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EFFECTS OF THIENORPHINE ON SYNAPTIC STRUCTURE AND SYNAPTOPHYSIN EXPRESSION IN THE RAT NUCLEUS ACCUMBENS

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Abstract—The partial opioid agonist thienorphine is currently in Phase II clinical trials in China as a candidate drug for the treatment of opioid dependence. However, its effect on synaptic plasticity in the NAc (nucleus accumbens) remains unclear. In the present study, we measured structural parameters of the synaptic interface to investigate the effect of thienorphine, morphine or a combination of both on synaptic morphology in the NAc of rats. Expression of synaptophysin was also examined. Ultrastructural observation showed that synaptic alterations were less pronounced after chronic thienorphine administration than after chronic morphine administration. Animals that received thienorphine had thinner postsynaptic densities and shorter active zones in the NAc compared with those in the saline group, but the active zone was larger, and the cleft narrower, than those in the morphine group. Furthermore, synaptophysin expression in the NAc was significantly greater after chronic administration of thienorphine, morphine, or both, than after saline. These results identified interesting differences between thienorphine and morphine in their effects on synaptic structure and synaptophysin expression in the rat NAc. Further study is deserved to investigate thienorphine as a new treatment for opioid dependence.

Key words: thienorphine, nucleus accumbens, synaptic structure, synaptophysin, synaptic plasticity.

INTRODUCTION

Drug abuse is a global social and medical problem. The partial opioid agonist buprenorphine has proved effective in long-term detoxification, alleviating the protracted withdrawal syndrome, and preventing drug craving (Cheskin et al., 1994). However, its poor oral absorption and potential for dependence hinder its suitability as a desirable agent to treat opioid dependence (Heel et al., 1979). A strategy in the development of new treatments for opioid addiction is to find other partial opioid agonists with a long duration of action but with high oral bioavailability. In a search for such compounds, thienorphine, a novel analog of buprenorphine, was synthesized by our institute (Liu et al., 2005). Thienorphine is a partial opioid agonist with high oral bioavailability, both antinociceptive activity and morphine antinociception-blocking activity, and persistently inhibits morphine-induced dependence (Zhao et al., 2004; Yu et al., 2006; Kong et al., 2007). It is being developed as a candidate to treat opioid dependence and is now in a Phase II clinical trial in China.

Long-lasting synaptic plasticity in the brain, especially in the nucleus accumbens (NAc), is thought to play a crucial role in the persistence of drug addiction (Lüscher and Malenka, 2011). Synaptic plasticity includes not only changes in efficacy of synaptic transmission but also changes in synaptic morphology. Structural plasticity and morphological changes are the basis for functional plasticity (Wilbrecht et al., 2010). Structural parameters of the synaptic interface, such as postsynaptic density (PSD) thickness, length of the active zone, width of the synaptic cleft and curvature of the synaptic interface, are reliable parameters for quantitative analysis of synaptic plasticity (Jing et al., 2004). Morphological changes in PSD and the active zone reflect receptor and ion channel alterations in the postsynaptic membrane, as well as changes in synaptic transmission efficacy. Morphological changes in the synaptic cleft also play an important role in the magnitude and kinetics of synaptic activity. To some extent, curvatures of changes in the synaptic interface represent the functional and active state of neurons (Kennedy, 2000; Takagi et al., 2000). Structural plasticity involves synapse formation, maturation, elimination, and maintenance, as well as the expression of synapse-associated proteins. Synaptophysin, a major synaptic vesicle protein, is a marker for synaptic activity and synapse formation during development (Thiele et al., 2000). Some findings strongly suggest that synaptophysin plays an important role in the regulation of mu-opioid receptor trafficking and signaling (Liang et al., 2007).

The effect of thienorphine on synaptic plasticity in the NAc remains unknown. Therefore, in the present study, we used electron microscopy to measure the structural parameters of the synaptic interface, in order to investigate the effects of thienorphine, morphine, or a combination of both, on the morphology and ultrastructure of the rat NAc. Finally, we also examined synaptophysin expression using Western blotting.

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Abbreviations: DMSO, dimethyl sulfoxide; NAc, nucleus accumbens; PSD, postsynaptic density; RER, rough endoplasmic reticulum; TEM, transmission electron microscopy.

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EXPERIMENTAL PROCEDURES

Animals

The experiments were carried out in male Wistar rats (weighing 180–200 g) supplied by the Beijing Animal Center (Beijing, China). The rats were acclimated to a colony room with ambient temperature (22 ± 1 °C), humidity (50 ± 10%), and a 12-h light/dark cycle with food and water available *ad libitum* for at least 3 days before the start of the experiment. All animal experiments were performed in strict accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985). The experimental procedures were approved by the local Committee on Animal Care and Use.

Laboratory reagents

Thienorphine hydrochloride was synthesized in our institute (Liu et al., 2005). Morphine hydrochloride was produced by Qinghai Pharmaceutical Factory (Xining, China). Rabbit anti-synaptophysin antibody was purchased from Abcam (Cambridge, MA, USA). Horseradish peroxidase-conjugated secondary antibodies were obtained from Zhongshan Biotechnology (Beijing, China). All other reagents were of analytical grade and purchased from commercial resources. Thienorphine was dissolved in dimethyl sulfoxide (DMSO), then diluted to desired concentrations in distilled water containing < 2% DMSO just before use. Morphine was dissolved in saline (0.9% NaCl). All drugs were injected in a volume of 2 ml/kg.

Drug treatment

For the assessment of NAc synaptic structures, rats received thienorphine (1 mg/kg, s.c.), morphine (escalating dose: 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 50, 50, 50, 50 mg/kg), thienorphine + morphine, or saline, three times daily for 2 weeks. For measurement of synaptophysin expression, rats received thienorphine (1 mg/kg, s.c.), morphine (10, 20, 20, 30, 30, 40, 40, 50 mg/kg, s.c.), thienorphine + morphine, or saline, three times daily for 8 days. Thienorphine was injected 30 min before morphine injection on each day in the thienorphine + morphine group.

Electron microscopy for the assessment of synaptic structure

Six hours after the last injection, rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). The chest was opened to expose the heart for left ventricle aortic cannulation. A total of 200-ml normal saline and 300-ml 4% paraformaldehyde/phosphate-buffered saline (PBS, pH 7.4) containing 1.5% glutaraldehyde were perfused. Brain tissue was collected and 1-mm^3 NAc tissue samples were harvested, according to the atlas of Paxinos and Watson (2007). The preparation of tissue for transmission electron microscopy (TEM) was performed as described previously (Xu et al., 1997). Electron micrographs of synapses were taken at a magnification of 15,000 for printing using a PHILIPS CM-120 transmission electron microscope (Philips, Eindhoven, Netherlands). Adobe Photoshop 7.0 (San Jose, CA, USA), was used to adjust contrast and brightness in the photomicrographs.

Morphometric measurement and analysis of synaptic structure

According to our previous method (Yong et al., 2013), Image-Pro Plus 6.0 image analysis software (Media Cybernetics, Inc. MD, USA) was used to analyze PSD thickness, cleft width, interface curvature, and active zone length on type Gray I synapses. The measurement method of Guldner (1980) was referenced for PSD thickness. A multi-point average measurement method was selected for synaptic cleft width. The Jones and Devon (1978) measurement method was referenced for synaptic interface curvature, as well as ratio of the arc length and chord length.

Western blot analysis for the expression of synaptophysin

Six hours after the last injection on days 3, 5 and 8, rats were deeply anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and sacrificed. The brain was rapidly removed from the cranium, placed on an ice-cold plate and the NAc dissected out, quickly frozen in liquid nitrogen, and transferred to a −80 °C freezer where it was stored until Western blot analysis. For analysis, samples were thawed, weighed, and homogenized in 10 volumes of extraction buffer (150 mM NaCl, 1.0% NP-40, 50 mM Tris HCl pH 8.0, 1 mM phenylmethylsulfonyl fluoride, 1 mg/ml aprotinin, 1 mM sodium vanadate and 1 mM EDTA pH 8.0). Samples were then centrifuged at 4 °C for 30 min at 16,000*g* and the supernatant removed. Protein content was determined using the method of Bradford (1976). The NAc samples were diluted in extraction buffer and proteins denatured in boiling water for 5 min. Equal amounts of each NAc protein were separated by 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred electrophoretically to nitrocellulose membranes. Nitrocellulose membranes in duplicate were then blocked with 5% non-fat milk in phosphate-buffered saline. The membranes were subsequently incubated with primary rabbit anti-synaptophysin antibody. Immunoreactivity was then detected by incubation with horseradish peroxidase-conjugated secondary antibody. Specific complexes were detected using an enhanced chemiluminescence kit (Millipore, MA, USA) according to the manufacturer's instructions. Western blots were scanned and analyzed with Image J software (U.S. National Institutes of Health, Bethesda, Maryland, USA).

Statistical analysis

Data are expressed as mean ± S.E.M. for four animals per group unless otherwise stated. Statistical analyses were performed by a one-way or two-way analysis of variance followed by Dunnett's *t* test. Student's *t* test was used