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The anthraquinone derivative emodin attenuates methamphetamine-induced hyperlocomotion and startle response in rats

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ABSTRACT

Abnormal signaling mediated by epidermal growth factor (EGF) or its receptor (ErbB) is implicated in the neuropathology of schizophrenia. Previously, we found that the anthraquinone derivative emodin (3-methyl-1,6,8-trihydroxyanthraquinone) inhibits ErbB1 signaling and ameliorates behavioral deficits of the schizophrenia animal model established by EGF challenge. In the present study, we assessed acute and subchronic effects of its administration on methamphetamine-triggered behavioral hyperactivation in rats. Prior subchronic administration of emodin (50 mg/kg/day, 5 days, *p.o.*) suppressed both higher acoustic startle responses and hyperlocomotion induced by acute methamphetamine challenge. In parallel, emodin also attenuated methamphetamine-induced increases in dopamine and its metabolites and decreases in serotonin and its metabolites. Emodin administered alone also had an effect on stereotypic movement but no influence on horizontal or vertical locomotor activity. In contrast to pre-treatment, post-treatment with emodin had no effect on behavioral sensitization to methamphetamine. Administration of emodin in parallel to or following repeated methamphetamine challenge failed to affect hyperlocomotion induced by methamphetamine re-challenges. These findings suggest that emodin has unique pharmacological activity, which interferes with acute methamphetamine signaling and behavior.

1. Introduction

Emodin (3-methyl-1,6,8-trihydroxyanthraquinone) is extracted and purified from rhubarb. This natural compound has been proposed to attenuate signal transduction of growth factors and cytokines, inhibiting ErbB2, src-family kinase, IkappaB kinase, and extracellular signal-regulated kinase (ERK) (Jayasuriya et al., 1992; Kaneshiro et al., 2006; Kumar et al., 1998; Li et al., 2005; Wang et al., 2007b; Zhang et al., 1999). Recent pharmacological studies indicate that the influence of this compound on the brain and on behavioral traits (Gu et al., 2005; Lu et al., 2007). Our latest study suggests that emodin has anti-psychotic activity in a schizophrenia model established by neonatal epidermal growth factor (EGF) challenge (Mizuno et al., 2008), although it is unknown whether this anti-psychotic activity can be generalized for other animal models of psychosis.

In humans, chronic use of methamphetamine (MAP) causes drug addiction/relapse and evokes psychiatric symptoms such as hallucination and delusions, which are indistinguishable from paranoid schizophrenia (Kalechstein et al., 2003; Nordahl et al., 2002). In rodents, repeated exposure to MAP results in a progressive and long-lasting facilitation of the locomotor response, namely behavioral sensitization, which is often used as a model for drug addiction or relapse (Robinson and Berridge, 1993). The neural mechanism underlying induction and expression of behavioral sensitization involves a complex interplay among various neurotransmitters (Pierce and Kalivas, 1997; Vanderschuren and Kalivas, 2000) and cytokines (Flores et al., 2000; Horger et al., 1999; Messer et al., 2000; Mizuno et al., 2004; Nakajima et al., 2004; Pierce et al., 1999). Several lines of evidence indicate involvement of the ERK pathway in the integration of extracellular signals and in the long-term effects of MAP abuse (Mizoguchi et al., 2004; Valjent et al., 2006). MAP-induced behavioral impairment in rodents may be useful as an animal model for psychosis as well as for schizophrenia.

In the present study, we investigated acute and subchronic effects of emodin on MAP-induced hyperlocomotion and/or sensitization using MAP-challenged rats as a model for psychosis (Niwa et al., 2008). As emodin influences ErbB1/2 signaling and the ERK cascade, we monitored the phosphorylation states of these kinases in the

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brain. Bearing in mind the potential therapeutic applications, we orally administered emodin prior to, in conjunction with, or following MAP challenge to assess the potential pharmaceutical application of emodin for MAP psychosis.

2. Materials and methods

2.1. Animals

Sprague–Dawley rats (7–8 weeks postnatal, all male) were purchased from SLC Co., LTD (Shizuoka, Japan) and were maintained in a regulated environment (23 ± 1 °C) under a 12-h light–dark cycle (7:00 on −19:00 off) with free access to food and water. All of the animal experiments described here were performed in accordance with the Animal Use and Care Committee guidelines of Niigata University and the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society.

2.2. Drug treatment

Emodin (>98% pure; Tokyo Chemical Industry Inc., Tokyo, Japan) was sonicated in a 10% lecithin solution (Wako Chemical Co., Osaka, Japan) at a concentration of 5 mg/ml. This emulsion of emodin (50 mg/kg) or vehicle (10% lecithin) was administered to rats with the aid of an oral zonde for rats (Natume Seisakusho Co., LTD, Japan), before or after MAP challenge. The given dose was optimized in our previous study (Mizuno et al., 2008) and set below the toxic amount of emodin (<80 mg/kg) reported by Jahnke et al. (2004). MAP was obtained as methamphetamine hydrochloride from Dainippon Pharmaceutical (Osaka, Japan) and dissolved in saline (1 mg/ml). In the paradigm (*A*) of the pre-treatment schedule, rats were given vehicle or emodin (*p.o.*) once daily for 5 days and then given MAP (1.0 mg/kg, *i.p.*) on day 6 (*A1*). To monitor the acute effect of emodin, alternatively, rats were subjected to another paradigm (*A*2): Rats were given emodin once and, 3 h later, were challenged with MAP (1.0 mg/kg). In the paradigm (*B*) of the co-treatment schedule, rats were given vehicle or emodin once daily for 5 days, then given vehicle plus MAP or emodin plus MAP for the following 5 days, and were finally challenged with MAP alone (1.0 mg/kg) on day 11. In the paradigm (*C*) of the post-treatment schedule, rats were given MAP (1.0 mg/kg) once daily for 7 days, then treated with vehicle or emodin daily for the following 7 days, and then challenged with MAP (1.0 mg/kg) on day 15. In paradigms (*A1*), (*B*) and (*C*), MAP challenge was performed more than 20 h after the last emodin treatment to minimize the acute effects of emodin.

2.3. Immunoblot analysis

Polyacrylamide gel electrophoresis (PAGE) and immunoblotting were performed as described previously (Mizuno et al., 2007). Cells were harvested, lysed, and sonicated in sample buffer (2% sodium dodecyl sulfate (SDS), 10 mM Tris–HCl, 150 mM NaCl, 2% SDS, 1 mM NaF, and 1 mM Na_3VO_4). After centrifugation, the supernatant was collected and the protein concentrations were determined. Equal amounts of protein were subjected to SDS-PAGE and transferred to PVDF membranes. Membranes were probed with anti-phosphorylated-ERK1/2 and anti-ERK1/2 (1:1000, Cell Signaling Tech, Danvers, MA, USA) and anti-β-actin antibodies (1:10,000, Chemicon Int, Temecula, CA, USA), followed by horseradish-peroxidase-conjugated anti-mouse IgG or horseradish-peroxidase-conjugated anti-rabbit IgG secondary antibodies (1:10,000, DAKO Cytomation, Glostrup, Denmark). Peroxidase activity was visualized by chemiluminescence (Western Lightning, Perkin Elmer, Tokyo, Japan) coupled with film exposure.

2.4. Quantification of monoamines and their metabolites

The assay for dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) is previously described (Futamura et al., 2003). One hour after MAP challenge, the striatum and frontal cortex were dissected and frozen on dry ice. The tissue samples were then weighed individually and stored at -80 °C. The samples were placed in 0.1 M perchloric acid containing isoproterenol (an internal standard). After sonication, the samples were vortexed and stored at 4 °C for 30 min. The samples were then centrifuged and the supernatant analyzed by high performance liquid chromatography with electrochemical detection (HPLC-ECD). The HPLC-ECD system consisted of a pump (model EP-10; EICOM, Kyoto, Japan) connected to an ODS column (model MA-5ODS; EICOM, 4.6 × 150 mm). Samples were separated with a mobile phase of 45 mM citric acid–50 mM sodium acetate buffer (pH 3.65), 285 mg/L octanesulfonic acid sodium salt, 0.13 mM EDTA, and 13% (v/v) methanol. Detection was performed electrochemically on a thin-layer cell with a glassy carbon-working electrode (model ECD-300; EICOM).

2.5. Measurement of acoustic startle responses

One hour after the last MAP challenge, acoustic startle responses were measured in a startle chamber (SR-Lab Systems, San Diego Instruments, San Diego, CA, USA) adapted for rats (Swerdlow and Geyer, 1998; Swerdlow et al., 2001). This paradigm was used to assess startle amplitude response with acoustic stimuli of 120 dB and background noise (white noise, 70 dB). Each rat was placed in the startle chamber and initially acclimatized for 5 min with background noise alone. The rats were then subjected to 16 trials, each trial repeated 8 times in a pseudorandom order: (i) a 40-ms 120-dB noise burst presented alone, or (ii) no stimulus (background noise alone). The inter-trial interval was 15 s. Analysis of acoustic startle response was based on the mean of eight trials.

2.6. Locomotor activity

We measured basal and MAP-induced locomotor activities of rats (120 min) in the afternoon. Locomotor activity was monitored before (60 min) and/or after MAP challenge (60 min) in a novel environment (Futamura et al., 2003; Mizuno et al., 2008). Each rat was placed in an open field box (45 cm length × 45 cm width × 30 cm height, MED Associates, St. Albans, VA, USA) under a moderate light level (400 Lx). Photo-beam sensors were used to measure line crossings and rearing counts (25-mm intervals for horizontal axis and 150-mm intervals for vertical axis).

2.7. Statistical analysis

Results are expressed as the means ± SEM. Behavioral data were analyzed by analysis of variance (ANOVA). When univariate data were obtained only from two groups, a two-tailed *t*-test was used for comparison. Locomotor scores were analyzed separately before and after MAP challenge using multiple repeated ANOVA with emodin administration (two levels) as between-subject factors, and time session for locomotor test (12 sessions) as a within-subject factor. Interaction of a within-subject factor with between-subject factors was estimated by MANOVA with Pilli tracing. Subsequently, a Fisher's LSD test was applied to absolute behavioral values as a *post-hoc* test of multiple comparisons. Alternatively, we performed the Student–Newman–Keuls multiple range test on multiple monoamine values to avoid type 2 error. A P value of less than 0.05 was regarded as statistically significant. Statistical analysis was performed using the SPSS software (version 11.5). N values in parentheses represent the number of animals used.

3. Results

3.1. Subchronic pre-treatment with emodin suppresses MAP-induced hyperlocomotion

To examine whether subchronic pre-treatment with emodin influences the effect of MAP on locomotor activity, rats that had previously been treated with various doses of emodin (0–50 mg/kg, *p.o.*) for 5 days were challenged with MAP (1.0 mg/kg, *i.p.*) (*Paradigm A1*). Rats were subjected to the locomotion test 1 h before MAP challenge. Subchronic pre-treatment with emodin reduced MAP-induced hyperlocomotion in a dose-dependent manner (60 min total: $F(3,16)=3.27$, $p=0.049$ by one-way ANOVA) (Fig. 1). *Post-hoc* analysis revealed that 20 and 50 mg/kg/day of emodin were sufficient to suppress MAP-induced hyperlocomotion. Accordingly, we used an emodin dose of 50 mg/kg/day for further studies.

Using a larger number of animals, we ascertained the effect of emodin on basal locomotor activity as well as on MAP-induced activity (Fig. 2). Subchronic pre-treatment with emodin alone (50 mg/kg/day, 5 days) did not influence basal horizontal locomotor activity in the novel environment (emodin, $F(1,13)=0.446$, $p=0.511$; time, $F(11,13)=10.1$, $p<0.001$; interaction of emodin and time, $F(11,13)=0.853$, $p=0.599$). Vertical movement (rearing) and ambulation scores were also indistinguishable between the emodin-pre-treated group and vehicle-pre-treated group: (emodin, $F(1,13)=2.10$, $p=0.161$; time, $F(11,13)=6.63$, $p=0.001$; interaction of emodin and time, $F(11,13)=1.68$, $p=0.185$). In contrast, emodin-pre-treatment significantly decreased stereotypic counts (emodin, $F(1,13)=5.80$, $p=0.024$; time, $F(11,13)=9.1$, $p<0.001$; interaction of emodin and time, $F(11,13)=2.88$, $p=0.037$).

Following 60 min of activity monitoring, we administered MAP to emodin- or vehicle-pre-treated rats (Fig. 2). MAP administration evoked hyperlocomotion in both groups but the magnitude of the behavioral enhancement was significantly lower in the emodin-pre-treated animals (emodin, $F(1,13)=12.27$, $p=0.002$; time, $F(11,13)=5.02$, $p=0.004$; interaction of emodin and time, $F(11,13)=2.06$, $p=0.108$). In parallel, emodin pre-treatment significantly attenuated vertical movement (emodin, $F(1,13)=4.91$, $p=0.037$; time, $F(11,13)=3.84$, $p=0.012$; interaction of emodin and time, $F(11,13)=2.07$, $p=0.107$) and ambulation scores (emodin, $F(1,13)=7.99$, $p=0.010$; time, $F(11,13)=3.77$, $p=0.013$; interaction of emodin and time, $F(11,13)=4.03$, $p=0.010$) induced by MAP. Stereotypic counts of emodin-pre-treated animals remained lower following MAP challenge (emodin, $F(1,13)=0.446$, $p=0.511$; time, $F(11,13)=9.1$, $p<0.001$; interaction of emodin and time, $F(11,13)=2.88$, $p=0.037$).

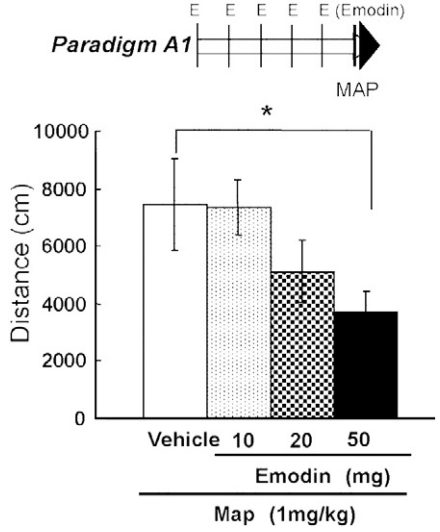

Fig. 1. Dose-dependent effects of emodin pre-treatment on MAP-induced hyperlocomotion in rats. Various doses of emodin (0, 10, 20, and 50 mg/kg weight) were orally administered to rats once a day for 5 days (i.e., days 1–5). Twenty to twenty-four hours after the final emodin treatment, MAP (1.0 mg/kg) was administered to the rats (*Paradigm A1*). Horizontal movement was monitored in a novel environment before and after MAP challenge. Bars indicate total distance of movement during the initial 60-min period (mean ± SE, $n=5$ each). $^*p<0.05$ by ANOVA.

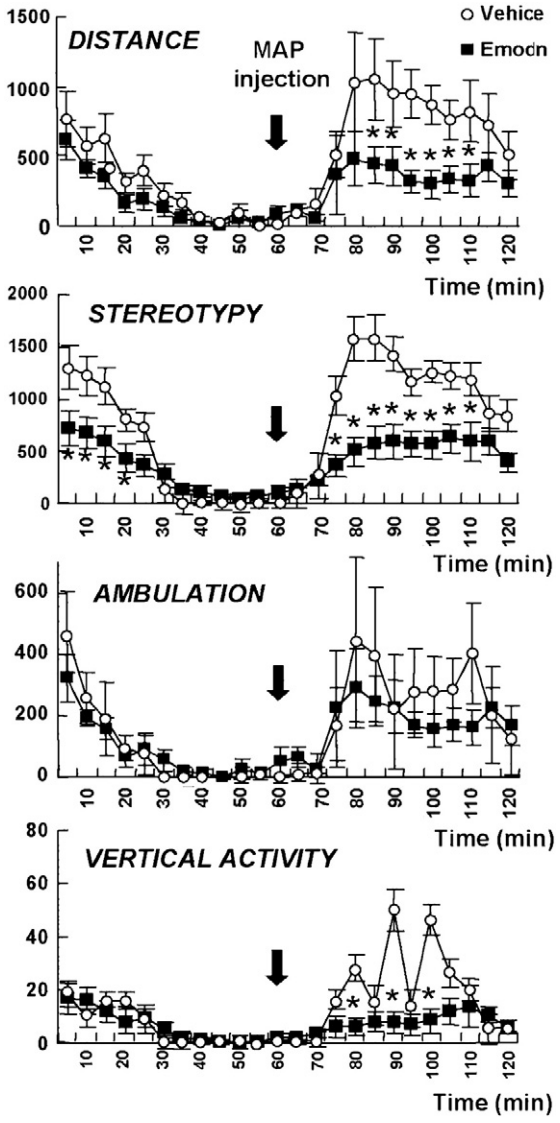

Fig. 2. Effects of subchronic pre-treatment with emodin on basal and MAP-induced locomotion in rats. Subchronic effects of emodin pre-treatment on distance (cm), ambulation (times), stereotypy (times), and vertical movement (times) of MAP-administered rats were examined with the experimental procedure detailed in Fig. 1 (*Paradigm A1*). Emodin was administered to rats once a day for 5 days (i.e., days 1–5). Twenty to twenty-four hours after the final emodin treatment, rats were subjected to an exploratory motor test before and after MAP challenge (1.0 mg/kg). Values indicate the mean ± SE ($n=7$–8). MAP-induced locomotion was compared between vehicle- and emodin-pre-treated groups by ANOVA, followed by *post-hoc*. $^*p<0.05$ vs vehicle-treated group.

3.2. Subchronic pre-treatment with emodin suppresses MAP-induced acoustic startle responses

MAP elevates the magnitude of the acoustic startle response (Davis, 1988). To examine whether emodin affects MAP-induced acoustic startle response, rats were similarly treated with various doses of emodin (0–50 mg/kg, *p.o.*) for 5 days and then challenged with MAP as described above (*Paradigm A1*). As the initial dose of MAP (1.0 mg/kg, *i.p.*) failed to affect acoustic startle responses (data

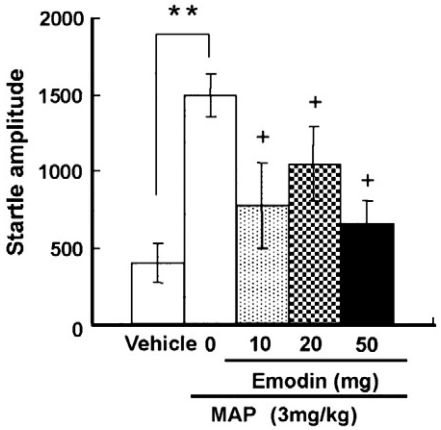

Fig. 3. Subchronic effects of emodin pre-treatment on MAP-induced hyperstartle responses. Various doses of emodin (10, 25, and 50 mg/kg/day) or vehicle were administered to rats once a day for 5 days (*Paradigm A1*). Twenty to twenty-four hours after the last emodin treatment, rats were challenged with MAP (3.0 mg/kg) and subjected to an acoustic startle test. Values indicate the mean ± SE (arbitrary unit; $n=5$ each). Startle responses to a 120 dB tone were compared among vehicle-treated and emodin-treated rats that were challenged with MAP. $^{**}p<0.01$ vs vehicle-treated group; $+p<0.05$ vs MAP-challenged naïve group.

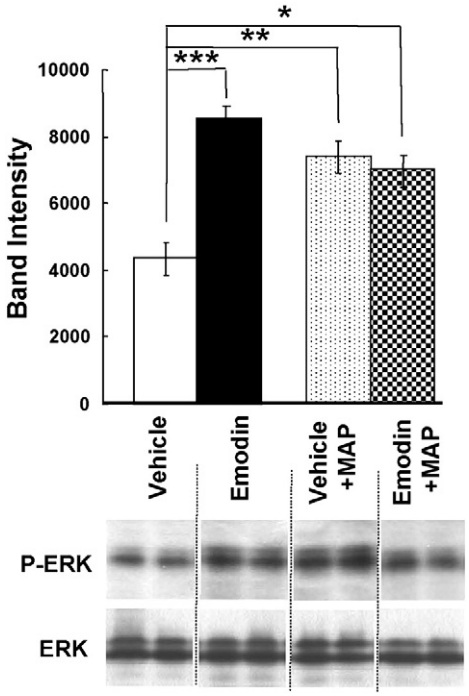

Fig. 4. Effect of emodin on phosphorylation of ERK1/2 in the striatum. Emodin or vehicle was administered to rats once a day for 5 days (days 1–5) (*Paradigm A1*). Rats were challenged with saline or MAP (1 mg/kg) on day 6 and killed 30 min after MAP challenge. Immunoblots of protein lysates from the whole striatum were probed with anti-phospho-ERK and anti-ERK antibodies. Values indicate the mean ± SE (arbitrary unit; $n=4$ each). Two representative lanes of immunoblots are displayed for each group. $^{*}p<0.05$. $^{**}p<0.01$, and $^{***}p<0.001$ vs the control group that was treated with vehicle but without MAP.

not shown), we increased the dose to 3.0 mg/kg (*i.p.*) and tested the startle responses to a 120-dB tone (Fig. 3). While acute administration of MAP significantly enhanced the startle responses, subchronic pre-treatment with emodin attenuated the MAP-induced hyperstartle response ($F(4,16)=8.13$, $p=0.005$ by one-way ANOVA). *Post-hoc* analysis revealed that an emodin dose of 10 mg/kg/day was sufficient to suppress MAP-induced acoustic hypersensitivity. In contrast to the above locomotion test results, the acoustic startle test did not display apparent emodin dose dependency.

3.3. Emodin alters ERK1/2 signaling, dopamine and serotonin metabolism

Emodin has been shown to attenuate growth factor signaling (Mizuno et al., 2008). MAP-induced behavioral impairment is associated with a disruption of extracellular receptor kinase1/2 (ERK1/2) signaling in the forebrain region (Kamei et al., 2006). To explore the contribution of ERK1/2 signaling, we examined the effects of emodin pre-treatment and after MAP challenge on ERK1/2 phosphorylation in the striatum of rats (Fig. 4). According to the above experimental protocol (*Paradigm A1*), rats were pre-treated with emodin or vehicle and then challenged with MAP (1 mg/kg) or saline. Consistent with previous studies, a significant increase in the level of phosphorylated ERK1/2 was observed following MAP challenge. Pre-treatment with emodin elevated basal phosphorylation of ERK1/2 and changed MAP-induced ERK expression and ERK phosphorylation ($F(1,12)=28.64$, $p<0.001$ for emodin, $F(1,12)=3.019$, $p=0.108$ for MAP but with an interaction; $F(1,12)=40.00$, $p<0.001$). However, the levels of total ERK1/2 did not differ among the groups examined ($F(1,12)=0.307$, $p=0.590$ for MAP and $F(1,12)=0.278$, $p=0.608$ for emodin without interaction $F(1,12)=0.024$, $p=0.880$).

Similarly, we monitored brain content of monoamine and its metabolites to assess the emodin effect on these neurotransmitters. Total monoamine was extracted from the frontal cortex and striatum of saline- and MAP-challenged rats that had been pre-treated with emodin or vehicle (Tables 1 and 2). We detected a significant increase in dopamine and its metabolites and a decrease in serotonin and its metabolites in both regions of the MAP-challenged rats. In contrast, subchronic treatment with emodin significantly attenuated MAP-induced increase in the content of dopamine and its metabolites in the striatum and also attenuated a MAP-induced decrease in the content of serotonin and its metabolites in the frontal cortex.

3.4. No effect of acute emodin treatment on MAP-induced hyperlocomotion

To examine whether acute intake of emodin affects MAP-induced hyperlocomotion, rats were given emodin once (50 mg/kg, *p.o.*) followed by MAP challenge (1.0 mg/kg, *i.p.*) with a 3-h delay (*Paradigm*

Table 1
Effects of emodin on dopamine metabolism in the frontal cortex and striatum.

Pre-treatment	Challenge	DA (pg/mg)	DOPAC (pg/mg)	HVA (pg/mg)
Frontal cortex				
Vehicle	SAL	4.20 ± 0.31a	1.15 ± 0.0,8a	1.30 ± 0.02a
Vehicle	MAP	5.75 ± 0.18a,b	2.53 ± 0.18a	1.52 ± 0.05a
Emodin	SAL	3.78 ± 0.31b	1.12 ± 0.10a	1.33 ± 0.02a
Emodin	MAP	5.45 ± 0.25a,b	2.26 ± 0.21a	1.89 ± 0.11a
Striatum				
Vehicle	SAL	364.29 ± 23.28a	46.19 ± 3.57a	5.81 ± 0.11a
Vehicle	MAP	417.68 ± 12.667a,b	90.89 ± 4.23a,b	12.69 ± 0.84a,b
Emodin	SAL	339.38 ± 10.37a,b	51.24 ± 6.94a,b	5.48 ± 0.60a,b
Emodin	MAP	366.77 ± 21.76b	72.80 ± 4.65b	11.07 ± 1.14b

Monoamines and their metabolites were extracted from frontal cortex and striatum 2 h after methamphetamine challenge (n = 6–7). Their concentrations were determined by HPLC-ECD. Abbreviations: DA; dopamine, DOPAC; 3,4-dihydroxyphenylacetic acid, HVA; homovanillic acid. Statistically homogeneous values are determined by the Student–Newman–Keuls multiple range test and marked with a or b.

Table 2
Effects of emodin on serotonin metabolism in the frontal cortex and striatum.

Pre-treatment	Challenge	5-HT (pg/mg)	5-HIAA (pg/mg)
Frontal cortex			
Vehicle	SAL	3.53 ± 0.28a	4.73 ± 0.79a
Vehicle	MAP	0.75 ± 0.11b	1.10 ± 0.08a
Emodin	SAL	1.58 ± 0.17b	2.90 ± 0.63a
Emodin	MAP	2.08 ± 0.11a,b	2.19 ± 0.08a
Striatum			
Vehicle	SAL	3.31 ± 0.28a	4.70 ± 0.27a
Vehicle	MAP	1.56 ± 0.17a,b	2.22 ± 0.13a,b
Emodin	SAL	1.61 ± 0.19a,b	2.33 ± 0.23a,b
Emodin	MAP	1.70 ± 0.16b	1.97 ± 0.16b

Monoamines and their metabolites were extracted from frontal cortex and striatum 2 h after methamphetamine challenge (n = 6–7). Their concentrations were determined by HPLC-ECD. Abbreviations: 5-HT; 5-hydroxytryptamine, 5-HIAA; 5-hydroxyindoleacetic acid. Statistically homogeneous values are determined by the Student–Newman–Keuls multiple range test and marked with a or b.

A2). One hour before MAP challenge, rats were subjected to the locomotion test. Acute pre-treatment with emodin had failed to alter MAP-induced hyperlocomotion (emodin, $F(1,14) = 1.36$, $p = 0.265$; time, $F(11,3) = 17.82$, $p = 0.018$; interaction of emodin and time, $F(11,3) = 2.38$, $p = 0.258$) (Fig. 5). MAP administration also failed to significantly alter other indices of ambulation, stereotypy and vertical movement (data not shown). Thus, acute treatment with emodin had no apparent action on MAP-induced hyperlocomotion.

3.5. Effects of parallel emodin treatment on MAP-induced behavioral sensitization

To examine whether emodin attenuates the establishment of MAP-induced behavioral sensitization, rats were pre-treated with emodin (50 mg/kg/day, *p.o.*) or saline for 5 days and then challenged with MAP (1.0 mg/kg/day, *i.p.*) with or without emodin (50 mg/kg/day, *p.o.*) for an additional 5 days. Parallel treatment with emodin failed to influence hyperlocomotion induced by the final MAP challenge (emodin, $F(1,13) = 2.09$, $p = 0.172$; time, $F(11,3) = 2.45$, $p = 0.250$; interaction of emodin and time, $F(11,3) = 0.458$, $p = 0.852$) (Fig. 6). Administration of emodin in parallel with MAP failed to significantly alter other indices of ambulation, stereotypy and vertical movement (data not shown).

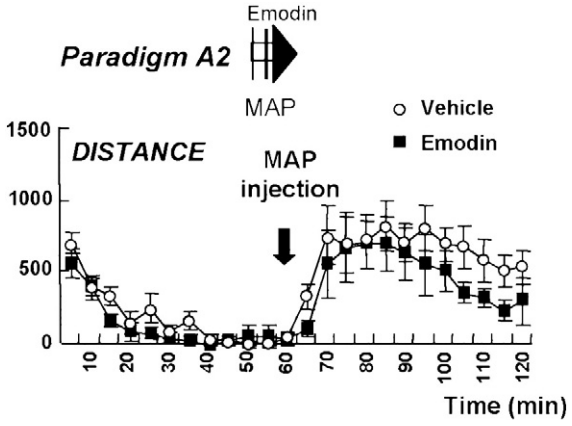

Fig. 5. Acute effect of emodin on MAP-induced hyperlocomotion. MAP-induced horizontal movement of rats was monitored 3 h after acute oral administration with emodin (50 mg/kg) (*Paradigm A2*). Values (cm) indicate the mean ± SE ($n = 8$ each). MAP-induced locomotion was compared between vehicle- and emodin-pre-treated groups.

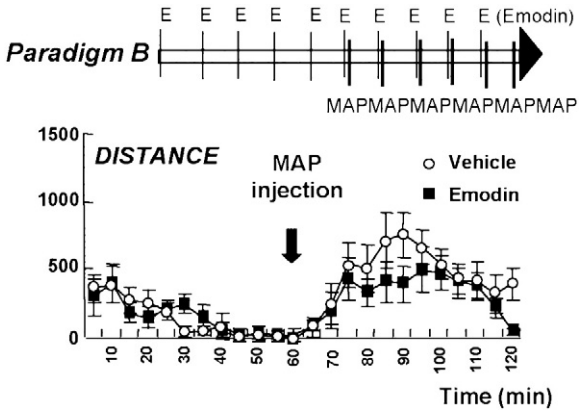

Fig. 6. Effect of parallel treatment with emodin and MAP. Emodin or vehicle was administered to rats once a day for 10 days (i.e., days 1–10). For the last 5 days, rats were additionally given MAP daily (1.0 mg/kg) (i.e., days 6–10). On day 11, all rats were re-challenged with MAP (1 mg/kg) to determine locomotor sensitization (*Paradigm B*). Locomotor activity (distance) was measured for 120 min before and after MAP treatment. Values (cm) indicate the mean ± SE ($n = 7$–8).

3.6. Influence of emodin on maintenance of MAP-induced behavioral sensitization

To test the potential therapeutic applications of emodin, we investigated the effect of emodin treatment on behavioral sensitization established by repeated MAP pre-exposure and compared that with behavioral activation triggered by acute MAP challenge alone. Rats were pre-exposed to MAP treatment (1.0 mg/kg daily for 7 days) followed by emodin- or vehicle-treatment for 7 days (*Paradigm C*). Alternatively, another group of rats received emodin- or vehicle-treatment alone (*Paradigm A1*). Both groups were challenged with MAP and their horizontal activity was monitored for 15 min (Fig. 7). Two-way ANOVA with subject factors of MAP pre-exposure and emodin treatment revealed a significant main effect of MAP pre-exposure ($F(1,21) = 24.9$, $p < 0.001$) but not of emodin ($F(1,21) = 0.202$, $p = 0.658$) without an interaction of emodin and MAP pre-exposure ($F(1,21) < 0.001$, $p = 0.99$). This statistical result suggests that MAP pre-exposure established MAP sensitization in locomotor activity, but emodin treatment had no effect on MAP sensitization.

4. Discussion

The anthraquinone compound emodin, which is purified from natural herbal extracts, inhibits ErbB2-dependent cancer proliferation as well as several inflammatory signaling pathways (Jayasuriya et al., 1992; Kaneshiro et al., 2006; Kumar et al., 1998; Li et al., 2005; Wang et al., 2007b; Zhang et al., 1999). We previously found that emodin ameliorates EGF-induced behavioral deficits, inhibiting the activation of ErbB1 and ErbB2 (Mizuno et al., 2008). Subchronic oral administration of emodin (50 mg/kg/day) ameliorates behavioral abnormality in the acoustic startle reaction as well as in PPI without influencing locomotor activity or learning performance (Mizuno et al., 2008). Furthermore, we had evidence that emodin significantly reduced body weight 1 day after the first administration. However, from the third administration onwards there were similar positive weight gains in the emodin-treated group and the untreated group (Mizuno et al., 2008). As there were no significant effects from emodin administration, it seems less likely that the physical impact of emodin's laxative activity was causative for the effects of emodin on behavior. In the present study, this compound also impacts MAP-triggered behavioral abnormality. We found that repeated pre-treatment with emodin significantly attenuated hyperlocomotion triggered by MAP challenge, although parallel- and post-treatments with emodin failed to influence behavioral sensitization to MAP.

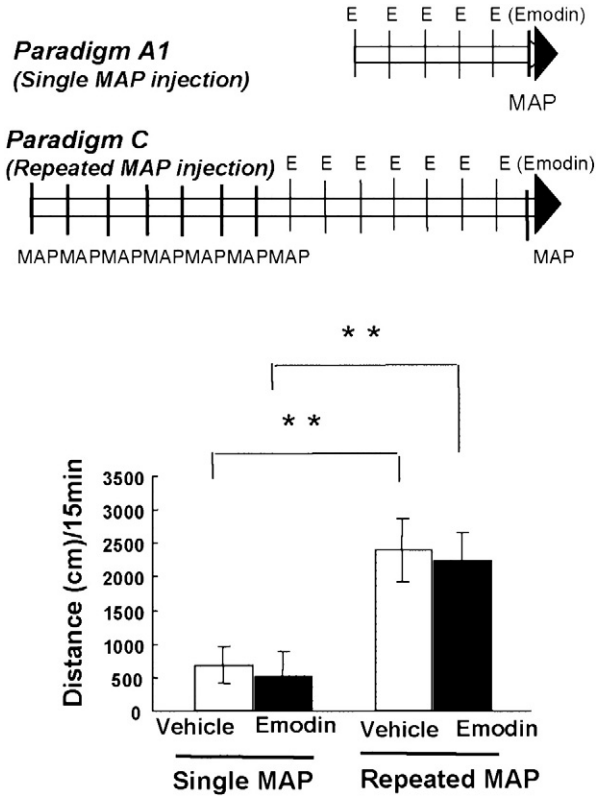

Fig. 7. Effect of emodin on the maintenance of MAP-induced sensitization in rats. Rats were challenged with MAP (1.0 mg/kg) daily for 7 days (i.e., days 1-7). For the next 7 days, rats were treated daily with emodin or vehicle (i.e., days 8–14). On day 15, all rats were re-challenged with MAP (1.0 mg/kg) (*Paradigm C*). In addition to *Paradigm C*, naïve rats were challenged with MAP in the presence or absence of emodin pre-treatment (*Paradigm A1*). Horizontal locomotor activity was also measured for 15 min after MAP challenge. Total distances were compared between acute and repeated MAP-injected groups or between emodin-treated and vehicle-treated group. Values indicate the mean ± SE ($n = 5–8$). $^{**}p < 0.01$ by *post-hoc*.

These findings suggest that pre-treatment with emodin alone is capable of ameliorating the psychotic behaviors triggered by MAP. The effects of emodin on MAP-induced behaviors temporally contrast with those of neonatal EGF challenge. In the EGF model, emodin treatment is performed at the adult stage, which is preceded by neonatal behavioral sensitization with EGF, and is effective on adult behavioral impairment.

Emodin has been shown to suppress EGF receptor (ErbB1/2) activation (Mizuno et al., 2008; Zhang et al., 1999). Activation of tyrosine kinase receptors triggers multiple intracellular signaling pathways including ERK (Oda and Kitano, 2006). ERK1/2 signaling is implicated in the rewarding effects of MAP (Kamei et al., 2006; Mizoguchi et al., 2004) as well as in behavioral sensitization of cocaine (Valjent et al., 2004). Inhibition of ERK1/2 by microinjection of PD98059, an ERK1/2 kinase inhibitor, into the ventral striatum abolishes MAP-induced ERK1/2 activation and decreases the expression of MAP-induced conditioned place preference (Mizoguchi et al., 2004). Accordingly, we tested the hypothesis that suppression of ERK1/2 phosphorylation might underlie the emodin effects on MAP-induced behavior. In the present experiment, pre-treatment with emodin abolished the MAP-triggered increase in ERK phosphorylation; however, it up-regulated basal phosphorylation levels of ERK instead of decreasing them. In this context, we cannot rule out the possibility that the emodin-triggered increase in ERK phosphorylation in the striatum might be a secondary influence of emodin. Further studies are necessary to clarify the mechanism by which emodin modulates ERK1/2 activation and attenuates MAP-induced behavioral activation (Kaneshiro et al., 2006).

There are several natural anthraquinone derivatives such as emodin, sennoside, chrysophanol, aloe-emodin, physcion and rheum (Huang et al., 2007; Wang et al., 2001). Extracts from Chinese herbs containing these compounds are most famous for promoting defecation but may also possess additional activities, including the regulation of various psychiatric conditions. Previous studies on emodin pharmacology suggest that emodin decreases the release of the excitatory neurotransmitter glutamate in rat hippocampus (Gu et al., 2005) and also attenuates glutamate excitotoxicity of brain neurons (Wang et al., 2007a). In the present investigation, we found that subchronic emodin treatment attenuated an acute MAP-triggered increase in dopamine, DOPAC and HVA content, and decreased 5-HT and 5-HIAA content in both regions of the fontal cortex and striatum. This latter observation appears to agree with the findings that serotonin content is decreased after methamphetamine injections into the striatum (Fumagalli et al., 1998), the nucleus accumbens and caudate nucleus (Wallace et al., 1999), and the hippocampus (Rau et al., 2006). It is reported that serotonin release in the frontal cortex is increased by methamphetamine challenge (Ago et al., 2006, 2008). These results of serotonin release are different from our content data most likely because serotonin content indicates that serotonin storage may decrease after MAP-induced potentiation of serotonin efflux.

Emodin prevents cycloheximide-induced amnesia in rats. Emodin is also involved in the serotonergic roles on memory ameliorating effects (Lu et al., 2007). Another anthraquinone derivative, hypericin, is synthesized from emodin and has potent anti-depressant activity (Caccia, 2005). One of its pharmacological activities is the modulation of serotonin metabolism and release (Calapai et al., 2001a,b). Furthermore, in contemporary screening studies, hypericin has inhibitory effects on various pharmaceutically important enzymes such as MAO (monoaminoxidase), PKC (protein kinase C), dopamine-beta-hydroxylase, reverse transcriptase, telomerase, and CYP (cytochrome P450) (Kubin et al., 2005). Thus, hypericin has a pharmacological activity similar to emodin. These findings suggest that emodin and its derivatives have psychomodulatory activity and might alleviate the symptoms of psychiatric illness.

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