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Transcriptional regulation of corticotrophin releasing factor gene by furocoumarins isolated from seeds of *Psoralea corylifolia*

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ABSTRACT

Dysregulation of the hypothalamic–pituitary–adrenocortical (HPA) system plays a causal role in the development and course of depression. Clinically effective antidepressant drugs normalize the disturbed activity of the HPA axis by inhibition of corticotrophin releasing factor gene promoter activity. Furocoumarins from *Psoralea corylifolia* have been demonstrated to possess potent antidepressant properties. In order to ascertain whether these coumarin components directly regulate corticotrophin releasing factor (CRF) gene transcription, we studied their effect on CRF promoter activity using the luciferase reporter assay in Neuro-2A cells. CRF promoter was cloned into firefly luciferase reporter vector and co-transfected into Neuro-2A cells with *Renilla* luciferase plasmid as internal control. CRF promoter transcription activity was induced by forskolin. We found that one of the components of *P. corylifolia*, psoralidin, strongly inhibited forskolin-induced CRF promoter activity. We further confirmed that psoralidin suppressed CRF gene transcription by quantitative reverse transcription polymerase chain reaction. Hence, down-regulation of CRF gene transcription by psoralidin may be involved in the molecular mechanism underlying its potent antidepressant effect.

Introduction

Major depression is frequently associated with hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis (Holsboer et al., 1985; Linkowski et al., 1985; Erhardt et al., 2006), whose proximal part is mediated by secretion of corticotrophin releasing factor (CRF) from the paraventricular nucleus of the hypothalamus. Antidepressant drugs as clinically effective therapeutics normalize the dysregulated HPA axis partly by decreasing CRF synthesis and secretion (Budzis-zewska et al., 2004).

Many studies attempted to identify herbal medicines and their extracts and evaluate their effectiveness in treating depression (He et al., 2004; Li et al., 2005; Luo et al., 2006; Mantle, 2002; Wu et al., 1999). Our preliminary data suggest that furocoumarins from *Psoralea corylifolia* possess potent antidepressant properties in an animal model (Kong et al., 2001; Chen et al., 2005; Qiao et al., 2006). Recently, we investigated the antidepressant-like effects of psoralidin isolated from the seeds of *P. corylifolia* in the forced swimming test (FST) in mice. Psoralidin significantly decreased immobility time and increased swimming behavior without altering climbing behavior in the mouse FST after oral administration for 3 consecutive days at dosage of 60 mg/kg (Yi et al., 2008). Furthermore, no toxicity was observed in mice after psoralidin treatment, suggesting it an effective yet safe antidepressant drug. Psoralidin also ameliorated the elevations in serum CRF induced by swimming stress in mice. However, the exact molecular mechanism has not been elucidated.

In the present investigation, we aim to test the effect of five furocoumarins isolated from *P. corylifolia*, including psoralen, isopsoralen, psoralidin, psoralenoside and isopsoralenside on transcriptional regulation of CRF gene. The effects of the compounds from *P. corylifolia* on CRF promoter activity and its mRNA level were investigated to explore the molecular mechanism underlying the antidepressant activity of the medicinal herb.

Materials and methods

Chemicals

Five furocoumarins (1 psoralenoside, 2 isopsoralenside, 3 psoralidin 4 psoralen, 5 isopsoralen) were isolated from *P. corylifolia* and purified as described in our previous reports (Yi et al., 2008). The purity

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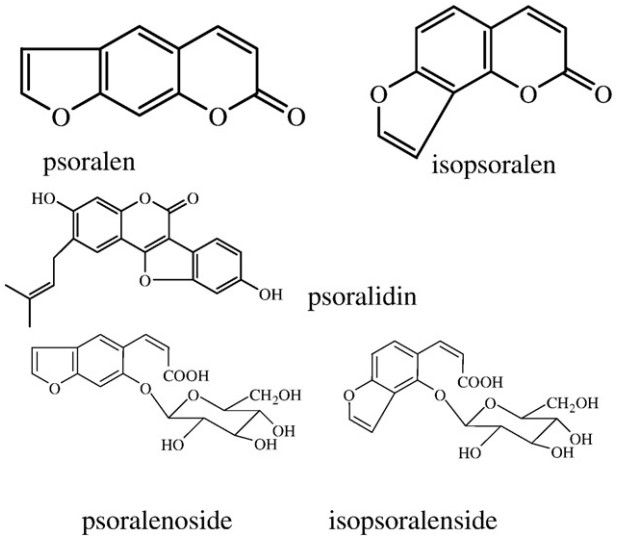

Fig. 1. Chemical structure of five furocoumarins from *Psoralea corylifolia*.

of the chemicals exceeded 98% as judged by HPLC analysis. Their chemical structures are shown in Fig. 1. Forskolin, fluoxetine hydrochloride and dimethylsulfoxide (DMSO) were purchased from Sigma. All drugs were dissolved in DMSO.

Cell culture and drug treatment

The mouse neuroblastoma cell line Neuro-2A (N2A) was purchased from American Type Culture Corporation (ATCC). N2A cells were cultured in Minimum Essential Medium (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen) and maintained at 37 °C in a humidified atmosphere of 5% CO_2. N2A cells (8×10^4 per well) were seeded in a 96-well culture plate 72 h prior to drug treatment. The five furocoumarins were incubated with the cells for 48 h at the indicated concentration. Afterwards, N2A cells were incubated with 15 μM forskolin or 0.5% DMSO for another 24 h.

Constructs and CRF promoter activity assay

Human DNA was extracted from the immortalized hepatocyte cell line LO2 by Wizard SV Genomic DNA Purification System (Promega). The regulatory region of human CRF gene (−663 to +124) was amplified by PCR using as forward primer CRF KpnI 5′ CGC GGT ACC GAG AGA CGT CTC CGG GGG C 3′ (28 bp, KpnI recognition site underlined) and as reverse primer CRF BglII 5′ GCG AGA TCT GGC TCA TAA CTC CTT TAT GTG CTT GC 3′ (35 bp, BglII recognition site underlined). The PCR reaction mixture consisted of 50 ng of human genomic DNA, 2 μl (10 μM) of each primer, 5 μl (10 mM) of dNTPs, 5 μl of 10×PCR buffer, 2 U of Taq polymerase. The fragment was amplified by initial denaturation at 94 °C for 5 min, 35 cycles at 94 °C for 30 s, 58 °C for 1 min and 72 °C for 1 min, and final extension at 72 °C for 10 min. Purified PCR fragment was sequenced, digested and then ligated into KpnI/BglII sites of pGL3-basic vector (Promega).

The promoter activity of the human CRF upstream region was analyzed using Dual-Luciferase Reporter Assay System (Promega); 0.25 μg of pGL3/hCRF plasmid and 1 ng of *Renilla* pRL-SV40 internal control plasmid were transiently transfected into N2A cells using Lipofectamine 2000 transfection reagent (Invitrogen). Twelve hours after transfection, N2A cells were treated with or without forskolin trigger for another 24 h, N2A cells were then lysed with 20 μl of PLB buffer. A 10-μl aliquot of the lysate was mixed with 10 μl of lysis buffer and 10 μl of Stop & Glo reagent in sequence. Firefly luciferase activity and *Renilla* luciferase activity were measured with a luminometer. As regarding for drug treatment, N2A cells were transfected with pGL3/hCRF plasmid, 12 h after transfection, N2A cells were exposed to the five furocoumarins at the indicated concentration for 48 h. Afterwards, N2A cells were incubated with 15 μM forskolin or 0.5% DMSO for another 24 h and luciferase activity was measured.

Quantitative real-time RT-PCR (QRT-PCR)

The QRT-PCR was carried out using SYBR green QRT-PCR Master Mix, 2-Step kit from ABI. Briefly, total RNA was extracted using Trizol reagent as described by the manufacturer (Invitrogen). The cDNA was generated using oligo dT primer mix and total RNA. The cDNA was PCR amplified using primers specific for mouse GAPDH and CRF. The PCR amplication was carried out in an ABI 7500 machine. The Ct (threshold cycle) value of CRF amplification was normalized to that of GAPDH control. The primers for QRT-PCR were— CRF forward: 5-GCT AAC TTT TTC CGC GTG TTG CTG-3; CRF reverse: 5-GGT GGA AGG TGA GAT CCA GAG AG-3; GAPDH forward: 5-AGG TGA CCG CAT CTT CTT GT-3; GAPDH reverse: 5-CTT GCC GTG GGT AGA GTC AT-3.

Determination of cell viability

Cell viability was determined using the methylthiazoletetrazolium (MTT) assay. MTT (Sigma) was dissolved in PBS and added to the culture to a final concentration of 0.5 mg/ml and incubated for 3 h. The formazan product was extracted with DMSO and detected with a UV spectrophotometer at 595 nm.

Statistical analysis

The data are presented as means±SEM of three independent experiments in triplicate wells, and the significance of differences between the means was evaluated using one-way analysis of variance (ANOVA). A value of $P<0.05$ was considered to be statistically significant in all cases.

Results

Effect of furocoumarins from P. corylifolia on forskolin-induced CRF gene promoter activity

We aimed to study the effect of the five furocoumarins from *P. corylifolia* (Fig. 1) on human CRF promoter activity. The regulatory region of human CRF gene was cloned into pGL3-basic plasmid and transfected into N2A cells. The cells were subsequently treated with the furocoumarins psoralenoside, isopsoralenside, psoralidin, psoralen, and isopsoralen, followed by triggering using 15 μM forskolin. As shown in Fig. 2, forskolin incubation increased promoter activity approximately 16 folds compared with untriggered cells. As shown in

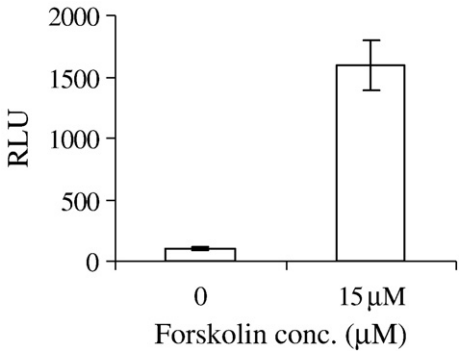

Fig. 2. Induction of CRF promoter by forskolin. N2A cells were transfected with PGL3/hCRFP and pRL-SV40 as internal control. Luciferase activity was measured after treatment with 15 μM forskolin.