Dopamine receptors oppositely regulate cocaine-induced transcription factor CREB activation

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Abstract: **Objective** To study the role of dopamine receptors in the regulation of the activity of transcription factor cAMP response element-binding protein (CREB) after cocaine treatment. **Methods** By using dopamine receptor antagonists SCH23390 and nafadotride, the activation of CREB by D1 and D3 dopamine receptors after cocaine treatment and role of extracellular signal-regulated kinase (ERK) in cocaine-induced CREB activation were examined by Western blotting, which was also employed for determination of the effect of SCH23390 and nafadotride on CREB activation. **Results** D1 receptor antagonist could inhibit cocaine-induced CREB activation, while D3 receptor antagonist enhanced cocaine-induced CREB activation. Dopamine receptor antagonists SCH23390 and nafadotride did not induce CREB activation. SL327, a MEK inhibitor, inhibited cocaine-induced CREB activation. **Conclusion** D1 and D3 dopamine receptors can oppositely regulate CREB activation after cocaine treatment and this regulation depends on ERK signaling pathway.

Key words: cocaine; dopamine receptor; cAMP response element-binding protein; extracellular signal-regulated kinase; gene expression

Repeated exposure to cocaine can induce long-term changes in the brain. Extensive studies have established an important role of the dopamine system in drug addiction [1] executed through dopamine receptors class D1 (D1 and D5) and D2 (D2, D3 and D4). D1 class receptors couple to Gs stimulatory proteins and activate adenylyl cyclase, while D2 class receptors couple to Gi inhibitory proteins and inhibit adenylyl cyclase[2].

Gene expression mediated by dopamine receptors after addictive drugs have been the subject of intense study. Cocaine can induce cAMP response element-binding protein (CREB) phosphorylation and expression of AP-1 immediate early genes in both the ventral and dorsal striatum [3-5]. CREB is a plasticity- associated transcription factor that regulates the expression of many downstream genes containing CRE elements and is phosphorylated at Ser-133 by multiple protein kinases, including MAPK [6]. Our recent work showed that cocaine-induced intracellular signaling and gene expression are oppositely regulated by dopamine D1 and D3 receptors and such regulation depends on proper ERK activation and c-fos function [7]. However, the role of dopamine receptors in regulating the activity of transcription factor CREB after cocaine treatment remains unclear. In order to understand if dopamine D1 and D3 receptors can coordinately regulate CREB activation after cocaine treatment, we conducted this study with D1 and D3 dopamine receptor antagonists and found that D1 and D3 dopamine receptors can oppositely regulate CREB activation after cocaine treatment in a ERK signaling pathway-dependent manner.

MATERIALS AND METHODS

Animals and reagents

Kunming mice weighing 18-22 g were housed in an animal housing room on a 12-hour light/dark cycle with food and water available *ad libitum*. The temperature and humidity of the room were controlled. Cocaine hydrochloride and SCH23390 (Sigma, St Louis, MO), the antagonist of D1 dopamine receptor, was dissolved in saline. Cocaine was used at 30 mg/kg and SCH23390 at 0.5 mg/kg. Nafadotride (Tocris Cookson, Ballwin, MO), the antagonist of D3 dopamine receptor, was also dissolved in saline and injected at the dose of 0.5 mg/kg. All the injections were administered intraperitoneally at 1 ml/100 g during the light phase of the light/dark cycle.

Treatments

Three paradigms were used in this study. For acute injections, the mice were divided into 3 groups (4 mice each), the first group was injected intraperitoneally with 30 mg/kg of cocaine, the second with SCH23390 (0.5

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mg/kg)+cocaine (30 mg/kg), and the third with nafadotride (0.5 mg/kg)+cocaine (30 mg/kg). SCH23390 and nafadotride were injected 15 min before cocaine injection. The mice were sacrificed 20 min after injection. For dopamine receptor antagonist injections, the mice were divided into 3 groups (4 mice each). The first group was injected intraperitoneally with saline, the second with SCH23390 (0.5mg/kg), and the third with nafadotride (0.5mg/kg). For MEK antagonist injections, the mice were divided into two groups (4 each) for intraperitoneal injection of cocaine (30 mg/kg) and SL327 (50 mg/kg) + cocaine (30 mg/kg), respectively. SL327 was injected 15 min before cocaine injection. Roughly equal numbers of male and female mice were used.

Protein extract preparation

The caudoputamen (CPu) tissues were isolated by gross dissection and the extracts were prepared from individual mouse brains as described [7]. The samples were homogenized in 300 μl buffer containing 50 mmol/L Tris-HCl (pH 7.5), 50 mmol/L NaCl, 5 mmol/L EDTA, 10 mmol/L EGTA, 2 mmol/L sodium pyrophosphate, 4 mmol/L paranitrophenylphosphate, 1 mmol/L sodium orthovanadate, 1 mmol/L phenylmethylsulfonyl fluoride, 2 μg/ml aprotintin, 2 g/ml leupeptin and 2 μg/ml pepstatin. Protein concentrations were determined by the Bradford method as before[7].

Western blotting

Twenty micrograms of the total protein were separated by 10% SDS-PAGE for phosCREB and CREB detection as described [7]. The resolved proteins were transferred onto PVDF membranes, and the blots were blocked in 5% nonfat dry milk, 10 mmol/L Tris-HCl (pH 7.5) and 0.1% Tween 20 and incubated in primary antibodies followed by horseradish peroxidase (HRP)-conjugated secondary antibodies. The signals were visualized using enhanced chemiluminescence. The antibodies to CREB and phospho-CREB (Cell Signaling) were used at a 1∶1 000 dilution and HRP-anti-rabbit conjugate (Santa Cruz) at 1∶5 000 dilution for CREB and phospho-CREB. All Western blot analyses were performed for at least 3 times and parallel results were obtained.

Quantification

Following phospho-CREB Western blotting, the membranes were stripped and re-probed with antibodies against total CREB. X-ray films were scanned. For each sample, the intensity of phospho-CREB was divided by the intensity of total CREB bands using Metamorph software. A two-way analysis of variance (ANOVA) was used to compare the expression of various genes under different treatment conditions between different groups. In all the cases, the significant level was set at $P<0.05$.

RESULTS

Opposite regulation of CREB by D1 and D3 dopamine receptors after cocaine treatment

In order to identify whether the opposite regulatory roles of D1 and D3 receptors in ERK activation extends to CREB activation by acute cocaine, we examined the effects of D1 and D3 dopamine receptor antagonists. Western blot analysis of CREB activation was performed using anti-phospho-CREB antibodies that recognized only the activated forms of CREB. As shown in Fig.1, CREB phosphorylation was pevented in the Cpu in mice pretreated with D1 receptor antagonist as compared with that in simply cocaine-treated mice. In contrast, CREB activation was enhanced in the Cpu in D3 receptor antagonist-pretreated mice in comparison with that in simply cocaine-treated mice. The optical density of the bands for D3 receptor antagonist -pretreated mice was 1.4-fold greater than that for simply cocaine-treated mice. These results indicate that dopamine D1 and D3 receptors execute opposite regulation of CREB activation.

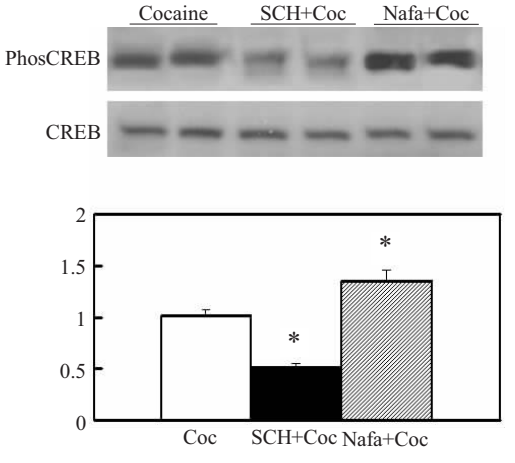

Fig.1 Opposite regulation of CREB by D1 and D3 dopamine receptors after cocaine treatment

The mice were treated with cocaine (30 mg/kg), SCH23390 (0.5 mg/kg) +cocaine (30 mg/kg), and nafadotride (0.5mg/kg)+cocaine (30 mg/kg), respectively. The protein extracts were isolated from the CPu of individual mouse and Western blotting was performed by using antibodies against phosphorylated CREB (Ser133) and total CREB, respectively. CREB phosphorylation level in exclusively cocaine-treated group mice was set as 1 for quantification. Equal amounts of protein were loaded in each lane. The data are represented as *Mean ±SE* of CREB phosphorylation in the CPu. **P*<0.05 *vs* exclusively cocaine-treated group.

Effect of SCH23390 and nafadotride on CREB activation

To further confirm that opposite regulation of CREB activation depends on functional dopamine D1 and D3 receptors, we examined the effect of dopamine receptor antagonists SCH23390 and nafadotride on CREB activation to eliminate the possibility that CREB phosphorylation was related with the dopamine receptor antagonists. The results showed that the phosphorylation levels of CREB were similar in saline, SCH23390 and nafadotride groups (Fig.2), indicating SCH23390 and nafadotride had no effects on CREB activation.

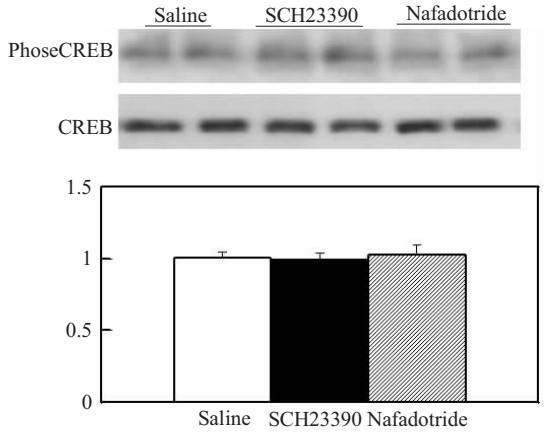

Fig.2 Effect of dopamine receptor antagonist SCH23390 and Nafadotride on CREB activation

CREB phosphorylation levels in the saline group were set as 1 for quantitative comparisons

Regulation of CREB activity by dopamine receptors depends on ERK

To determine whether the opposite regulation of CREB phosphorylation by D1 and D3 receptors depends on the ERK pathway, we treated the mice with a selective MEK inhibitor SL327 before cocaine treatment. SL327 is a potent and selective small-molecule MEK inhibitor. Systemic administration of SL327 has been shown to selectively inhibit ERK activation in the brain[8]. Our results showed that SL327 inhibited CREB phosphorylation in the CPu 20 min after cocaine treatment (Fig.3). Our recent studies demonstrated that the induction of c-Fos protein by acute cocaine administration depends on ERK signaling pathway [6]. We found that SL327 also attenuated CREB phosphorylation in the CPu in cocaine-treated mice. These evidences suggest that the activation of CREB by acute cocaine administration depends on ERK signaling pathway.

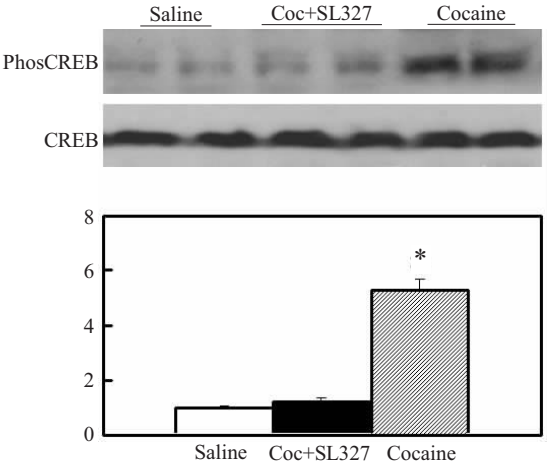

Fig.3 Induction of CREB phosphorylation depends on ERK activation

Western blot analyses were performed in the absence or presence of SL327. SL327 was injected 15 min before cocaine treatment and the protein extracts from the CPu (n=3) were isolated 20 min after cocaine injections. Equal amounts of protein were loaded in each lane. The data are represented as *Mean*±*SE* of CREB phosphorylation in the CPu. The levels of saline-injected group was set as 1 for quantifications. **P*<0.05 *vs* saline group.

DISCUSSION

CREB is a transcription factor activated by phosphorylation through the cAMP pathway and other intracellular signaling cascades[9]. The signaling pathways and gene expression changes associated with various DA receptors have been suggested to play a critical role in drug-induced neuroadaptations in the brain. Cocaine induces CREB phosphorylation and expression of the immediate early genes in D1 re ceptor-expressing neurons in both the NAc and CPu [7, 10]. The D3 receptors also regulate gene expression after cocaine challenges[11]. MAPKs are critical for cells to respond to physical and chemical changes of the environment [12]. Three major groups of MAPKs exist in mammals, including ERKs, c-Jun N-terminal kinases (JNKs) and p38. Acute and chronic exposure to abused drugs, such as cocaine and morphine, can also induce MAPK activation in the DA system and such activation may contribute to the development of drug-induced persistent changes in the brain[13].

CREs and CREB protein have proved to be involved in the transcription and regulation of many genes, including *c-fos*. CREB and *c-fos* have been implicated as two key transcriptional regulators in drug

treatment [7, 14]. Our recent study showed that *c-fos* is oppositely regulated by D1 and D3 dopamine receptors[7]. In the current study, we found that CREB phosphorylation was not induced in the Cpu in D1 receptor antagonist-pretreated mice after acute cocaine injection. Interestingly, CREB activation was enhanced in the Cpu in D3 receptor antagonist-pretreated mice as compared with that in exclusively cocaine-treated mice. These results indicate that D1 and D3 receptors also oppositely regulate acute cocaine-induced CREB activation in the Cpu.

ERK can be activated by an acute cocaine treatment in the striatum and blockade of ERK activation abolishes the rewarding effects of cocaine[15]. Previously, we found that the opposite regulatory roles of D1 and D3 receptors in c-fos induction depended on ERK. Our present study demonstrated that SL327, a MEK selective inhibitor, can also inhibit the CREB phosphorylation by acute cocaine treatment in the CPu, indicating that the activation of CREB by acute cocaine administration depends on ERK signaling pathway.

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多巴胺受体在可卡因诱导的转录因子 CREB 活化中的作用

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摘要:目的 研究多巴胺受体在可卡因诱导的转录因子 CREB 磷酸化活化中的调控作用。**方法** 采用 D1 和 D3 多巴胺受体抑制剂,应用 Western blotting 检测 D1 与 D3 多巴胺受体在可卡因诱导的 cAMP 反应元件结合蛋白(CREB)磷酸化活化中的作用及 D1 和 D3 多巴胺受体抑制剂本身对 CREB 磷酸化活化的影响,进一步应用 Western blotting 检测细胞外信号调节激酶(ERK)在 CREB 磷酸化活化中的作用。**结果** D1 多巴胺受体抑制剂阻止可卡因诱导的 CREB 磷酸化活化,而 D3 多巴胺受体抑制剂促进可卡因诱导的 CREB 磷酸化活化,D1 和 D3 多巴胺受体抑制剂本身不能诱导 CREB 磷酸化活化。MEK 的特异性抑制剂 SL327 可以抑制可卡因诱导的 CREB 磷酸化活化。**结论** D1 和 D3 多巴胺受体对 CREB 的磷酸化活化起反式调控作用,并且这种反式调控作用依赖于 ERK 信号通路。

关键词:可卡因;多巴胺受体;cAMP 反应元件结合蛋白;细胞外信号调节激酶;基因表达

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