 1), unlike its 3α,5β isomer or its structurally similar cortisol analog 3α,5α-cortisol.

4. Discussion

Cortisol and its precursor 11-deoxycortisol are considered the primary regulators of the stress response in humans. Fluctuations in cortisol levels and its metabolite levels are associated with a wide spectrum of disease states from psychiatric disorders including depression and alcoholism to obesity and other peripheral diseases (Andrew et al., 1998; Chin et al., 2000; Iranmanesh et al., 1989).

3α,5β-Cortisol inhibits chloride flux mediated by both the GABA and neurosteroid recognition sites, while 3α,5α-cortisol inhibits neurosteroid effects on GABA$_A$ receptor activity with less potency. 3α,5β-11-Deoxycortisol is also a weak negative modulator of chloride flux, while its 3α,5α

isomer has no activity at GABA$_A$ receptors. These data suggest that cortisol and 11-deoxycortisol metabolites have distinct and separate effects on GABA$_A$ receptors. Several authors have suggested the existence of at least two separate neurosteroid recognition sites (Akk et al., 2004; Morrow et al., 1990; Park-Chung et al., 1999). A 3α,5α-reduced neuroactive steroid was recently shown to have different effects on recombinant GABA$_A$ receptor channel kinetics than its 3α,5β-reduced counterpart (Akk et al., 2004), in correlation with our present data on 3α,5α- and 3α,5β-reduced cortisol. Our data further support the hypothesis of multiple neuroactive steroid recognition sites on GABA$_A$ receptors.

3α,5β-Cortisol possesses negative modulatory activity at both the GABA and neurosteroid recognition sites on GABA$_A$ receptors similar to pregnenolone sulfate. Pregnenolone sulfate inhibits recombinant GABA$_A$ receptor single-channel openings at concentrations of 10 and 50 μM (Akk et al., 2001) and inhibits GABA-induced current in chick spinal cord neurons with an EC$_{50}$ of 7.2 μM (Park-Chung et al., 1999). 3α,5β-Cortisol inhibits GABA$_A$ receptor activity with an IC$_{50}$ of 13.4 μM, which corresponds with these studies.

Blood concentrations of C$_3$- and C$_5$-reduced cortisol metabolites reach physiologically relevant levels in humans so these steroids should be measured in future studies of stress steroid levels in human diseases. Morning plasma cortisol levels in humans average 10–12 μg/dl with a range of 3–20 μg/dl (Miller and Tyrrell, 1995). After intravenous administration of radioactively labeled cortisol, urinary concentration of the 3α,5α- and 3α,5β-reduced cortisol derivatives were 6% and 14%, respectively, of all cortisol metabolites (Miller and Tyrrell, 1995). In disease states such as polycystic ovarian syndrome and obesity, the levels of these cortisol metabolites are increased above normal levels (Andrew et al., 1998; Chin et al., 2000). Specifically, the total amount of 3α,5β-reduced cortisol in these diseases would increase due to increased production of total urinary cortisol metabolites coupled with a shift in concentration from 5α-

reduced to 5β-reduced cortisol metabolites. 3α,5β-Reduced cortisol can alter GABA$_A$ receptor activity with an IC$_{50}$ of 13.4 μM, which could ultimately lead to decreases in GABA-mediated inhibition as well as adaptations in central nervous system function. Because these neuroactive steroids have differential effects on GABAergic activity, the disease state could be altered by changes in the balance between the steroids. For instance, in normal individuals, 3α,5α-cortisol is the primary urinary derivative. However, in diseased states such as alcoholism, there is a shift to favor 3α,5β-cortisol metabolism (Cronholm et al., 1994). The 3α,5β-cortisol is a more potent negative modulator of GABA$_A$ receptor activity than 3α,5α-cortisol and could contribute to the down-regulation of the overall GABA tone in the central nervous system.

Previous studies suggest that neuroactive steroids can target more than one neurotransmitter system. Allopregnanolone and tetrahydrodeoxycorticosterone are potent positive modulators of GABA$_A$ receptors at low nanomolar concentrations but also modulate sigma, NMDA, and serotonin receptors at high micromolar concentrations (Rupprecht and Holsboer, 1999). In the rodent paraventricular nucleus, cortisol does not alter whole cell GABA current amplitudes or GABA$_A$ single-channel kinetics, but inhibits voltage-gated potassium channels (Zaki and Barrett-Jolley, 2002). 3α,5β-Cortisol is a negative modulator of GABA$_A$ receptors similar to pregnenolone sulfate, but pregnenolone sulfate also inhibits NMDA receptors (Ceccon et al., 2001). Hence, future studies on the effects of cortisol metabolites on other neurotransmitter systems are warranted.

Acknowledgements

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