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Brief communication

Increase of glutamate/*N*-methyl-D-aspartate receptor immunodensity in the dentate gyrus of rats following pseudoephedrine administration

S. Nudmamud-Thanoi [a,*], S. Thanoi [a], P. Sobhon [b]

[a] *Faculty of Medical Science, Naresuan University, Phitsanulok 65000, Thailand*
[b] *Department of Anatomy, Faculty of Science, Mahidol University, Bangkok 10400, Thailand*

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Abstract

Pseudoephedrine is a sympathomimetic drug in which its structure is similar to amphetamine. Although pseudoephedrine is not as potent as amphetamine, it has been reported that the actions of pseudoephedrine on the central nervous system via dopamine release resemble to amphetamine. Changes of dopamine function can induce malfunction of glutamatergic system because there are well-documented interactions between glutamate/*N*-methyl-D-aspartate (NMDA) receptors and dopaminergic system. Therefore, the aim of this study was to investigate the effects of acute and chronic pseudoephedrine administration on NMDA receptors in hippocampal formation. Immunohistochemistry was used to determine the alteration of NMDA receptor density in rat hippocampus and dentate gyrus following acute and chronic pseudoephedrine administration. The density of NMDA receptors was increased significantly ($p < 0.005$) in the dentate gyrus of animals treated with pseudoephedrine chronically when compared with the acute and control groups. Similarly, the density of NMDA receptors in an acute group was also higher than the control group ($p < 0.01$). These results indicate that pseudoephedrine could induce an increase of NMDA receptors in the dentate gyrus. This might be a compensatory effect of NMDA receptor in response to the degeneration or loss of glutamatergic neurons.

Keywords: Pseudoephedrine; NMDA receptor; Hippocampus; Dentate gyrus; Immunohistochemistry

1. Introduction

Pseudoephedrine is a diastereoisomer of ephedrine and both are classified as sympatomimetic drugs (Will, 1997). Pseudoephedrine is commonly found in over-the-counter decongestants, anorectic agents and as an amphetamine substitute (Blosser et al., 1987; Blum, 1981). The sympathomimetic agents can produce psychoactive effects such as pleasant perceptual changes, euphoria and mental stimulation as same as amphetamine, a psychostimulant drug, if taken in large doses because of its related structure (Will, 1997). However, there appears to be very few reports on the effect of pseudoephedrine in the nervous system. Pseudoephedrine has been reported to induce an immediate early gene Fos immunoreactivity in the nucleus accumbens and striatum (Kumarnsit et al., 1999) and mediate via dopaminergic mechanism (Kumarnsit et al., 1999; Zarrindast, 1981). In the study of drug discrimination, pseudoephedrine was shown both partial substitution (20 mg/kg) and full substitution (40 mg/kg) for amphetamine (1 mg/kg) (Tongjaroenbuangam et al., 1998). Amphetamine, dopamine agonist, can produce long-term behavioral changes including sensitization, tolerance, and dependence (reviewed by Robinson and Becker, 1986). In addition, the administration of methamphetamine, the derivatives of amphetamine, can damage neurotoxicity to dopaminergic and serotonergic nerve terminals in several brain areas (O'Callaghan and Miller, 1991; O'Dell et al., 1991). Not only acute methamphetamine administration can decrease dopamine transporter function (Fleckenstein et al., 1997), but the loss of dopamine transporter sites also demonstrates following chronic methamphetamine administration (Nakayama et al., 1993). Changes of dopamine function can induce malfunction of glutamatergic system because there are well-documented interactions between glutamate/NMDA receptors and dopaminergic systems (Murase et al., 1993; Verma and Moghaddam, 1996). There is an evidence indicated the role of glutamate in the development of methamphetamine toxicity. Methamphetamine enhances glutamate release (Abekawa et al., 1994), while NMDA-receptor

* Corresponding author. Tel.: +66 5526 1000x4517; fax: +66 5526 1197.
E-mail address: sutisat@nu.ac.th (S. Nudmamud-Thanoi).

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antagonist has been reported to protect against methamphetamine neurotoxicity (Sonsalla et al., 1998). Moreover, methamphetamine can produce alterations of striatal and cortical glutamate/NMDA receptors (Eisch et al., 1996). Although glutamate abnormalities have not to date been elucidated after exposure to sympatomimetic agents, ephedrine has been reported to play a critical role of glutamate release in subcortical region indicating dysfunction of CNS glutamatergic pathways (Bowyer et al., 2000). It is very interesting to study mechanisms of glutamatergic system after pseudoephedrine, exposure as the glutamate/NMDA receptors seem to be critically involved in synaptic formation and plasticity of the CNS (Cline et al., 1987; Udin and Scherer, 1990) as well as in aspects of long-term potentiation (Coan et al., 1987; Davies et al., 1989). Therefore, in the present study, we aimed to investigate the effects of acute and chronic pseudoephedrine administration on the alteration of glutamate/NMDA receptor density in rat hippocampus and dentate gyrus.

2. Materials and methods

Male Sprague-Dawley rats (250–280 g) were obtained from the National Animal Center, Mahidol University, Thailand. The animals were housed 3–6 per cage and maintained at room temperature under a 12 h light/dark cycle with free access to water and food. All animals were handled for at least 1 week before experiment. All animal procedures were carried out in compliance with Mahidol University Code of Practice and the National Institutes of Health (USA) Guidelines for treatment of laboratory animals. Eight animals were treated for each group of experiment.

Psedoephedrine was received from the Health Sciences Research Institute, Naresuan University, Thailand. Animals were divided into the following three groups: control group, acute group and chronic group. To examine the effects of acute pseudoephedrine administration, animals were administered intragastically with vehicle for 14 days and administered with pseudoephedrine at a dose of 320 mg/kg on the last day once before sacrificed. The chronic effects of pseudoephedrine were examined after treated intragastrically at the dose of 160 mg/kg, once daily for 15 days. For the control group, animals were administered intragastically with vehicle. Rats were sacrificed 2 h later the last dose and brains were removed for the immunohistochemical analysis of glutamate/NMDA receptor subunit1 (NMDAR1). Drug doses for these studies were chosen based on the dose that pseudoephedrine can produce a discriminative stimulus resembling that of amphetamine (Glennon and Young, 2000; Tongjaroenbuangam et al., 1998).

Brains were fixed in 4% paraformaldehyld and embedded in paraffin wax and subsequently sectioned at a thickness of 5 μm, then mounted onto 3-aminopropyltriethoxysilane (APES) coated glass slides. Sections were deparaffinized in xylene, rehydrated in grade alcohol, then heated in microwave oven on full power (650 W) for three periods of 5 min in phosphate buffer saline (PBS; 0.01 M phosphate buffer, 0.9% NaCl,) pH 7.4, to aid antigen retrieval. The sections were incubated for 30 min in a solution of 0.6% H_2O_2 in 10% methanol and 0.1% Triton X in PBS pH 7.4 to inhibit endogeneous peroxidase activity and then washed for 3× 5 min in PBS. Non-specific binding was minimized by incubation for 1 h in 5% normal rabbit serum in PBS and incubated overnight at 4 °C with a polyclonal antibody against the NMDAR1 (Sigma) at a dilution of 1:1000 in protein blocking solution. The sections were washed for 3× 5 min in PBS before incubation for 2 h at room temperature with biotinylated secondary antibody (anti-rabbit IgG) diluted 1:200 in protein blocking solution. This was followed by incubation for 2 h at room temperature with avidin–biotinylated horseradish peroxidase complex (purchased with secondary antibody as a Vectorstain ABC kit, Vector Laboratories, Burlingame, CA) after which the sections were washed for 3× 5 min in PBS. The sections were washed for a further 3× 5 min. Then the protein immunoreactivity was visualized using the chromogen diaminobenzidine (DAB), intensified with nickel chloride. The sections were dehydrated and mounted. Immunoreactivity was not present in control sections in which the primary antibody was omitted from the staining protocol.

Immunoreactivity was quantified by optical density (OD) of NMDAR1 immunoreactivity in the subregions of hippocampus and the dentate gyrus. The optical measurements were made blindly to the animal groups. OD analysis was performed on high resolution and analyzed with Scion Image Software based on NIH image (v. beta 3b; www.scioncorp.com; 1998). The software was used to obtain the integrated optical density (IOD) of the region. The value is the sum of the optical densities of all pixels in the region divided by number of pixels. Background values were obtained from the neighboring white matter. The average of values from three sections for each subject was used for statistical analysis. Statistical analysis was performed using ANOVA with Dunnett post hoc tests.

3. Results

Immunohistochemistry demonstrated NMDAR1 immunoreactive cells in all principal neuronal populations of the hippocampus, namely pyramidal neurons in cornu ammonis fields 1–3 (CA1–3), granule cells in the dense cell layer of the dentate gyrus (DG) (Fig. 1). Immunoreactivity was strongly limited to pyramidal cells of CA1–3, and in granule cells of DG. Neurons in other hippocampal subareas (e.g. molecular layer) and surrounding areas (white matter) were less immunoreactive. Therefore, the data was analyzed by selected the area of interest in pyramidal cell layer in CA1–3 and granule cell layer in dentate gyrus. Within each region, NMDAR1 immunodensity in three experimental groups were compared by ANOVA with Dunnett post hoc tests. NMDAR1 immunodensity was significantly increased above control in dentate gyrus in acute ($p < 0.01$) and chronic ($p < 0.005$) pseudoephedrine administration, respectively (Fig. 2A). However, NMDAR1 immunodensity was not significantly different between control and treated groups in hippocampus (CA1–3) (Fig. 2B).