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Acupuncture attenuates cocaine-induced expression of behavioral sensitization in rats: Possible involvement of the dopaminergic system in the ventral tegmental area

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ABSTRACT

Acupuncture is widely used for the treatment of many functional disorders, such as substance abuse, and has the suppressive effect on the central nervous system. Many studies have suggested that behavioral sensitization by repeated injections of cocaine produce an increase in locomotor activity and an increase in the expression of tyrosine hydroxylase (TH), in the central dopaminergic system. In order to investigate the effects of acupuncture on the repeated cocaine-induced neuronal and behavioral sensitization alternations, we examined the influence of acupuncture on the repeated cocaine-induced locomotor activity and the expression of TH in the brain using immunohistochemistry. Male SD rats were given repeated injections of cocaine hydrochloride (15 mg/kg, i.p. for 10 consecutive days) followed by one challenge injection on the 4th day after the last daily injection. Cocaine challenge produced a large increase in the locomotor activity and the expression of TH in the ventral tegmental area (VTA). Treatment with acupuncture bilaterally at the Shenman (HT7) points for 1 min significantly inhibited the increase of locomotor activity as well as the TH expression in the VTA. Our data demonstrated that the inhibitory effects of acupuncture on cocaine-induced expression of behavioral sensitization were closely associated with the reduction of dopamine (DA) biosynthesis and the postsynaptic neuronal activity. These results provide evidence that acupuncture may be effective for inhibiting the behavioral effects of cocaine by possible modulation of the central dopaminergic system.

Cocaine is a potent and widely abused psychostimulant that exerts behavioral and neuropharmacological effects. These effects may be mediated by the mesolimbic dopamine (DA) systems. The mesolimbic dopaminergic pathway from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) and prefrontal cortex is thought to play a major role in the reinforcing effects and behavioral sensitization in drugs abuse [4,33]. Therefore, repeated exposure to cocaine can produce behavioral sensitization, as evidenced by an enhanced motor-stimulant response that occurs with repeated exposure to such psychostimulants as cocaine or amphetamine. This behavioral sensitization has been implicated in the development of drug addiction behaviors and in drug-induced psychosis [14,29].

Many recent studies have demonstrated that the reinforcing and sensitizing effects of cocaine produce sensitization of extracellular DA levels in the nucleus accumbens and behavioral sensitization in rats, as evidenced by an increased dopamine release in the brain, and increased locomotor activity [5,25]. In addition, the behavioral effects of cocaine have been shown to be related to the blocking of the DA reuptake systems [31], and the consequent increases in the binding of DA to its postsynaptic receptors in several brain nuclei such as the NAc, the striatum, the VTA and the medial prefrontal cortex [4,8,11,15,16]. Several pieces of evidence suggest that repeated exposure to cocaine produces the expression of tyrosine hydroxylase (TH) enzyme activity for DA biosynthesis in the mesolimbic DA pathways [3,19,35,36].

Acupuncture, as an alternative medical treatment, has been widely used for the treatment of many functional disorders including substance abuse and mental illness in Asian countries [7]. In addition, it is well known that acupuncture contributes to the biochemical balance in the central nervous system and the maintenance or recovery of homeostasis [37,38]. A few studies using animal models have recently shown that acupuncture can suppress the morphine withdrawal syndrome and alcohol-drinking behavior [39,41]. Furthermore, some studies have shown that acupuncture

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at a specific acupoint (Shenmen (HT7)) has attenuated ethanol-induced DA release in the nucleus accumbens through the GABAb receptor [12,40,42], and suppressed c-fos expression in the nucleus accumbens and ventromedial striatum following a nicotine challenge in rats sensitized to nicotine [9]. In Asian medicine, studies have suggested that the Shenmen point, on the heart channel, has been useful clinically for the treatment of drug abuse as well as other mental and psychiatric disorders. In addition, the Neiguan (PC6) point is an acupuncture point of the pericardium channel that is related to psychosomatic disorders including cardiac disorders [34], as described previously [42].

However, there are still many questions about the basic mechanism underlying acupuncture's effect as therapeutic intervention for cocaine addiction. In addition, the effects of acupuncture on the cocaine-induced neurochemical and behavioral alterations have not been investigated in animal models. The purpose of this study was to investigate whether acupuncture could affect the repeated cocaine-induced behavioral sensitization. In addition, the expression of TH in the VTA was also examined by using immunohistochemical methods in order to determine a possible mechanism underlying the suppressive effects of acupuncture on the cocaine-induced behavioral sensitization in rats.

The subjects were male Sprague-Dawley rats, weighing 260–270 g at the start of the experiment. Upon arrival, the animals were randomly divided into several groups and housed for at least 7 days prior to initiating the experimental procedures. The rats were kept on a 12 h light, 12 h dark cycle in individual home cages with food and water available ad libitum. The experimental procedures were carried out according to the animal care guidelines of the NIH and the Kyung Hee University Institutional Animal Care and Use Committee.

The experiment consisted of three phases: a 10 day developmental phase, a 3 day withdrawal phase and a 1 day challenge phase. The rats were divided into 11 groups. One group was pretreated with saline (0.9% NaCl, i.p., SAL group; $n = 6$) or cocaine (15 mg/kg, i.p., COC group; $n = 7$) once daily for 10 consecutive days, after which the rats were challenged with the same dose of saline and cocaine, 72 h after the last treatment, respectively. The acute cocaine treated group (1 mg/kg, i.p., ACT group; $n = 6$) received saline for 10 days, after which time the rats were challenged with cocaine. The other control group (15 mg/kg, RCT group; $n = 5$) received cocaine for 10 days, after which the rats were challenged with saline. The other groups received saline once daily for 10 consecutive days, after which rats were challenged with saline along with HT7 point stimulation (HT/O group; $n = 5$) and after which the rats were challenged with cocaine along with HT7 point stimulation (HT/S group; $n = 5$). Another group received cocaine once daily for 10 consecutive days, after which the rats were challenged with saline along with HT7 point stimulation (HT/C group; $n = 5$). Following 72 h of cocaine withdrawal, acupuncture was applied at bilaterally at the Shenmen (HT7, HT + COC group; $n = 7$) points for 1 min after the systemic challenge with cocaine. For the other control group (TA + COC group; $n = 6$) the tail points were used as a stimulation control sites to determine the effects of mechanical stimulation at non-acupoints. In another group of rats (PC6, PC + COC group; $n = 6$) bilateral PC6 points were used as nonspecific control acupoints.

Stainless-steel needles with a diameter of 0.18 mm and a length of 20 mm were inserted vertically to a depth of 3 mm into the acupoints of rats lightly restrained by human hands. The rats were also restrained in the same manner and prehandled for 2 min/day for 3 consecutive days prior to the acupuncture treatments to reduce stress and facilitate handling as described previously [42]. The acupuncture stimulation was manually delivered by twisting the acupuncture needles at a frequency of twice per second for a total of 2 s of stimulation while the needles were inserted and withdrawn from the acupoints. The anatomical locations of the stimulated acupuncture points in the rats were equivalent to the acupoints in man as described by Stux and Pomeranz [34] and in the animal acupuncture atlas [32]. The HT7 point is anatomically located on the transverse crease of the wrist of the forepaw, radial to the tendon of the m. flexor carpi ulnaris. The PC6 point is located between the tendons of the m. palmaris longus and the flexor carpi redialis, 4 mm proximal to the transverse crease of the wrist of the forepaw. In addition, needles were placed into non-acupoints 0.2 (1/5 tail length) from the proximal region of the tail to avoid the two tail acupoints (proximal tail and tip to the tail). These non-acupoints are distal to the proximal tail acupoints.

Their locomotor activity was measured for 1 h after every injection of cocaine or saline. The rats were individually housed prior to the behavioral testing. The locomotor activity was measured in a rectangular container ($40 \times 40 \times 45\ cm^3$) that was equipped with a video camera above the center of the floor as described previously [9]. The walls and floor were made of clear plastic and they were painted black. The locomotor activity was monitored by a video-tracking system using the S-MART program (PanLab, Barcelona, Spain). The animals were allowed to adapt for 1 h in the container and the distance they traveled was recorded every 10 min during a 1 h baseline and during 1 h after treatment. The measurement of locomotor activity was indicated in cm.

One hour after the last behavioral testing, the animals were deeply anesthetized with a sodium pentobarbital (80 mg/kg, i.p.) and then they were perfused through the ascending aorta with normal saline (0.9%) followed by 800 ml of 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS). The brains were removed, post-fixed overnight and cryoprotected in 20% sucrose. The brains were cut by a cryostat in 30 μm coronal sections. The sections (AP -5.2, ML 1.0) were obtained according to the rat atlas of Paxinos and Watson [26], and they were stored in PBS solution for immunocytochemical processing. The sections were immunostained for the TH protein by the avidin–biotin–peroxidase method. The sections were rinsed three times for 5 min each in PBS. They were then incubated for 72 h at 4 °C with a primary polyclonal antiserum (sheep anti-TH; Chemicon, Temecula, CA, USA) at a titer of 1:2000 in PBST. The sections were washed for 5 min in PBST and then incubated for 120 min at a 1:200 dilution (Vector Laboratories, Burlingame, CA, USA). Following a 90 min incubation in the Elite standard vecta stain avidin–biotin complex (ABC) reagent (Vector Laboratories, Burlingame, CA, USA), the sections were again washed three times for 5 min each time in PBS, then they were incubated in a medium containing 0.05% 3′-diaminobenzidine tetrahydrochloride (DAB; Sigma, St. Louis, MO, USA) with 0.01% H_2O_2 for 1 min to reveal the immunoreactivity. Finally, the tissues were rinsed in PBS; this was followed by a brief rinse in dH_2O and then the tissues were individually mounted onto slides. After the slides were allowed to air dry, they were cover-slipped. The sections were viewed at 400× magnification and the number of TH-like immunereactive cells was quantified in the ventral tegmental area. Counts of TH-labeled cells within square grids of defined size (100 μm × 100 μm) that were placed over each area, was performed by an observer that was blind to the treatment. TH-labeled cells were counted only if they reached a defined darkness above the background. Counts from the ventral tegmental area were obtained according to the stereotaxic atlas [26]. The cells within the areas were counted on each of three sections per each animal.

The experimental results were expressed as means ± S.E. The behavioral data were analyzed by one-way ANOVA using the SPSS program (Version 13.0). Statistical differences among groups were further analyzed using Tukey's post hoc test. The immunohistological data were calculated and analyzed by one-way ANOVA followed