Acupuncture Stimulates the Release of Serotonin, but Not Dopamine, in the Rat Nucleus Accumbens

KANJI YOSHIMOTO, FUMIHIKO FUKUDA, MASAFUMI HORI, BAKU KATO, HIDEAKI KATO, HIROYUKI HATTORI, NAOKI TOKUDA, KINYA KURIYAMA, TADASHI YANO and MASAHIRO YASUHARA

Department of Forensic Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan, Department of Health Promoting Acupuncture and Moxibution, Meiji University of Oriental Medicine, Kyoto, Japan, and Department of Psychiatry, Kyoto University School of Medicine, Kyoto, Japan

YOSHIMOTO, K., FUKUDA, F., HORI, M., KATO, B., KATO, H., HATTORI, H., TOKUDA, N., KURIYAMA, K., YANO, T. and YASUHARA, M. Acupuncture Stimulates the Release of Serotonin, but Not Dopamine, in the Rat Nucleus Accumbens. Tohoku J. Exp. Med., 2006, 208 (4), 321-326 —— Acupuncture has been introduced as one of the available therapies widely used in alternative medicine, but it has not achieved widespread acceptance with scientific evidence. Furthermore there are still many unanswered questions about the basic mechanisms of acupuncture. To investigate the neuropharmacological mechanisms of oriental acupuncture, we studied the acupuncture-induced changes of in vivo monoamine release in the rat brain. A microdialysis guide cannula was implanted into the nucleus accumbens (ACC), which plays an important role in the brain reward system. Acupuncture treatment at the unilateral or bilateral Shenshu (bladder urinary channel 23) acupoints, located on the both sides of the spinous processes on the lower back, was carried out for 60 min in freely moving rats, and the dopamine (DA) and serotonin (5-HT) contents of the microdialysates in the ACC were measured simultaneously. In rats subjected to acupuncture at bilateral Shenshu acupoints, increases of 5-HT release in the ACC were observed at 20 min of acupuncture treatment and continued until 40 min after acupuncture was ended. Acupuncture at a unilateral Shenshu acupoint increased the release of 5-HT at 20 min compared with that in the sham-control group. Five-HT release returned to the baseline level at 120 min. The effects of acupuncture at bilateral Shenshu acupoints on the release of 5-HT in the ACC were greater than that of unilateral acupuncture treatment. In contrast, DA release in the ACC was not changed following acupuncture treatment. Effective acupuncture increased and prolonged the activity of serotonergic neurons in the reward system pathway of the brain. This suggests that oriental acupuncture therapy may be effective for the treatment of emotional disorders, drug abuse and alcoholism. ——— acupuncture; integrative medicine; nucleus accumbens; rat; monoamines

© 2006 Tohoku University Medical Press
Acupuncture has been widely clinically applied for the treatment of neurological disorders. By correcting the body’s balance, acupuncture aims to cure diseases involving an imbalance caused by the strain of internal and external stresses. Acupuncture therapy has been used for many types of neuronal disorders as well as for pain control in oriental medicine. The beneficial effects of acupuncture on psychological parameters have recently been emphasized in various experiments in both animals and humans (Yano et al. 1993; Han et al. 1999; Yoshimoto et al. 2001).

To clarify the mechanism underlying the efficacy of acupuncture, most studies have been focused only on its analgesic effects. Acupuncture induces relaxation, both psychologically and physiologically, by affecting the emotional state and evokes euphoria. Acupuncture is also widely used for the treatment of mental dysfunction, including drug and alcohol abuse (Han 1989). Which acupuncture method is the most effective for each type of disease or dysfunction remains unknown, and further scientific research will be required to answer this question.

Recently, neurochemical studies on the relationship between the contents of dopamine (DA), serotonin (5-HT) and their metabolites in the central nervous system (CNS) and application of acupuncture were reported (Kato 2000; Yano et al. 2004). However, the effects of acupuncture on the in vivo release of DA and 5-HT in the CNS are still unclear. The present study was carried out to examine the effects of acupuncture application on the release of DA and 5-HT in the rat nucleus accumbens (ACC) of freely moving rats, which plays an important role in the brain reward system.

**Materials and Methods**

**Animals**

Adult male Wistar rats weighing between 260 and 300 g were used. All animals were housed in groups of three or four with continuous access to food and water ad libitum and were maintained on a 12 hrs light/dark cycle at 23°C.

**Brain microdialysis and acupuncture**

For surgical implantation of the guide cannula, rats were anesthetized with chloral hydrate (400 mg/kg). The guide cannula was implanted stereotaxically into the ACC with dental acrylic cement anchored to the skull with stainless steel screws. All rats were chronically implanted with a microdialysis guide cannula placed 1 mm above the ACC at the position with following stereotaxic coordinate (anterior-posterior [AP], 1.2 mm anterior to the bregma; L, 2.6 mm; and dorsal-ventral [DV], 5.5 mm from the surface of the skull) (Paxinos and Watson 1986).

Animals were allowed 4 - 6 days to recover from the surgery before insertion of the microdialysis probe into the ACC (2.0 mm membrane, 500 μm o.d., CMA Co., BAS, W. Lafayette, IN, USA) through the guide cannula. Experiments were initiated approximately 4 hrs after insertion of the probe.

Following the establishment of stable baseline values for DA and 5-HT in the ACC dialysates, acupuncture groups underwent acupuncture (Needle: No. 36, 0.19 mm × 10 mm, Seirin Kasei, Co. Ltd., Shizuoka) at bilateral (n = 8) or unilateral (n = 8) Shenshu (bladder urinary channel [BU] 23) point, located on the both sides of the spinous processes on the lower back. This point is used to treat mental and psychosomatic disorders and is known clinically to produce a sedation effect. We chose the acupoint BU 23 to analyze the extracellular 5-HT and DA levels in the ACC in unanesthetized, freely moving, adult rats. During the acupuncture, any stress was not induced into the animals. The control groups (n = 8) underwent the sham acupuncture; needles were inserted bilaterally (n = 5) and unilaterally (n = 3) at distance from Shenshu in the Tai-Yang axis which made up the urinary bladder channel and the small intestine channel. Experiments were carried out in the same plastic containers in which the rats were housed individually. Animals were allowed to move freely during the course of the experiments.

Artificial cerebrospinal fluid (in mM: 145 NaCl; 2.7 KCl; 1.0 MgCl2; 1.2 CaCl2; 0.2 ascorbate; and 0.2 NaH2PO4; with the pH adjusted to between 7.3 and 7.4 with 2 mM NaPO4 buffer) was circulated through the brain microdialysis probe at a flow rate of 0.8 μl/min (CMA/100, CMA Co., Stockholm, Sweden).

**High performance liquid chromatography (HPLC) analysis**

Samples were collected every 20 min into micro-
centrifuge tubes containing 5 μl of 0.1 N HCl and 5 μl of 0.1 M ethylenediamine tetraacetate (EDTA)-2Na and analyzed simultaneously.

Aliquots (5 μl) were assayed for DA and 5-HT using the previously described procedure modified for small-bore HPLC-electrochemical detection (ECD) to increase sensitivity (Yoshimoto et al. 1991). The mobile phase consisted of 15% (v/v) methanol, 5% (v/v) acetonitrile, 0.2 mM SOS, and 0.12 mM EDTA-2Na (pH adjusted to 2.8 with phosphate). A C₁₈ reversed-phase column (Waters Nova-pak, octadecylsila, 2.0 mm × 150 mm, 5 μm, Waters, NC, USA) was used at a flow rate of 0.25 μl/min (EP-300, Eicom Co., Kyoto). The ECD-300 (Eicom) used for electrochemical detection was usually set at a sensitivity of 0.1 nA/V, with a 0.4 V potential on the glassy carbon electrode.

After the experiments, the rats were anesthetized and decapitated, the brains were removed and frozen at −70°C. Coronal sections (40 μm thick) were cut using a cryostat microtome and stained with cresyl violet. The placement of the microdialysis probe in the ACC was verified histologically under a microscope. Data were not used unless placement of the probe was in the ACC.

All experimental procedures were in accordance with the guideline of the Committee for Animal Research, Kyoto Prefectural University of Medicine, Kyoto, Japan.

Statistical analysis

Statistical analyses of the effects of acupuncture treatment were performed by using one-way ANOVA (StatView 5.5, Abacus Concept, Berkley, CA, USA). Student’s t-test was used to determine significance of differences among three groups, as compared with the sham-control groups.

RESULTS

The time-course of changes in 5-HT level with acupuncture treatment for 60 min is shown in Fig. 1. Unilateral or bilateral acupuncture treatment increased the release of 5-HT (F [2, 118] = 6.881, p < 0.0001). Acupuncture at the bilateral or unilateral Shenshu points increased the release of 5-HT at 20 min (p < 0.05). Acupuncture treatment at the bilateral Shenshu point resulted in the maintenance of elevated 5-HT release for 40 min after the end of acupuncture stimulation (Fig. 1). The increased ACC
5-HT level in the unilateral acupuncture at Shenshu acupoint group returned to the basal values at approximately 60 min after starting acupuncture.

Fig. 2 shows the time-course of the extracellular ACC DA level after acupuncture treatment ($F_{[2, 118]} = 0.096$, $p > 0.05$). Neither unilateral nor bilateral Shenshu acupuncture treatment significantly stimulated the release DA in the ACC in this animal model.

**DISCUSSION**

Stimulation of an acupuncture point induces the neural activity related to acupuncture analgesia. Numerous studies in the rat have shown that acupuncture initiated by stimulation of small diameter nerves in muscle excites cells in periaqueductal gray matter (PAG) through the spinoreticular tract neurons (Oliveras et al. 1981; Chandler et al. 1989). The raphe nuclei are essentially located in the PAG, and in particular the dorsal raphe nuclei (DRN) are located in the ventral part of the PAG. The DRN located in the ventral part of the PAG projects onto the ACC, which is involved in the brain reward system (Koob and Bloom 1988). The 5-HT neurons projecting to the ACC from the DRN can be activated by the excitatory amino acid receptor, and the DRN 5-HT receptor regulates DA pathways from the ventral tegmental area to the ACC by stimulation of 5-HT$_{1A}$ receptors in the DRN (Yoshimoto and McBride 1992). Therefore, acupuncture stimulation of the Shenshu (BU 23) acupuncture induces the activation of ACC 5-HT neurons neuropharmacologically.

Acupuncture at the Zusanli (stomach channel [ST] 36) acupoints induces weight reduction and increases the 5-HT level and 5-HT/5-Hydroxyphenylacetic acid (5 - HIAA) ratio in the DRN (Wei and Liu 2003). This result showed that acupuncture at Zusanli (ST 36) stimulated the serotonergic neurons in the DRN involved in the PAG. A large number of acupuncture points can be allocated to point categories. Thirteen categories of points are known in the traditional Chinese medicine. Sheshu (BU 23) and Zusanli (ST 36) are categorized into the “Back Shu” points of urinary bladder channel and “He-Sea” points of stomach channel, respectively (Pomeranz and Berman 2003). Our results provide strong evidence that acupuncture acted on the serotonergic neurons in the brain reward system, and suggest that it could be useful for the treatment of neural disease involving an imbalance caused by the strain of internal or external stress. Acupuncture may compensate for neural abnormalities via acupuncture-induced 5-HT release and/or acupuncture-induced enhancement of neural availability in the ACC.

Acupuncture stimulates peripheral nerves in the muscle (e.g., at Shenshu in the back), and these nerves send physiological messages to the CNS to release neurochemicals (e.g., endorphins; morphine-like peptides in the brain). Acupuncture thus induces analgesia by blocking the transmission of painful messages (Yi et al. 1977; Pomeranz and Beruman 2003). Acupuncture treatment itself is different from a stimulation of needle, because several neural actions to acupuncture are dependent on the oriental acupoints in the body. Furthermore, the effects of acupuncture are achieved by exciting the PAG in the midbrain through the spinoreticular tract, which releases endorphins to excite the raphe nucleus neurons. The disinhibited or excited raphe nucleus neurons send neural message to the spinal cord through the reticulospinal tract to inhibit firing of the spinothalamic tract neurons (Hans and Terenius 1982). Dense of immunofluorescence-positive networks are seen between the PAG and the DRN, and the ascending NE pathway exerts a modulatory role on acupuncture via forebrain structures such as the habenula, PAG, or ACC (Ungerstedt 1971; Hoekfelt et al. 1984). Eventually these excited raphe neurons send neural messages to the ACC.

Using the technique of microdialysis coupled with small-bore HPLC, we studied the effects of microinfused l-glutamate, serotonin, (+)-8-hydroxy-2-(di-N-propylamine) tetrinal (8-OHDPAT, a 5-HT$_{1A}$ agonist), and muscimol (a GABA$_A$ agonist) into the DRN on the extracellular levels 5-HT, DA and their metabolites in the ACC. It was shown that 5-HT neurons projecting
to the ACC from the DRN are networked, and that these neurons can be activated by excitatory amino acid receptors and inhibited by stimulation of \(5-HT_{1A}\) autoreceptors (Yoshimoto and McBride 1992).

Bilateral microinjection of 5,6-dihydroxytryptamine into the medial forebrain bundle of the rat brain causes a selective decrease of the cerebral 5-HT content and attenuation of the effect of acupuncture (Han et al. 1979). The importance of ascending 5-HT fibers from the dorsal raphe nucleus in the action of acupuncture is demonstrated by the fact that lesioning of the DRN interferes with the effect of acupuncture (Liang et al. 1981). As shown by the evidence mentioned above, activation of the central 5-HT system from the DRN to the ACC potentiates the effect of acupuncture.

Changes of behavior or locomotion activity were not observed in the present study. Stimulation with acupuncture at Bai-Hui point increased the spontaneous locomotion activity of rats measured in ANIMEX meter (Cheng et al. 1996). However this point can be used in every acupuncture treatment because of its general psychological and harmonizing effect. It is not well known the relationship with the spontaneous locomotion activity and acupuncture effects. It is considered that an activation of the serotonergic neurons in the nucleus accumbens by bilateral acupuncture at Shenshu may influence on the consummatory behavior, e.g., feeding or mounting.

Increases of the central content of 5-HT and its metabolic product 5-hydroindolacetic acid were found in rats treated with electroacupuncture (EA) for 15–60 min (Kato 2000). Prolonging the EA stimulation period caused a corresponding increase in the 5-HT level. It was shown that acupuncture seems to accelerate the rate of synthesis and utilization of 5-HT in the CNS. Increased utilization of 5-HT may be related to the increased release of 5-HT in the ACC observed in this study (Fig. 1), and the increased release is also a plausible explanation for the increase of steady-state levels in the brain. In the present study, acupuncture at the Shenshu acupuncture, but not at sham acupuncture, significantly increased the release of 5-HT in ACC, but not DA (Fig. 1). The actions of acupuncture at bilateral Shenshu acupoints showed more potent effect on the 5-HT release compared to unilateral acupuncture treatment (Fig. 1). Bilateral Shenshu stimulation causes significant and long lasting increase of 5-HT release in the ACC. On the other hand, unilateral Shenshu stimulation increases 5-HT releases only 20 min after starting the acupuncture (Fig. 1). This may result from different thresholds and/or lower potentials to activate the release of 5-HT in the nervous terminals of the ACC, because the 5-HT release returns to the control level during the acupuncture stimulation on the unilateral Shenshu is continued. Thus, acupuncture at bilateral acupoints shows more effective of neural activity in the CNS than that at unilateral acupoint.

Alcoholic patients who undergo acupuncture are more likely to complete the course of clinical therapy. In accord with increased serotonergic activity in the CNS, patients treated with acupuncture had substantially fewer drinking episodes and admissions to a detoxification center (Bullock et al. 1987, 1989). There are 2 subtypes of the model of alcoholism. Type 1 affects both genders, has a later onset, and appears to be much more related to environmental and developmental factors. On the other hand, Type 2 is seen only in men, begins before age 25, and results in a more severe, more treatment refractory illness. Lower levels of brain 5-HT metabolite in the cerebrospinal fluids were confirmed in the Type 2 patients (Devor and Cloninger 1989; Higgin et al. 1992). Acupuncture can be applied clinically for the treatment of addictive disorders associated with the brain reward system. These clinical effects are in accord with our observation that Shenshu-stimulated neurophysiological messages induced increased 5-HT release in the ACC, presumably via the firing of the spinoreticular tract neurons. 5-HT has been implicated in practically every type of behavior, such as appetitive, emotional, motor, cognitive and autonomic (Jacobs and Fornal 1993; Blier and DeMontigny 1994). These led to the hypothesis that decreased serotonergic activity is related to the mental disorder.
Our findings suggest that acupuncture activates the neurons of the DRN in the midbrain through the spinoreticular tract, and then the excited neurons in the DRN induce an increase of 5-HT release in the ACC through 5-HT$_{1A}$ receptor regulation. The mechanisms of acupuncture-induced relaxation may be primarily caused by the firing and prolonged activity of serotonergic neurons in the brain Rewarding system pathways. Acupuncture at Shenshu acupoints affecting serotonergic neurons and their receptors may be used treat diseases such as depression or anxiety disorders.

In conclusion, acupuncture at the Shenshu acupoint caused a significant increase of 5-HT release in the ACC. Although there is a gap between an animal study and a human clinical trial, the combined use of acupuncture and the usual western medicine may improve the treatment of drug dependence, e.g., alcoholism.

Acknowledgments

This study was supported by a Grant-in-Aid from the Ministry of Education, Culture, Sciences and Technology (#C-15590584 and #B-17390203).

References


