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Blockade of ERK Phosphorylation in the Nucleus Accumbens Inhibits the Expression of Cocaine-induced Behavioral Sensitization in Rats

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Repeated administration of psychostimulants such as cocaine leads to the development of behavioral sensitization. Extracellular signal-Regulated Kinase (ERK), an enzyme important for long-term neuronal plasticity, has been implicated in such effects of these drugs. Although the nucleus accumbens (NAcc) is the site mediating the expression of behavioral sensitization by drugs of abuse, the precise role of ERK activation in this site has not been determined. In this study we demonstrate that blockade of ERK phosphorylation in the NAcc by a single bilateral microinjections of PD98059 (0.5 or 2.0 μg/side), or U0126 (0.1 or 1.0 μg/side), into this site dose-dependently inhibited the expression of cocaine-induced behavioral sensitization when measured at day 7 following 6 consecutive daily cocaine injections (15 mg/kg, i.p.). Acute microinjection of either vehicle or PD98059 alone produced no different locomotor activity compared to saline control. Further, microinjection of PD98059 (2.0 μg/side) in the NAcc specifically lowered cocaine-induced increase of ERK phosphorylation levels in this site, while unaffecting p-38 protein levels. These results indicate that ERK activation in the NAcc is necessary for the expression of cocaine-induced behavioral sensitization, and further suggest that repeated cocaine evokes neuronal plasticity involving ERK pathway in this site leading to long-lasting behavioral changes.

Key Words: Cocaine, Behavioral sensitization, PD98059, U0126, Nucleus accumbens

INTRODUCTION

Repeated exposure to psychomotor stimulant drugs such as cocaine produces behavioral sensitization, which most often demonstrated in mouse and rats as a progressive and enduring augmentation of locomotor responses to the drug [1]. This phenomenon has been proposed to underlie the ability of psychomotor stimulants to elicit craving by enhancing the incentive motivational value of these drugs [2,3]. Thus, elucidating the molecular mechanisms that involve in behavioral sensitization will provide a better understanding about the critical steps that mark the transitional shift to compulsive drug use among human addicts.

Extracellular signal-regulated kinase (ERK) is a protein serine/threonine kinase that plays an important role in various types of neuronal adaptive responses as well as in learning and memory [4,5]. Several lines of evidence indicate that ERK pathway also importantly involves in both acute and long-term adaptive processes by drugs of abuse. For example, chronic administration of cocaine or morphine increases ERK activity in the ventral tegmental area (VTA), the region in the ventral midbrain that contains dopaminergic cell bodies [6]. In the nucleus accumbens (NAcc), which receives dopaminergic axonal projections from the VTA, ERK activation is induced by acute cocaine [7] and it is augmented in cocaine-sensitized rats [8]. Behavioral studies also show that the systemic injection of SL327, a selective inhibitor of ERK kinase (MEK), abolishes the development of locomotor sensitization as well as conditioned place preference by cocaine [7,9].

Evidence indicates that the development of behavioral sensitization is initiated in the VTA, while its expression is due to drug actions in the NAcc [10,11]. Recent research shows that the development of sensitization to cocaine was prevented by pre-treatment of a MEK inhibitor prior to each drug administration during the induction phase [9,12], while its expression was not affected when challenged later

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ABBREVIATIONS: ERK, extracellular signal-regulated kinase; MEK, ERK kinase; NAcc, nucleus accumbens; VTA, ventral tegmental area; IP, intra-peritoneum.

with cocaine in the presence of the same inhibitor [9]. However, these results were all obtained with systemic injections of a MEK inhibitor SL327, which crosses the blood-brain barrier and affects various brain regions (for example, see [9]), so that it was not clear in which area of brain ERK actually contributes to sensitization. Although it was previously observed that the initiation of behavioral sensitization to cocaine was blocked by intra-VTA microinjection of another MEK inhibitor PD98059 [13], the role of ERK in the expression of sensitization has not been examined yet directly in the NAcc. Thus, we investigated in the present study whether a specific blockade of ERK phosphorylation by microinjection of PD98059 into the NAcc produces a differential effect to the expression of behavioral sensitization to cocaine.

METHODS

Animals and surgery

Male Sprague-Dawley rats weighing 220~250 g on arrival were obtained from Orient Bio Inc. (Seongnam-si, Korea). They were housed three per cage in a 12-hr light/dark cycle room with food and water available at all times. All animal use procedures were conducted according to an approved IACUC protocol. Rats were anesthetized with intraperitoneal (IP) ketamine (100 mg/kg) and xylazine (6 mg/kg), placed in a stereotaxic instrument with the incisor bar at 5.0 mm above the interaural line and implanted with chronic bilateral guide cannulas (22 gauge; Plastics One, Roanoke, VA) aimed at the NAcc (A/P, +3.4; L, ±1.5; D/V, −7.5 mm from bregma and skull) [14]. Cannulas were angled at 10° to the vertical, positioned 1 mm above the final injection site, and secured with dental acrylic cement anchored to stainless steel screws fixed to the skull. After surgery, 28 gauge obturators were placed in the guide cannulas, and rats were returned to their home cages for a 7-day recovery period.

Drugs and intracranial microinjections

PD98059 and U0126 (Sigma, St. Louis, MO) were dissolved in 70% and 20% DMSO, respectively, and small aliquots were stored at −20°C. Immediately before use, frozen aliquots of each drug were diluted to concentrations of either 1.0 or 4.0 μg/μl in 70% DMSO (PD98059) and of either 0.2 or 2.0 μg/μl in 20% DMSO (U0126). Cocaine hydrochloride (Belgopia, Belgium) was dissolved in sterile 0.9% saline. Bilateral intracranial microinjections into the NAcc were made in the freely moving rat. Injection cannulas (28 gauge) connected to 1 μl syringes (Hamilton, Reno, NV) via PE-20 tubing were inserted to a depth 1 mm below the guide cannula tips. Injections were made in a volume of 0.5 μl per side over 30 sec. After 1 min, the injection cannulas were withdrawn and the obturators were replaced.

Locomotor activity

Locomotor activity was measured in a bank of 6 activity boxes (35×25×40 cm) (IWOO Scientific Corporation, Seoul, Korea) made of translucent Plexiglas and individually kept in larger PVC plastic sound attenuating cubicles. The floor of each box consisted of 21 stainless steel rods (5 mm diameter) spaced 1.2 cm apart center-to-center. Locomotor activity was counted by two infrared light photocells (Med Associates, Inc., St. Albans, VT, USA), positioned 4.5 cm above the floor and spaced evenly along the longitudinal axis of each box.

Design and procedure

Experiment 1: Six different groups of rats were randomly assigned and half of them were administered once a day with saline and the other half with cocaine (15 mg/kg, IP) for 6 consecutive days. Injections were made in the activity boxes on day 1 and 6, and at home-cages for the rest of days (day 2 to 5). On day 7, animals were first habituated to the activity boxes for 1 hour, then microinjected bilaterally into the NAcc with either vehicle (70% DMSO) or each dose of PD98059 (0.5 and 2.0 μg/0.5 μl/side). Microinjections were performed only a single time and animals remained in the activity boxes for further 30 min until they were all cocaine (15 mg/kg, IP) challenged, then their locomotor activity was measured for 2 hours.

Additional four groups of drug-naïve rats were first habituated to the activity boxes for 1 hour, then microinjected only a single time with either saline or vehicle, one of two doses of PD98059 (0.5, 2.0 μg/side) and remained in the activity boxes for 30 min. Then, they were all saline (IP) challenged and their locomotor activity measured for 1 hour.

Experiment 2: Four different groups of rats were randomly assigned and half of them were microinjected with vehicle and the other half with a single dose of PD98059 (2.0 μg/side). Then, each group was subdivided into two and administered with either saline or cocaine (15 mg/kg, IP), respectively. Animals were decapitated 10 min after saline or cocaine IP injection, brains were rapidly removed and coronal sections (1.0 mm thick extending 1.60~2.60 mm from bregma) were obtained with an ice-cold brain slicer. Tissue punches (1.2 mm diameter) were obtained in both the NAcc region (covering most medial part of the core and some portion of the shell near the border of the two) and the dorsal striatum on an ice-cold plate, immediately frozen on dry ice and stored at −80°C. They were prepared bilaterally and pooled for each individual animal's protein isolation.

Experiment 3: Six different groups of rats were randomly assigned and half of them were administered once a day with saline and the other half with cocaine (15 mg/kg, IP) for 6 consecutive days. Injections were made in the activity boxes on day 1 and 6, and at home-cages for the rest of days (day 2 to 5). On day 7, animals were first habituated to the activity boxes for 1 hour, then microinjected bilaterally into the NAcc with either vehicle (20% DMSO) or each dose of U0126 (0.1 and 1.0 μg/0.5 μl/side). Microinjections were performed only a single time and animals remained in the activity boxes for further 30 min until they were all cocaine (15 mg/kg, IP) challenged, then their locomotor activity was measured for 1 hour.

Western blotting

Tissues were homogenized in lysis buffer containing 0.32 M sucrose, 2 mM EDTA, 1% SDS, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1mM phenylmethylsulfonyl fluoride. The concentration of protein was determined by using Pierce BCA protein assay kit (Pierce, Rockford, IL). Samples were then boiled for 10 min and subjected to SDS-polyacrylamide gel electrophoresis. Proteins were transferred

electrophoretically to nitrocellulose membranes (Bio-Rad, Hercules, CA), which were then blocked with 5% skim milk in PBS-T buffer [10 mM phosphate-buffered saline plus 0.05% Tween-20]. Specific antibodies against phospho-ERK 1/2, phospho-p38 (1 : 1,000 dilution in PBS-T with 5% bovine serum albumin; Cell Signaling, Beverly, MA) and ERK1/2, p38 (1 : 1,000 dilution in PBS-T with 5% skim milk; Cell Signaling) kinases were used to probe the blots. Primary antibodies were detected with peroxidase-conjugated secondary antibodies (1 : 2,000 dilution in PBS-T with 5% skim milk; KOMA Biotech, Seoul, Korea) followed by enhanced chemiluminescence (ECL) reagents (Amersham Biosciences, Arlington Heights, IL) and exposure to X-ray film. Band intensities were quantified based on densitometric values using Fujifilm Science Lab 97 Image Gauge software (version 2.54). Antibodies on the membrane were removed by using Restore™ Western Blot Stripping Buffer (Pierce, Rockford, IL, USA) and re-probed with anti-β-actin antibody (1 : 10,000 dilution in PBS-T with 5% skim milk; Abcam, Cambridge, UK).

Histology

After completion of behavioral experiments, animals were anesthetized and perfused via intracardiac infusion of saline and 10% formalin. Brains were removed and further post-fixed in 10% formalin. Coronal sections (40 μm) were subsequently stained with cresyl violet for verification of cannula tip placements. Only rats with injection cannula tips located bilaterally in correct placements were included: a total of 117 rats (38, 20, 30 and 29 for Fig. 1~4, respectively) out of 135 initially prepared.

RESULTS

Blockade of ERK phosphorylation by a single microinjection of PD98059 in the NAcc inhibits the expression of cocaine-induced locomotor sensitization

Fig. 1A shows the locomotor activity counts obtained in both saline and cocaine pre-exposed rats in response to an IP cocaine challenge injection following a single NAcc microinjection of either vehicle or each dose of PD98059 (0.5 and 2.0 μg/side). The two-way between (microinjections) - within (pre-exposures) ANOVA conducted on these data found an approached significance for different microinjections [$F_{2,32}$=2.95, $p<0.068$] and multiple significant effects of different pre-exposures [$F_{1,32}$=35.34, $p<0.001$], and a microinjections X pre-exposures interaction [$F_{2,32}$=3.71, p

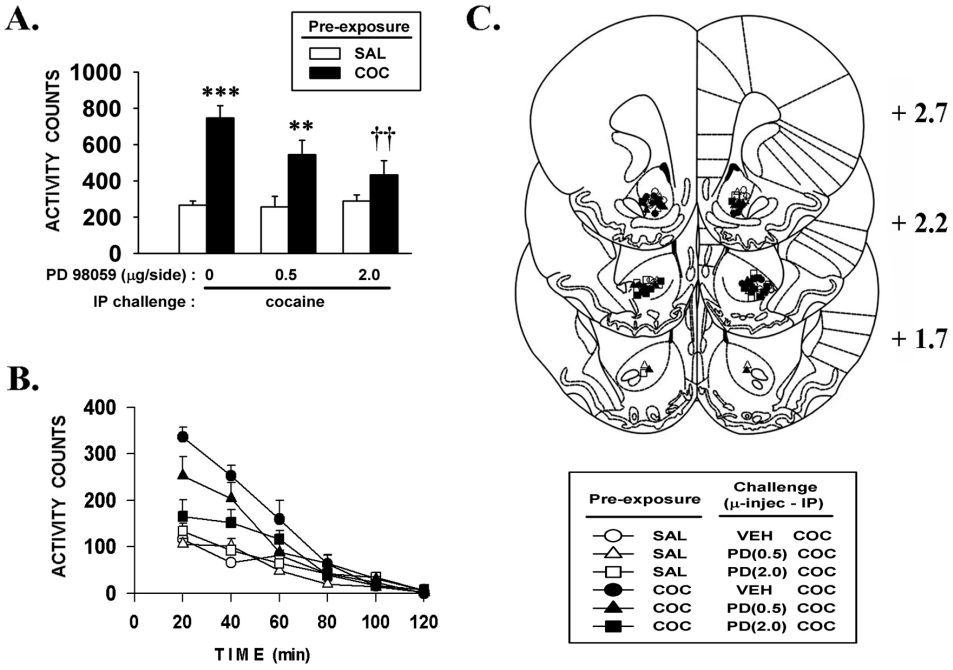

Fig. 1. Blockade of ERK phosphorylation by a single microinjection of PD98059 in the NAcc inhibits the expression of cocaine-induced locomotor sensitization. (A) Animals were either saline or cocaine pre-exposed for 6 days. At day 7, their locomotor activity was observed after cocaine (15 mg/kg, IP) challenge injections preceded 30 min earlier by an acute single bilateral microinjection of either vehicle or PD98059 (0.5, 2.0 μg/side) into the NAcc. Enhanced increase of locomotor activity in cocaine compared to saline pre-exposed animals was dose-dependently inhibited by PD98059 microinjection into the NAcc. Data are shown as group mean (+S.E.M.) total locomotor activity counts observed during the first 1 hour. Numbers of rats in each group are 6~7. Symbols indicate significant differences as revealed by *post-hoc* Scheffé comparisons following two-way between (microinjections) - within (pre-exposures) ANOVA. ***$p<0.001$, **$p<0.01$; significantly more counts in cocaine relative to saline pre-exposed animals. $^{\dagger\dagger}p<0.01$; significant differences in cocaine pre-exposed animals when PD98059 (2.0 μg/side) compared to vehicle was microinjected. (B) Time-course data are shown as group mean (+S.E.M.) locomotion activity counts for every 20 min time-bins obtained during the 2-hr test immediately after cocaine challenge injection. (C) Location of the microinjection cannula tips in the NAcc of rats included in the data analyses. Only rats with injection cannula tips located bilaterally in this site were included. No neuronal damage was observed other than that produced by the insertion of the cannulae. The line drawings are from Paxinos and Watson [15]. Numbers to the right indicate millimeters from bregma.

<0.037]. As expected, daily cocaine pre-exposed rats compared to saline showed a sensitized locomotor activity in response to an IP cocaine challenge injection when vehicle was microinjected into the NAcc ($p<0.001$, by *post-hoc* Scheffé comparisons). This effect of cocaine, however, was inhibited in a dose-dependent manner by a single microinjection into the NAcc of PD98059 ($p<0.01$ in a high dose, 2.0 μg/side, compared to vehicle). In saline pre-exposed rats, PD98059 had no effect on locomotor activity in response to a cocaine challenge indicating that NAcc microinjection of PD98059 produces its effect on sensitized rather than acute locomotor activity by cocaine. The time-course data and location of injection cannula tips in the NAcc of rats that were included in this experiment are illustrated in Fig. 1B and C, respectively.

Acute microinjection of vehicle or PD98059 alone into the NAcc produces no different levels of locomotor activity compared to saline control

To further examine the specific ability of NAcc PD98059 to inhibit the expression of locomotor sensitization to cocaine, additional rats were just acute saline challenged following microinjection of either vehicle or PD98059 and their locomotor activity were measured (Fig. 2). The one-way ANOVA conducted on these data found no significant differences between groups [$F_{3,16}=0.07$, $p<0.976$], indicating that either vehicle or any dose of PD98059 used in the present experiments does not produce the change of basal locomotor activity consistent with previous findings [16].

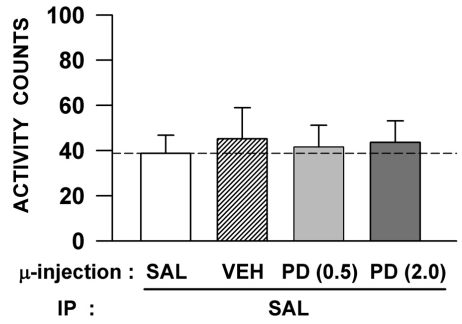

Fig. 2. Acute microinjection of vehicle or PD98059 alone into the NAcc produces no different levels of locomotor activity compared to saline control. Locomotor activity is observed after saline (IP) injections following bilateral microinjections of either saline or vehicle (70% DMSO), PD98059 (0.5, 2.0 μg/side) into the NAcc. Data are shown as group mean (+S.E.M.) total locomotor activity counts observed for 1 hour (n=5/group).

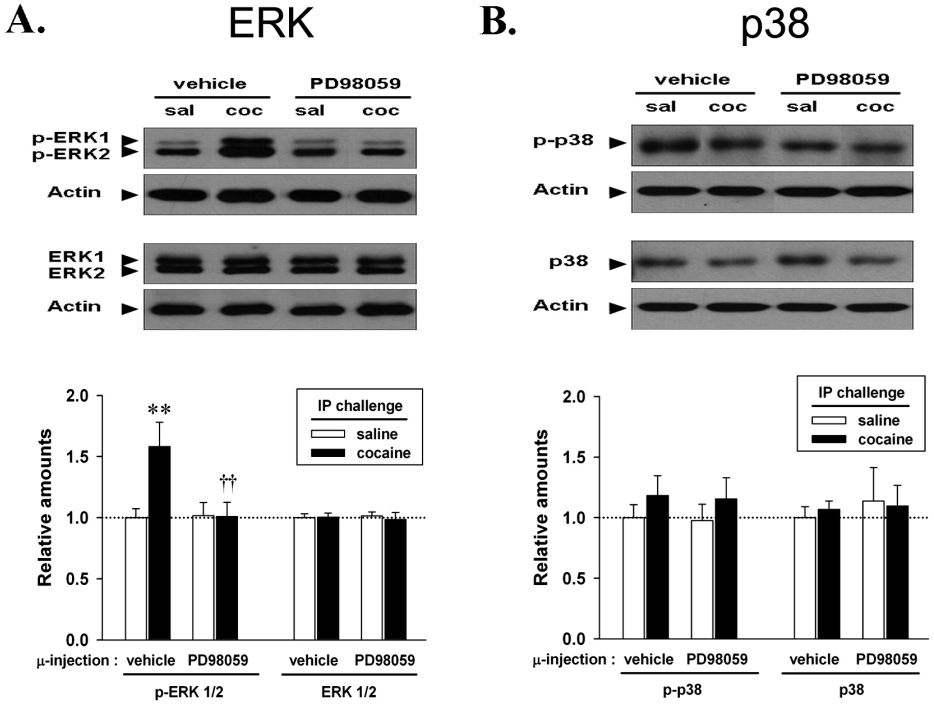

Fig. 3. PD98059 in the NAcc lowers cocaine-induced increase of ERK phosphorylation levels in this site. Animals were either saline or cocaine (15 mg/kg) IP challenged following 30 min earlier microinjection of either vehicle or a single dose of PD98059 (2.0 μg/side). After 10 min, brains were removed and the NAcc tissues were punched out. Representative Western blots labeled with antibodies against phosphorylated and total ERK1/2 (A), and p-38 (B), were shown. Blots were scanned and the band intensities were quantified using densitometer. Values are normalized to β-actin and expressed as mean (+S.E.M.) (n=7 to 8/group) transformed to relative amounts of control (vehicle microinjection - saline IP) values. Symbols indicate significant differences as revealed by *post-hoc* Scheffé comparisons following two-way between (IP challenges) - within (microinjections) ANOVA. **$p<0.01$; significantly higher levels of p-ERK1/2 in cocaine relative to saline challenged animals. $^{\dagger\dagger}p<0.01$; significant differences in cocaine challenged animals when PD98059 (2.0 μg/side) compared to vehicle was microinjected. Other proteins examined showed no differences between groups.

PD98059 in the NAcc lowers cocaine-induced increase of ERK phosphorylation levels in this site

Because we used microinjection techniques, in order to confirm the ability of PD98059 to lower phosphorylated ERK1/2 levels directly in the tissue, both the NAcc and dorsal striatum were taken out from the animals either saline or cocaine IP injected following microinjection of either vehicle or a high dose of PD98059 and their protein levels were analyzed (Fig. 3). Either phosphorylated (pERK1/2 and p-p38) or total (ERK1/2 and p38) protein kinase levels were examined on separate gels and normalized to β-actin protein levels. The 42- and 44-kDa forms of ERK were quantified together. The increased levels of phosphorylated ERK1/2 by cocaine in the NAcc were significantly lowered in PD98059 (2.0 μg/side)- compared to vehicle-microinjected animals as revealed by *post-hoc* Scheffé comparisons ($p<0.01$) following two-way between (IP challenges) - within (microinjections) ANOVA (for microinjections [$F_{1,26}=4.03$, $p<0.055$], IP challenges [$F_{1,26}=4.33$, $p<0.047$], and a microinjections X IP challenges interaction [$F_{1,26}=4.60$, $p<0.042$]). Total ERK1/2 and both phosphorylated and total p38 levels showed no differences between groups. All protein levels examined in dorsal striatum were unaffected by PD98059 microinjection into the NAcc (data not shown). These results clearly indicate that microinjection of PD98059 into the NAcc selectively lowers ERK phosphorylation levels only in this site.

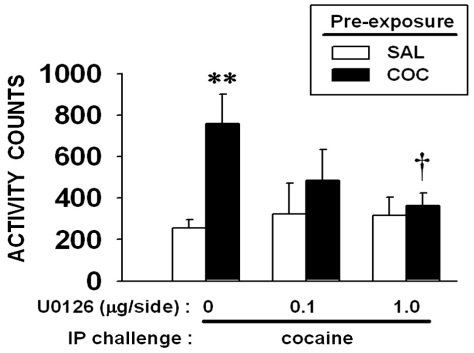

Fig. 4. Blockade of ERK phosphorylation by a single microinjection of U0126 in the NAcc inhibits the expression of cocaine-induced locomotor sensitization. Animals were either saline or cocaine pre-exposed for 6 days. At day 7, their locomotor activity was observed after cocaine (15 mg/kg, IP) challenge injections preceded 30 min earlier by acute bilateral microinjections of either vehicle or U0126 (0.1, 1.0 μg/side) into the NAcc. Enhanced increase of locomotor activity in cocaine compared to saline pre-exposed animals was dose-dependently inhibited by a single U0126 microinjection into the NAcc. Data are shown as group mean (+S.E.M.) total locomotor activity counts observed during the 1 hour. Numbers of rats in each group are 4~6. Symbols indicate significant differences as revealed by *post-hoc* Scheffé comparisons following two-way between (microinjections) - within (pre-exposures) ANOVA. **$p<0.01$; significantly more counts in cocaine relative to saline pre-exposed animals. $^{\dagger}p<0.05$; significant differences in cocaine pre-exposed animals when U0126 (1.0 μg/side) compared to vehicle was microinjected.

Blockade of ERK phosphorylation by a single microinjection of U0126 in the NAcc inhibits the expression of cocaine-induced locomotor sensitization

Finally, we further examined the specific ability of p-ERK blockade in the NAcc on the inhibition of the expression of locomotor sensitization to cocaine by using another MEK inhibitor, U0126. Fig. 4 shows the locomotor activity counts obtained in both saline and cocaine pre-exposed rats in response to an IP cocaine challenge injection following a single NAcc pre-infusion of either vehicle or each dose of U0126 (0.1 and 1.0 μg/side). The two-way between (microinjections) - within (pre-exposures) ANOVA conducted on these data found a significant effect of different pre-exposures [$F_{1,23}=6.90$, $p<0.016$]. Again, rats pre-exposed to daily cocaine compared to saline showed a sensitized locomotor activity in response to an IP cocaine challenge injection when vehicle was microinjected into the NAcc ($p<0.01$, by *post-hoc* Scheffé comparisons). However, this effect of cocaine, similar to PD98059, was inhibited in a dose-dependent manner by a single microinjection into the NAcc of U0126 ($p<0.01$ in a high dose, 1.0 μg/side, compared to vehicle). In saline pre-exposed rats, U0126 had no effect on locomotor activity in response to a cocaine challenge indicating that NAcc microinjection of U0126 also produces its effect on sensitized rather than acute locomotor activity by cocaine.

DISCUSSION

The present results are the first demonstration, to our knowledge, that a direct bilateral microinjection into the NAcc of PD98059, or U0126, MEK inhibitors, dose-dependently inhibits the expression of cocaine-induced behavioral sensitization. This effect was clearly observed when challenged by cocaine following the acute single microinjection of PD98059, or U0126, in cocaine pre-exposed animals, while it was not in saline pre-exposed. Further, microinjection of PD98059 into the NAcc produced no effect on the basal locomotor activity, while it specifically lowered cocaine-induced increase of phosphorylated ERK levels in this site. These results illustrate the importance of ERK activation in the NAcc for the expression of cocaine-induced behavioral sensitization.

It has recently been shown that the development of sensitization to cocaine is prevented by systemic pre-treatment of a MEK inhibitor, SL327, prior to each drug administration during the induction phase [9,12]. These effects were further supported by earlier results that the initiation of behavioral sensitization to cocaine was blocked by intra-VTA microinjection of another MEK inhibitor, PD98059 [13]. These results indicate that consistent ERK activation, especially in the VTA, during the induction phase is necessary for the development of behavioral sensitization by cocaine. However, the role of ERK activation in the expression of behavioral sensitization, which is mediated through a different neuronal substrate (i.e., the NAcc) [10,11], remained undefined. Although recent results also showed that the blockade of ERK pathway did not alter the expression of behavioral sensitization to cocaine and amphetamine [9], these results were all obtained with systemic injections of SL327. This drug crosses the blood-brain barrier and reduced ERK phosphorylation levels in various brain regions [9], which thereby makes it unclear whether

the NAcc ERK actually contributes to the expression of behavioral sensitization to psychomotor stimulants. In the present results, we used a single direct microinjection into the NAcc of different types of MEK inhibitors, PD98059, or U0126, and found that ERK activation specifically in this site is actually necessary for the expression of behavioral sensitization. The NAcc exists as a central part of a complicated neuronal circuit mediating rewarding and motor behaviors and its activity is regulated by signals coming from other brain regions. The difference in our results compared from previous findings that SL327 have no effect on the expression of behavioral sensitization may have come out because we inhibited ERK phosphorylation directly in the NAcc, whereas others inhibited it simultaneously in many brain regions causing its effect in the NAcc to be compromised by signals coming from other regions.

The delivery vehicle we used for MEK inhibitors was DMSO. Microinjection of this vehicle alone (up to 70% for PD98059) compared to saline didn't affect basal locomotor activity in response to IP saline injection. Similar to and consistent with our findings, others have also reported that various dose ranges (50～100%) of DMSO were microinjected into the NAcc as well as into the VTA without evident disturbances of locomotor activity and neuronal damage [8,13,16]. Our present results also showed that microinjection into the NAcc of PD98059 had no effect on the locomotor activity produced by either acute cocaine (saline pre-exposed and cocaine challenged in Fig. 1A) or saline injection (Fig. 2). These results indicate that the inhibitory effect of PD98059 was rather specific to the expression of locomotor sensitization to cocaine and further support and extend its role in the regulation of neuronal plasticity induced by drugs of abuse found in various aspect of addiction [8,9,12,16,17].

It has been reported that the levels of ERK phosphorylation in the NAcc increased by cocaine challenge [8,18]. In order to verify that this increase of cocaine-induced ERK phosphorylation levels is actually lowered by our microinjection procedure, we measured protein levels in both the NAcc and dorsal striatum after either vehicle or PD98059 microinjection. Although a recent finding using knockout mice suggests that ERK 1 and 2 may have different roles in cocaine-induced plasticity [12], it remains to be further explored, so that we decided in the present experiments to quantify both proteins together. As shown in Fig. 3, our microinjection procedure specifically lowered cocaine-induced increase of phosphorylated ERK1/2 levels in the NAcc, but not affected the levels of total ERK1/2 and another MAP kinase p38, both phosphorylated and total. These effects were not observed in dorsal striatum (data not shown). These results support that our behavioral findings were actually obtained correlated with the specific blockade of ERK phosphorylation in the NAcc and further suggest that specific ERK activation in this site may play an important role in neuronal plasticity contributing to the expression of sensitization.

Evidence shows that the interaction of both dopamine and glutamate in the NAcc is necessary for the expression of behavioral sensitization by psychomotor stimulants [19-21] and previous exposure to psychomotor stimulants increases dopamine and glutamate overflows in the NAcc [22,23]. Interestingly, it has been shown that cocaine-induced ERK activation is also dependent on both dopamine and glutamate activation probably via DARPP-32 mediated signaling pathways [7,18,24]. Together with our present findings, this leads to the hypothesis that the increase of dopamine and glutamate levels by cocaine challenge may subsequently result in increase of ERK phosphorylation, and thereby contributes to the expression of behavioral sensitization. However, it remains to be further explored.

Finally, in the present experiments, most microinjections of PD98059 were made into the core of the NAcc. However, in consideration with the increasing knowledge of the differential involvement in addiction-related behaviors of the two sub-regions, core and shell, of the NAcc [25,26], a more thorough systematic assessment of the effects of PD98059 in these different sub-regions of the NAcc is in order.

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