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Antidepressant Effects of Abscisic Acid Mediated by the Downregulation of Corticotrophin-Releasing Hormone Gene Expression in Rats

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Background: Corticotrophin-releasing hormone (CRH) is considered to be the central driving force of the hypothalamic-pituitary-adrenal axis, which plays a key role in the stress response and depression. Clinical reports have suggested that excess retinoic acid (RA) is associated with depression. Abscisic acid (ABA) and RA are direct derivatives of carotenoids and share a similar molecular structure. Here, we proposed that ABA also plays a role in the regulation of CRH activity sharing with the RA signaling pathway.
Methods: [$_3$H]-ABA radioimmunoassay demonstrated that the hypothalamus of rats shows the highest concentration of ABA compared with the cortex and the hippocampus under basal conditions.
Results: Under acute stress, ABA concentrations increased in the serum, but decreased in the hypothalamus and were accompanied by increased corticosterone in the serum and c-fos expression in the hypothalamus. Moreover, chronic ABA administration increased sucrose intake and decreased the mRNA expression of CRH and retinoic acid receptor alpha (RARα) in the hypothalamus of rats. Furthermore, ABA improved the symptom of chronic unpredictable mild stress in model rats, as indicated by increased sucrose intake, increased swimming in the forced swim test, and reduced mRNA expression of CRH and RARα in the rat hypothalamus. In vitro, CRH expression decreased after ABA treatment across different neural cells. In BE(2)-C cells, ABA inhibited a series of retinoid receptor expression, including RARα, a receptor that could facilitate CRH expression directly.
Conclusions: These results suggest that ABA may play a role in the pathogenesis of depression by downregulating CRH mRNA expression shared with the RA signaling pathway.

Keywords: abscisic acid, corticotrophin-releasing hormone, depression, retinoic acid receptor alpha, stress response

Introduction

The hypothalamic-pituitary-adrenal (HPA) axis is considered to be the final common pathway in the stress response and the symptoms of depression (Swaab et al., 2005; Bao et al., 2008).

Corticotrophin-releasing hormone (CRH) in the paraventricular nucleus of the hypothalamus plays a central role in the regulation of HPA axis activity (Swaab, 1995; Bao et al., 2006). Evidence

from abundant clinical case reports uncovered the association between retinoic acid (RA) treatment and depressive symptoms (Bremner and McCaffery, 2008; Bremner et al., 2012). Recently, we have demonstrated that retinoic acid receptor alpha (RARα) contributes to the etiology of affective disorder through co-localized and upregulated CRH gene expression (Chen et al., 2009). Moreover, chronic RA administration induces HPA axis hyperactivity and depression-like behavioral changes in young rats (Cai et al., 2010). Direct intracerebroventricular injection of RA induces potent HPA axis activation and typical depression-like behaviors in rats (Hu et al., 2013). Furthermore, abnormal endogenous retinoid signaling has also been found in the brains of depressed patients (Chen et al., 2009; Qi et al., 2013). These findings suggest that the RA signaling pathway may be involved in the pathophysiology of depression. Interestingly, abscisic acid (ABA) and RA are direct derivatives of carotenoids (Vershinin, 1999; Nambara and Marion-Poll, 2005), and both substances share a similar molecular structure: the free carboxyl group at the end of the isoprene-composed side chain, which is a critical part of their bioactivity (Bremner and McCaffery, 2008; Moise et al., 2009). Whether ABA also plays a role in regulating the activity of CRH sharing with RA signaling pathway remains unclear.

ABA is a crucial phytohormone that exists and functions not only in plants, fungi, cyanobacteria, and algae, but also in a wide range of lower animals and mammals, including various human tissues and cells (Cowan and Rose, 1991; Bruzzone et al., 2007; Nagamune et al., 2008; Li et al., 2011; Bruzzone et al., 2012). Recently, ABA was identified as an endogenous hormone in mammals, which acts as a stress signal and regulates different cell functions, including inflammatory processes, stem cell expansion, insulin release, and glucose uptake (Bruzzone et al., 2008; Scarfi et al., 2008; Bodrato et al., 2009; Bruzzone et al., 2012). Moreover, dietary ABA ameliorates glucose tolerance and obesity-related inflammation in db/db mice (Guri et al., 2007; Guri et al., 2010a), decreases intestinal inflammation in mouse models of inflammatory bowel disease (Guri et al., 2010b; Guri et al., 2011), and reduces pulmonary inflammation in mouse models of influenza virus–associated disease (Hontecillas et al., 2012). Interestingly, Le Page-Degivry et al. (1986) first reported the presence of ABA in the mammalian brain and other tissues; clearly, the brain contained much more ABA than any other types of tissue. Despite these findings, further insight into ABA functions in the brain and the central nervous system (CNS) is lacking. The distribution of ABA in the brains of rodents and its role in the stress response are still not well understood.

Here, we propose that ABA may be a candidate for the negative regulation of CRH mRNA expression, and that ABA/RA is a pair of CRH regulators involved in the regulation of HPA axis activity. Furthermore, ABA may share the capacity to regulate the transcriptional expression of CRH with the RA signaling pathway.

Methods

Animals and Drugs

All Sprague Dawley (SD) rats were male and maintained with free access to food and water on a 12h light/dark cycle (lights on 0700h) at a room temperature of 22±1°C with 50–60% relative humidity. Rats were handled daily for 5min for at least four days before initiating stress or drug administration. The Animal Resource Center of the University of Science and Technology of China reviewed and approved this study.

For the animals that received an intraperitoneal injection, (±)-cis, trans-ABA (Sigma), and fluoxetine hydrochloride (Sigma) were dissolved in a vehicle of sterile saline solution (0.9% w/v sodium chloride) with dimethyl sulfoxide at a ratio of 1:1 (v/v).

Acute Stress

Two-month-old SD rats (250–300g) were randomly divided into two groups: the handled control (n=20) and the forced swim test (FST; n=20) groups. The 15-min FST for the rats was conducted by placing each rat in a cylinder (60cm x 30cm) containing water at 22±2°C with a depth of 35cm. The control rats were simultaneously handled for 5min and kept away from the FST to avoid stress. The rats were anesthetized and decapitated immediately after the FST.

Chronic ABA Administration in SD Young Rats and the Behavior Test

The four-week-old SD rats received a daily injection of 20mg/kg ABA (n=7) or the vehicle (control, n=6) treatment; the animals were continuously treated between 0800 and 1000h for six weeks before the behavioral test. All rats were housed individually for 24h prior to the sucrose intake test. The rats were deprived of water and food for 12h and then given one bottle of 1% sucrose and one bottle of water at the same time; the positions of the bottles were random. The amount of each solution consumed was determined by weighing the bottles before and after the 12h consumption period. The sucrose intake was calculated as the sucrose consumed (g)/the body weight (kg) of each rat (Duncko et al., 2001).

ABA Administration in the CUMS Model Rats and the Behavior Test

The chronic unpredictable mild stress model was used as a validated animal model of depression. The three weeks CUMS protocol and the behavior tests to measure the depression behaviors were performed as described previously (Wu et al., 2007; Ge et al., 2013). Forty-five SD rats (seven weeks old) were randomly split into four groups, including an undisrupted control group (vehicle control, n=12), a chronic unpredictable mild stress group (vehicle + CUMS, n=11), a fluoxetine-treated group (2mg/kg/day Flu + CUMS, n=10), and an ABA-treated group (40mg/kg/day ABA + CUMS, n=12). Briefly, after one week of stress, the CUMS-treated rats received fluoxetine, ABA, or vehicle on days 8–21; the control rats were administered an undisrupted vehicle treatment. After the CUMS, depression behaviors were evaluated using the sucrose intake, the open field test, and the forced swim test. All behavioral tests and the drug administration were performed in a soundproof room with a neutral environment between 0800 and 1400h.

Tissue Preparation

Blood samples were collected and centrifuged at 4°C (4 000rpm, 10min). Subsequently, the serum was separated for the determination of corticosterone (Corticosterone EIA Kit, Enzo Life Sciences) and ABA concentrations. The hypothalamus, the hippocampus, and the cortex of the brains were isolated immediately after death for the ABA assay and quantitative real-time PCR (Q-PCR).

ABA Purification and Determination

The extraction procedures used to identify ABA in the rat tissues are commonly used to extract ABA from plant tissues (Quarrie et al., 1988; Jia et al., 2001). To prevent ABA degradation, all steps were performed in the dark. The rat brain tissues were homogenized in a RNase-free aqueous 80% methanol (extract buffer) containing 1 mM 2,6-dibutyl-4-methyl phenol (Sigma) and held at 4°C overnight. The samples were centrifuged twice at 4°C (5 000 rpm, 5 min), and the supernatant was then dehydrated using nitrogen blowing and stored at -80°C for ABA detection. The precipitation of the extraction was re-suspended in TRIzol (Pufei) for RNA isolation.

ABA levels were detected using a radioimmunoassay as previously described (Quarrie et al., 1988; Perata et al., 1990) with minor modifications. The monoclonal antibody Mac252 (ab50594, Abcam), which is specific for (+)-cis, trans-ABA, was used in the radioimmunoassay detection (Barrieu and Simonneau, 2000); it was diluted to 1:8 000 in a phosphate-buffer saline (PBS, pH 6.0) containing 5 mg/ml of bovine serum albumin and 4 mg/ml of soluble polyvinylpyrrolidone (Sigma). DL-cis, trans-[G-^{3}H]-ABA (ARC) was diluted to 1:111 in water and then diluted to 1:50 in PBS containing 5 mg/ml of bovine-γ-globulin serum (Calbiochem). The reaction system contained 100 µl of sample (dissolved in 50% PBS), ABA standard, or water (for B_{max} determination) and 150 µl 50% PBS, 100 µl diluted antibody solution, and 100 µl [^{3}H]-ABA (approximately 16 000 cpm) solution. Non-specific binding (B_{min}) was determined using 10 times the standard ABA from the assay mixture. The reaction mixture was incubated at 4°C for 45 min and then 500 µl saturated ammonium sulfate (Sigma) was added at room temperature for 30 min to end the reaction. The samples were centrifuged (12 000 rpm, 5 min) to obtain the precipitated pellet, washed with 50% saturated ammonium sulfate, and centrifuged again. The pellet was dissolved with 250 µl of distilled water and 1.5 ml of scintillation liquid (Sigma). Finally, the bound radioactivity was measured in the precipitated pellets using a liquid scintillation analyzer (PerkinElmer).

Cell Culture

The human neuroblastoma cell lines BE(2)-C and SHSY-5Y were cultured with DMEM/F12 DMEM=1:1 (Sigma) supplied with 10% fetal bovine serum (GIBCO) at 37°C in a 5% CO$_2$ humidified atmosphere. Prior to treatment with the 1-µM all-trans-RA (Sigma) or 20-µM ABA, the medium was replaced with a phenol red-free medium and supplemented with 10% dextran coated charcoal–treated fetal bovine serum. Primary cortical neurons were isolated from the SD rats (E18) following a standard enzyme treatment protocol and cultured as previously described (Zhang et al., 2011).

RNA Isolation and Quantitative Real-Time PCR

The total RNA from the cells and brain tissues was extracted using the TRIzol (Pufei) method and evaluated by a One Drop OD-1 000 spectrophotometer. Total RNA (500 ng) was then reverse transcribed (TaKaRa) into cDNA and analyzed via Q-PCR using a SYBR Green PCR Master Mix (TaKaRa) on a StepOne platform (Applied Biosystems). A Q-PCR system was applied in a 25-µl volume for 40 cycles (15 s at 95°C and 1 min at 60°C). The gene expression levels were evaluated using the 2$^{-\Delta\Delta Ct}$ method. The primers used are shown in Supplementary Tables S1 and S2.

Western Blot Analysis

The protein of the BE(2)-C cells was extracted using whole cell lysates via a radioimmunoprecipitation assay buffer containing a protease inhibitor cocktail (Roche). The protein samples were separated on a 12%-SDS-polyacrylamide gel electrophoresis plate and detected using antibodies against RARα (ab28767, Abcam), RARβ (sc-552, Santa Cruz), and either β-actin-HRP (KC-5A08, KangChen) or GAPDH-HRP (KC-5G5, KangChen) as an internal control. These samples were then processed with an appropriate HRP-conjugated secondary antibody with regard to the protein of interest. The protein levels were analyzed using ImageJ (Wayne Rasband) and normalized relative to the internal control.

Data Analyses

Data analyses were performed using SPSS Statistics 19.0 (IBM). The values are expressed as the means ± SEM. The differences between the two groups (e.g., the protein levels of RARα with or without ABA treatment) were tested using student's t-test. The differences between the three groups or above (e.g., the differences in the ABA concentrations between the different rat brain regions) were tested using one-way analyses of variance (ANOVAs), followed by a LSD post-hoc test. P-values less than 0.05 were considered significant.

Results

The Hypothalamus had the Highest Concentration of ABA in the Rat Brain

To reveal the distribution pattern of ABA in the rat brain, the ABA levels in different brain regions that regulate the stress response were detected using an [^{3}H]-ABA radioimmunoassay. The results showed that the levels of ABA ranged from 2.81 ± 1.60 ng/g to 4.34 ± 2.23 ng/g (the weight of the tissue is the wet weight) in the measured brain regions, with the highest levels in the hypothalamus compared with the hippocampus and the cortex (F[2, 57] = 4.805, P = 0.012; hypothalamus, 4.34 ± 2.23 ng/g; hippocampus, 3.00 ± 1.05 ng/g; cortex, 2.81 ± 1.60 ng/g; Figure 1).
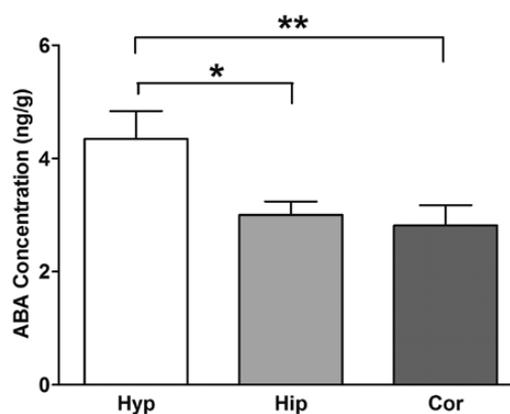
Figure 1. In the normal rats, the concentrations of ABA in the hypothalamus (Hyp), the hippocampus (Hip), and the cortex (Cor) were extracted and detected by a [^{3}H]-ABA radioimmunoassay (the weight of the tissue is the wet weight). Data are shown as the means ± SEM; *P < 0.05 and **P < 0.01 via an ANOVA with a LSD post-hoc test, n = 20.

Acute Stress Enhanced ABA Levels in the Serum and Decreased ABA Levels in the Hypothalamus

The high concentration of ABA in the hypothalamus encouraged us to determine the relationship between the levels of ABA and the stress response in the rats; thus, a 15-min FST was used as an acute stress model. The FST successfully caused an acute stress response as confirmed by the twofold increase of serum corticosterone levels (F[1, 38] = 4.344, P < 0.001; Figure 2A) and a remarkable increase in c-fos mRNA expression in the hypothalamus (F[1, 38] = 0.609, P < 0.001; Figure 2B). Serum ABA concentrations also increased from a basal value of 0.47±0.15 ng/ml to 0.59±0.17 ng/ml (F[1, 38] = 0.925, P = 0.022; Figure 2C). Interestingly, the ABA levels in the hypothalamus decreased from a basal value of 4.34±2.23 ng/g to 2.78±1.06 ng/g (F[1, 38] = 2.095, P = 0.008; Figure 2D).

Chronic ABA Administration Enhanced Sucrose Intake and Downregulated HPA Axis Activity

The involvement of ABA in the stress response urged us to detect the possible effects of chronic ABA administration in the SD young rats (Figure 3A). First, the sucrose intake test showed that the ABA-treated rats had a significantly higher sucrose consumption (corrected for body weight) compared with the control rats (F[1, 11] = 9.377, P = 0.047; Figure 3B). Moreover, the ABA-treated rats had lower serum corticosterone concentrations compared with the control rats (F[1, 11] = 1.247, P = 0.056; Figure 3C). It is worth noting that the ABA rats exhibited both a decreased CRH mRNA expression (F[1, 11] = 1.148, P = 0.012; Figure 3D) and a downregulated RARα mRNA expression (F[1, 11] = 4.060, P = 0.018; Figure 3E) in the hypothalamus.

ABA Administration Ameliorated Depression-Like Behaviors and Decreased the Hyperactivity of the HPA Axis in the Rat CUMS Model

The combination of enhanced sucrose intake and decreased corticosterone and mRNA expression of CRH in the ABA-treated rats prompted us to explore whether ABA could be a possible antidepressant. A CUMS model was established to detect the antidepressant effects of ABA (Figure 4A). During the three consecutive weeks of unpredictable mild stress, the body weights of the CUMS rats were significantly lower compared with the control rats (Supplementary Figure S1A), and this decrease was not improved by fluoxetine or ABA treatment. A difference in sucrose intake was found between the control, CUMS, ABA, and fluoxetine groups (F[3, 41] = 8.990, P < 0.001; Figure 4B). The sucrose intake in the CUMS group was significantly decreased compared with the control group (P < 0.001). Remarkably, ABA reversed the decreased sucrose intake induced by CUMS (P = 0.045), but fluoxetine had no effect (P = 0.775).

In the FST, the CUMS rats exhibited an increased immobility compared with the control rats (P = 0.009; Figure 4C). There were, however, no differences in immobility between the fluoxetine- or ABA-treated rats compared with the controls (F[3, 41] = 2.654, P = 0.061). Moreover, the ABA rats increased their swimming compared with the CUMS rats and the controls (F[3, 41] = 4.008, P = 0.014). Nevertheless, the decreased climbing in the CUMS rats was not ameliorated by fluoxetine or ABA treatment.

In the open field test, CUMS rats showed a decreased number of central square crossings (P = 0.002; Supplementary Figure S1B), but neither fluoxetine nor ABA could reverse this behavior (F[3, 41] = 5.744, P = 0.002). Although CUMS rats showed a trend toward an increased duration ratio of the peripheral to the central area than controls (P = 0.175), there were no significant differences between the four groups (F[3, 41] = 0.680, P = 0.569;
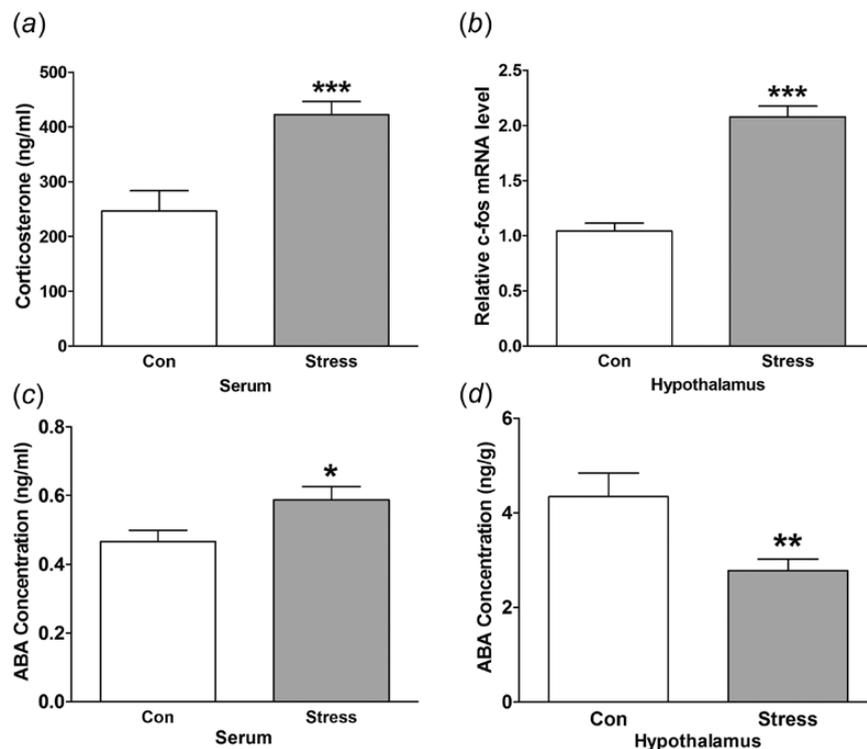
Figure 2. A 15-min forced swim test was used as an acute stress model. The serum corticosterone concentrations (A) and the c-fos mRNA expression in the hypothalamus (B) were measured after acute stress. Stress-induced ABA concentrations changed in the serum (C) and the hypothalamus (D) compared with the normal rats. Data are shown as the means ± SEM; *P < 0.05, **P < 0.01, and ***P < 0.001 via student's t-test, n = 20.
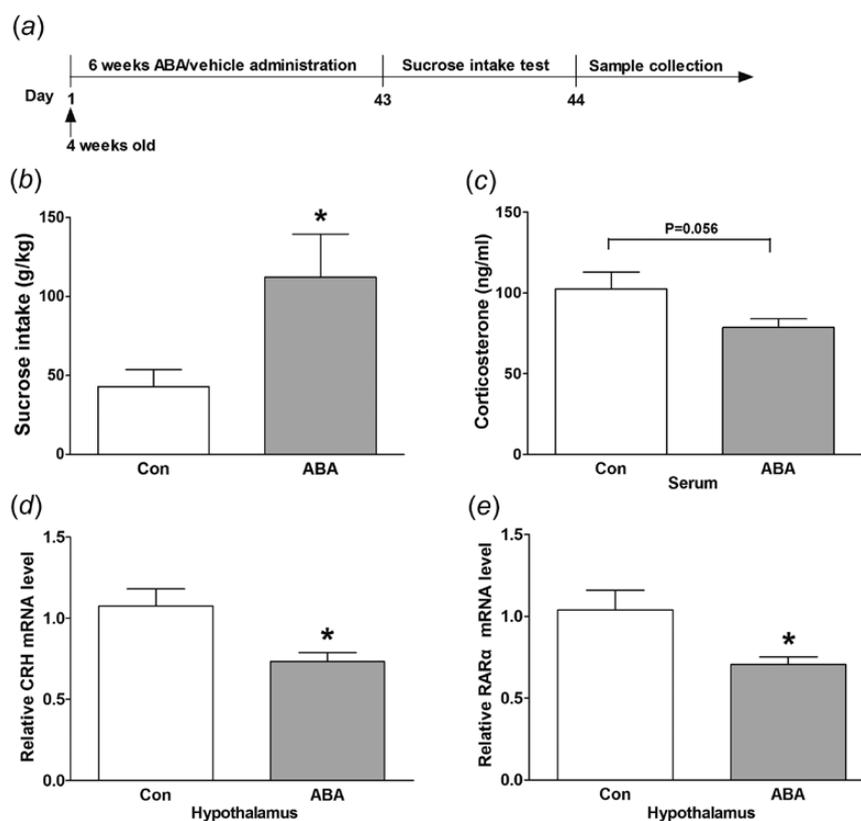
Figure 3. The schedule of six weeks chronic ABA administration in SD young rats (A). The behavioral performances of the ABA (20 mg/kg/day, n = 7) and control (vehicle, n = 6) rats were evaluated using sucrose intake test, which was expressed as the amount of sucrose solution ingested corrected for body weight (B). The corticosterone concentrations in the serum (C) and the mRNA expression of CRH (D) and RARα (E) in the hypothalamus of the rats were detected. The data are shown as the means ± SEM; *P < 0.05 via student's t-test.

Supplementary Figure S1C). Additionally, neither fluoxetine nor ABA alleviated the decreased rearing number in the CUMS rats (Supplementary Figure S1D).

As expected, significantly different corticosterone concentrations were found between the four groups (F[3, 41] = 3.543, P = 0.023; Figure 4D). A post-hoc analysis revealed the corticosterone concentration in the CUMS rats was higher than controls (P = 0.003), and no differences were found between the fluoxetine and control rats (P = 0.053). Compared with the CUMS rats, a decreased tendency of corticosterone levels was observed in the ABA rats (P = 0.287).

Consistent with the previous report, CUMS resulted in increased CRH (P < 0.001; Figure 4E) and RARα (P = 0.042) mRNA expression in the hypothalamus. Interestingly, ABA normalized the CUMS-induced elevation of CRH (P= 0.017; ABA vs. CUMS) and RARα (P = 0.036; ABA vs. CUMS) expression. Fluoxetine showed a decreased tendency of CRH (P = 0.131) and RARα (P = 0.216) levels compared with CUMS.

Consistent with the changes in the ABA concentration during acute stress, the serum ABA levels increased from the basal condition, 0.15 ± 0.12 ng/ml, to the CUMS condition, 0.78 ± 0.92 ng/ml (P = 0.047). The ABA levels in the hypothalamus showed a decreased tendency from the basal condition, 11.09 ± 4.976 ng/g, to the CUMS condition, 8.29 ± 4.62 ng/g (P = 0.462).

ABA Downregulated CRH mRNA Expression in Different Neural Cells

To confirm the effects of ABA on the transcriptional activity of CRH, the mRNA expression of CRH was measured in different neural cell lines. The dose- and time-dependent effects of ABA on CRH expression were first tested in the BE(2)-C cells (Supplementary Figure S2). In light of the effective concentration of ABA on the dose and time concerning CRH mRNA expression, we chose a 20-μM ABA and a 24 h treatment for additional studies. In the BE(2)-C cells, the 20-μM ABA treatment resulted in a 50% decrease in the expression of CRH mRNA (F[2, 15] = 53.209, P < 0.001; Figure 5A). As a positive control, however, the CRH mRNA expression doubled after a 1-μM RA treatment. The downregulation effect of ABA on CRH mRNA expression was repeated in the SHSY-5Y cells (P = 0.016; Figure 5B) and the rat primary cortical neurons (P = 0.005; Figure 5C). A MTT measurement in the BE(2)-C cells did not show changes in cell viability after ABA treatment, which confirmed that the ABA-reduced CRH mRNA expression was not due to the toxicity of ABA to the cells (Supplementary Figure S3).

ABA Reduced the Expression of RA Signaling Molecules in BE(2)-C Cells

To determine whether the retinoid-signaling pathway mediated the ABA reductions in CRH mRNA expression, the mRNA expression of a series of receptors and binding proteins involved in this pathway were measured (Figure 5C). After 24 h of treatment, ABA decreased the expression of CRH (F[2, 15] = 60.829, P < 0.001), RARα (F[2, 15] = 113.613, P < 0.001), and RARγ (F[2, 15] = 18.997, P < 0.001); in contrast, as a positive control, RA significantly increased their expression. Additionally, ABA suppressed PPARβ expression (F[2, 15] = 30.310, P < 0.001) and showed no effect on the other receptors (i.e., RARβ, RXRβ, and PPARγ). Furthermore, although RA increased CRABP II expression (F[2, 15] = 204.440,
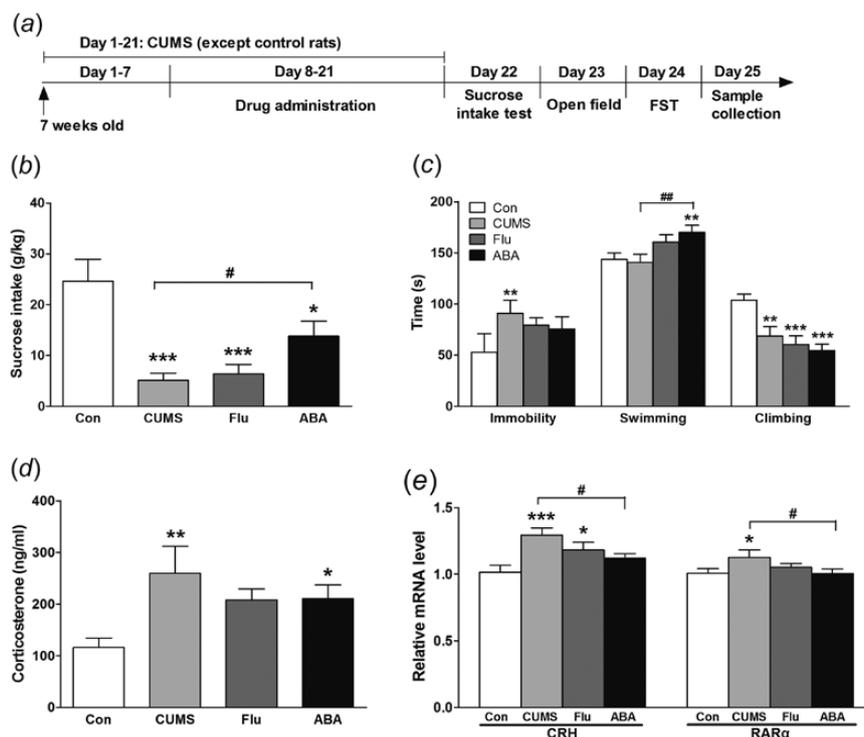
Figure 4. The schedule of ABA administration in the CUMS model rats (A). The study included four groups: the control group (vehicle treated, n = 12); the CUMS group (CUMS + vehicle, n = 11); the Fluoxetine group (CUMS + 2 mg/kg/day Fluoxetine, n = 10); and the ABA group (CUMS + 40 mg/kg/day ABA, n = 12). The sucrose intake was calculated as the sucrose consumed (g)/the body weight (kg) of each rat (B). The time spent on immobility, climbing, and swimming in the FST was measured (C). The corticosterone concentrations in the serum (D) and the mRNA expression of CRH and RARα in the hypothalamus (E) were detected. The data are shown as the means ± SEM; *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared with the control group and #$P < 0.05$ and ##$P < 0.01$ compared with the CUMS group, using ANOVAs with a LSD post-hoc test.

$P < 0.001$) and decreased FABP5 expression (F[2, 15] = 7.166, $P < 0.007$), ABA showed no effect with regard to these binding proteins. Interestingly, both ABA and RA inhibited the expression of RXRα ($P < 0.05$ in both cases). Western blot confirmed the changes in the protein levels of RARα and RARβ (Figure 5E and F). ABA significantly reduced the expression of RARα protein levels ($P = 0.031$), but no effect was observed with regard to RARβ ($P = 0.697$). As a positive control, RA increased the protein levels of RARα ($P = 0.009$).

Discussion

The existence of ABA in the brains of rats and pigs was first reported by Le Page-Degivry et al. in 1986. Specifically, there is a greater abundance of ABA in the brain compared with other tissues, which predicts a potential function of ABA in the CNS. Moreover, the researchers confirmed that ABA was produced and released by the brain itself (Le Page-Degivry et al., 1986), such as microglial cells (Bodrato et al., 2009). In line with the previous finding, we confirmed the existence of ABA in the rat brain. Importantly, an asymmetric distribution of ABA was found in the different brain regions, and the hypothalamus showed the highest concentration.

The hypothalamus is the key brain structure involved in the central regulation of the stress response in mammals (Swaab, 1995; Bao et al., 2006). The dominant distribution of ABA in the rat hypothalamus prompted us to examine the potential role of ABA in the stress response. By using the FST as an acute stress model, we found that a 15-min FST led to a two-fold increase in the serum corticosterone concentration and elevated the c-fos mRNA expression in the hypothalamus. The

release of corticosterone is the best characterized HPA axis marker for responses to stress (Foley and Kirschbaum, 2010), and stimulus-induced c-fos expression is also widely used as a marker in studies of neuronal activation after stress (Emmert and Herman, 1999). Interestingly, the ABA concentration significantly increased in the serum, and it was accompanied by elevated corticosterone levels. To our surprise, a decreased ABA concentration was found in the hypothalamus of the rats under acute stress. Nevertheless, it confirmed our speculation that ABA may play a role in the stress response. Moreover, the lower ABA levels in the hypothalamus prompted us to hypothesize that ABA may work as an inhibitory factor in the regulation of CRH transcription.

The HPA axis is the final common pathway in the stress response (Swaab et al., 2005; Bao et al., 2008). A previous study has demonstrated that chronic RA administration induces HPA axis hyperactivity in young rats (Cai et al., 2010). Here, six weeks of chronic ABA administration in SD young rats showed a remarkable downregulation in the CRH mRNA expression in the hypothalamus. Moreover, lower corticosterone concentrations in the serum were found after ABA treatment. These results indicate that ABA inhibits HPA axis activity under physiological conditions. In addition, it suggests that RA and ABA may act as a pair of regulators in the balance of CRH activity. Interestingly, the chronic ABA-treated rats also showed a higher sucrose intake compared with the control rats. Sucrose intake is a measure of motivation to seek out a pleasurable experience, which represents the capacity to feel interest or pleasure in mammals (Der-Avakian and Markou, 2012). The higher sucrose intake and the downregulated HPA axis activity suggest that ABA may play a role in the pathogenesis of depression.
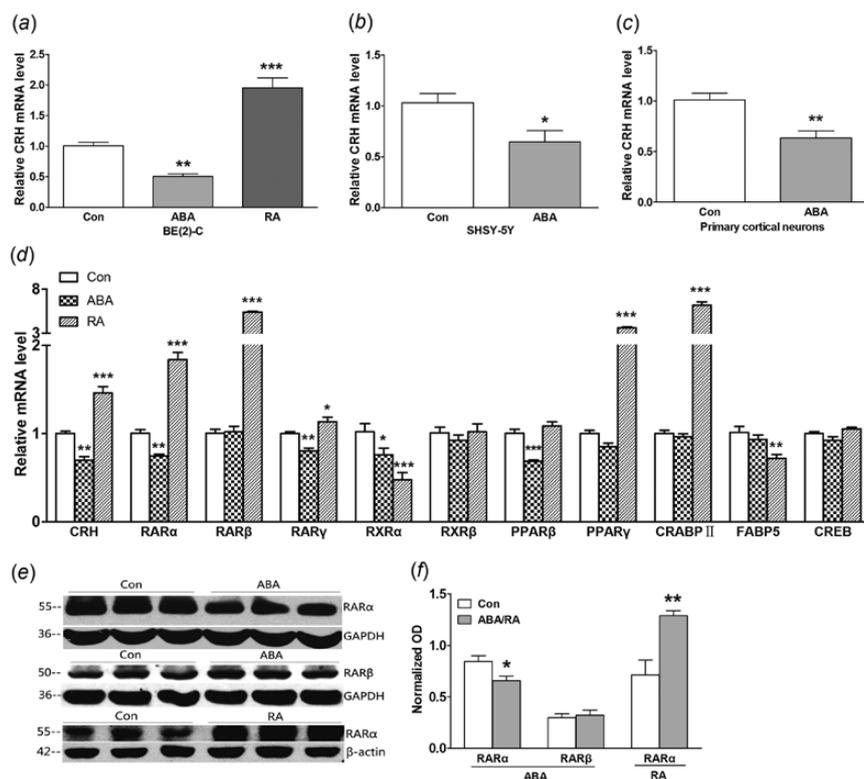
Figure 5. CRH mRNA expression was determined in BE(2)-C cells (A), SHSY-5Y cells (B), and primary cortical neurons (C) after treatment with ABA, RA, or vehicle for 24 h. A series of receptors (RARα, RARβ, RARγ, RXRα, RXRβ, PPARβ, and PPARγ) and binding proteins (CRABPII, FABP5, and CREB) were analyzed after ABA treatment in the BE(2)-C cells (D). Western blot assessed RARα, RARβ, β-actin, and GAPDH levels after treatment with ABA or RA in the BE(2)-C cells. The left side of the gel image displays the protein sizes (KDa), and the protein names are shown on the right (E). The normalized data of RARα and RARβ to β-actin or GAPDH were analyzed using Western blotting (F). The data are shown as the means ± SEM; *P < 0.05, **P < 0.01, and ***P < 0.001 using ANOVAs with a LSD post-hoc test or student's t-test, n = 6.

CUMS, which is an established effective model that mimics the pathogenesis of depression (Willner, 1997), was utilized in the present study to investigate the antidepressant effects of ABA. Consistent with the results of previous studies (Wu et al., 2007; Chen et al., 2008; Ge et al., 2013), CUMS successfully decreased sucrose intake and increased immobility in the FST in rats. Excitingly, ABA improved these depression-like behaviors. Anhedonia is a core symptom of depression and is most commonly used to assess depression-like behavior in rodents using sucrose intake (i.e., the sucrose solution ingested corrected for body weight) or sucrose preference (i.e., the sucrose solution/total liquid; Duncko et al., 2001; Der-Avakian and Markou, 2012). In this study, ABA reversed the decreased sucrose intake in the CUMS rats, although ABA could not normalize the sucrose intake levels as the controls. The FST, which is a behavioral model for testing antidepressant efficacy (Slattery and Cryan, 2012), showed that the ABA-treated rats spent a longer time swimming compared with the CUMS rats. However, ABA-treated rats could not alleviate anxiety-related behaviors, as indicated by the decreased numbers of central square crossings, increased ratio of time spent in the peripheral to the central area, and reduced rearing in open field test. Consistent with the results of the behavior tests, ABA normalized the CRH mRNA expression in the hypothalamus to the control levels, and showed a decreased tendency in serum corticosterone levels. These results demonstrate that the effects of ABA occur at the level of the hypothalamus to inhibit HPA axis drive and corticosterone output.

In both the acute stress and CUMS conditions, we found similar decreases of ABA in the hypothalamus and increases of ABA in the serum. Moreover, ABA-treated rats showed a trend toward increased ABA levels in both the hypothalamus and serum compared with the CUMS rats (data not shown), which suggest that the peripheral delivery of ABA may rescue or prevent the decreased hypothalamic ABA levels under stress conditions.

Although the acute stress and CUMS data suggested that hypothalamic ABA changes occur independently of ABA in circulation, ABA pharmacokinetic analysis revealed that the peripheral ABA was rapidly taken up into the hypothalamus (data not shown), and peripheral 20 mg/kg ABA administration significantly decreased CRH mRNA expression in the hypothalamus. These results suggest that peripheral injections of ABA may actually access CRH-expressing neurons in the hypothalamus.

ABA treatment inhibited the HPA axis activity by decreasing CRH mRNA under both normal and CUMS conditions with different doses of ABA. The dose of ABA was based on reports in which a dose of 100 mg/kg ABA of diet was used (Guri et al., 2007; Guri et al., 2010a, b). Using the formula for dose translation based on body surface area and bioavailability, a 40 mg/kg intraperitoneal injection of ABA was administered to the CUMS rats under pathological conditions. The *in vitro* results of Guri et al. (2007) led to speculation that a diet containing lower than 100 mg/kg ABA might also be effective; thus, a 20 mg/kg intraperitoneal injection of ABA was used in the normal young rats under physiological conditions. Importantly, all results in the rat models demonstrated that ABA had an antidepressant effect.

The possible mechanism that underlies the antidepressant effects of ABA was explored in different neural cells. The negative regulatory effect of ABA on CRH expression was further confirmed in the BE(2)-C cells, the SHSY-5Y cells, and the primary cortical neurons, which differed from the positive effect

of RA. As mentioned above, ABA and RA are analogue derivatives of carotenoids and share a similar molecular structure (Vershinin, 1999; Nambara and Marion-Poll, 2005). Our group has demonstrated that RA upregulates CRH transcriptional activity through elevated RARα expression (Chen et al., 2009; Cai et al., 2010). We proposed that ABA might share the same signaling pathway as RA, which regulates CRH gene expression. Our data showed that ABA decreased the mRNA expression of a series of retinoid receptors: RARγ, PPARβ, RXRα, and RARα. RARs, RXRs, and PPARs have specific localizations in the central nervous system (i.e., RARγ was not detected in any part of the CNS, although it was decreased in the BE(2)-C cells after ABA treatment; Zetterstrom et al., 1999). PPARγ is required for the anti-inflammatory efficacy of ABA in different mouse models and cells (Guri et al., 2010a, b; Guri et al., 2011), but ABA significantly decreased PPARβ and had no effect on PPARγ. These findings suggest that the effects of ABA in the periphery and the CNS may act through different signaling pathways, although the role of PPARβ in the CNS is unclear. RXRα is usually associated with PPARs or RARs to form heterodimers, which bind to regulatory regions of specific target genes and modulate transcriptional rates (Chambon, 1996; Schug et al., 2007); thus, we focused on RARα expression. Previously, data has shown that RARα, which is a key sensor in the RA signaling pathway, regulates CRH gene expression by its recruitment to the CRH promoter (Chen et al., 2009). Interestingly, RARα gene expression was downregulated after ABA treatment in the BE(2)-C cells with respect to both mRNA and protein levels. In accordance with this *in vitro* data, ABA administration reduced RARα expression in the normal rats and normalized the elevated RARα mRNA expression of the CUMS rats to the control levels, which is consistent with the changes of CRH expression. These results suggest that the negative regulation of ABA on CRH transcriptional activity may occur through the decreased RARα expression. However, it remains difficult to speculate whether ABA directly inhibits RARα or mediates it via other transducers (e.g., cAMP/PKA/RARα; Saito et al., 2010; Bassaganya-Riera et al., 2011).

The present study is the first to provide the evidence that ABA plays an antidepressant role in rats by downregulating CRH mRNA expression shared with the RA signaling pathway. The substantial role that ABA plays in regulating HPA axis activity and antidepressant behaviors highlights new functions for ABA in the central nervous system and may propose novel therapeutic strategies for depression. Building upon our previous findings of multiple paired receptors involved in the regulation of CRH transcription (Bao et al., 2005; Bao et al., 2006; Chen et al., 2008; Wang et al., 2008; Wang et al., 2010; Liu et al., 2011; Hu et al., 2013), ABA and RA may serve as a pair of signaling molecules in the balance of CRH regulation (Supplementary Figure S4).

Supplementary Material

For supplementary material accompanying this paper, visit http://www.ijnp.oxfordjournals.org/

Acknowledgments

Statement of Interest

None

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