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Differential effects of spinal 5-HT_{1A} receptor activation and 5-$HT_{2A/2C}$ receptor desensitization by chronic haloperidol

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Abstract

The effects of 7- and 21-day haloperidol treatment on the spinal serotonergic system were examined *in vivo* in acutely spinalized adult rats. Intravenous administration of a selective 5-$HT_{2A/2C}$ receptor agonist, (±)-2,5-Dimethoxy-4-iodoamphetamine hydrochloride (0.1 mg/kg) significantly increased the excitability of spinal motoneurones as reflected by increased monosynaptic mass reflex amplitude. This was significantly reduced in rats treated with haloperidol (1 mg/kg/day, i.p.) for 7 and 21 days. Administration of a 5-$HT_{1A/7}$ receptor agonist, (±)-8-Hydroxy dipropylaminotetraline hydrobromide (0.1 mg/kg, i.v.) significantly inhibited the monosynaptic mass reflex. This inhibition was greatly prolonged in haloperidol treated animals. These results demonstrate that the effects of haloperidol on the activation and desensitization of 5-HT_{1A} and 5-$HT_{2A/2C}$ receptors respectively, may be mediated via intracellular mechanisms shared by these receptors with dopamine D_2 receptors in the mammalian spinal cord. The above serotonergic mechanisms may be partly responsible for haloperidol-induced extrapyramidal motor dysfunction.

Keywords: Activation; Desensitization; Haloperidol; Motoneurones; Serotonin receptors; Spinal reflex

1. Introduction

Antipsychotic drugs, such as haloperidol, are widely used in the treatment of schizophrenia and in treating psychoses related to neurodegenerative disorders. However, repeated administration of haloperidol produces extrapyramidal motor dysfunction, including Parkinsonian-like symptoms such as akathesia and dystonia. Long-term treatment with haloperidol often elicits tardive dyskinesia in patients with schizophrenia (Kane and Smith, 1982). These motor dysfunctions definitely appear to be related to the dopaminergic system in the spinal cord structures (Gajendiran et al., 1996; Langer et al., 1979; Maitra et al., 1992a) in addition to involvement of supraspinal structures. The extrapyramidal motor symptoms are postulated to result from the repeated blockade and supersensitization of dopamine D_2 receptors (Burt et al., 1977) in the central nervous system. Indeed, clinical studies (Chouinard et al., 1988) suggest that extrapyramidal symptoms in the form of akathesia and Parkinsonism are associated with increased risk of later development of tardive dyskinesia. Moreover, muscular rigidity is one of the cardinal symptoms characteristic of Parkinson's disease, and this also occurs as a side effect of treatment with haloperidol in humans (McEvoy et al., 1986). Previous studies have demonstrated the involvement of spinal cord components in the development of neuroleptic-induced extrapyramidal motor dysfunction (Langer et al., 1979; Lorenc-Koci et al., 1996). However, the precise mechanisms by which changes in spinal function are mediated remains to be revealed. The pathophysiology of extrapyramidal motor dysfunction seems to be complex, and it is manifested not only in the supersensitization of dopaminergic system following chronic haloperidol treatment, but also in its anatomical, neurochemical, and functional impact on non-dopaminergic neurotransmitter systems (Naidu and Kulkarni, 2001; Scatton et al., 1986; Wolf et al., 2005).

Abbreviations: DOI, (±)-2,5-Dimethoxy-4-iodoamphetamine hydrochloride; IP_3, inositol 1,4,5-triphosphate; 8-OH-DPAT, (±)-8-Hydroxy dipropylaminotetraline hydrobromide.

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However, several studies suggested that chronic haloperidol treatment did not change the mRNA or the binding site densities of 5-HT_{1A} and 5-$HT_{2A/2C}$ receptors in the rat brain (Ase et al., 1999; Burnet et al., 1996a,b; Meltzer and Nash, 1991).

A disturbed balance between spinal dopaminergic and serotonergic systems, apart from supraspinal structures, has been suggested to be responsible for the symptoms of Parkinson's disease (Scatton et al., 1986), one of the classical extrapyramidal motor dysfunctions, as well as neuroleptics-induced Parkinsonism (Langer et al., 1979; Maitra et al., 1992a; Neal-Beliveau et al., 1993). To test this hypothesis, the involvement of the spinal serotonergic system was examined in the genesis of extrapyramidal motor dysfunction following 7- and 21-day haloperidol treatment.

2. Methods

2.1. Animals and drug treatments

All animal procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals adopted by the National Institutes of Health, USA, and Animal Care and Use Committee of the Science University of Tokyo approved all the research protocols. Adult male Wistar rats at the beginning of drug treatment were weighed about 175–200 g. The weights of animals in the different groups did not differ significantly throughout the treatment periods. They were housed in groups of 3–4 with food and water freely available. Artificial lighting was provided from 07:00 h to 19:00 h. One group of animals ($n=16$) was injected with haloperidol, 1 mg/kg/day, i.p. for 7 consecutive days. Another group of animals ($n=16$) was injected with the same dose of haloperidol for 21 consecutive days. Haloperidol was dissolved in 50 μl of lactic acid (0.15%), and then diluted with 0.9% saline (1 mg of haloperidol per ml of final solution). For the vehicle treated groups, animals received injections of saline with the appropriate volume of lactic acid (0.15%) for 7 ($n=16$) or 21 days ($n=16$), for the respective groups. Rats were weighed once a week and the dosage was adjusted accordingly. The effect of intravenously administered saline (0.9%; 1 ml/kg) alone on the monosynaptic mass reflex was referred to as saline control. Separate saline controls ($n=5$, for each group) were undertaken for each group of haloperidol treated animals.

2.2. Electrophysiological recordings

All the procedures and maintenance of animals were carried out essentially as previously described (Gajendiran et al., 1996; Seth et al., 1997). The *in vivo* electrophysiological experiments were performed 5 days after the last injection of haloperidol to eliminate residual haloperidol and its metabolites from the system (Burt et al., 1977; Maitra et al., 1992a). Animals were anaesthetized with sodium pentobarbitone (35 mg/kg, i.p.), supplemented at 5 mg/kg/h, i.v. throughout the surgery and recording sessions. The animals were spinally transected at the C1 level. Briefly, laminectomy was performed at the C1 level to expose spinal cord and the meninges were removed before spinal transection under lidocaine anesthesia (4%, 50 μl). The completeness of transection was achieved by placing a piece of stuffed cotton at the place of transection to separate the spinal cord from the supraspinal structures. The spinal transection was performed to eliminate any drug induced supraspinal influences on spinal monosynaptic mass reflex. The cervical spinal transection provides a stable monosynaptic mass reflex, which was remained unaltered after C1 spinal transection (Hino et al., 1984). In addition to that spinal sympathetic neural circuits (in anaesthetized rats) are capable of generating sympathetic nerve activity even after C1 spinal transection helps to maintain the core body temperature and stable cardiovascular functions (Foreman, 2000; Kenney et al., 2000; Simon, 1974; Taylor and Schramm, 1987). Trachea, femoral vein and left carotid artery were cannulated, for artificial respiration, drug/saline administration and monitoring blood pressure, respectively. The depth of anaesthesia was assessed by regular heartbeat, a stable blood pressure (80–100 mm Hg) and the absence of change of these parameters in response to peripheral noxious stimuli. Animals were paralyzed initially with *d*-tubocurarine (2 mg/kg, i.v.) followed by repeated administration of the drug (1 mg/kg/h). After fixing the animal in a spinal stereotaxic frame, dorsal laminectomy was performed in the lumbo-sacral (L1-S1) region. The right (contralateral) hind limb was denervated as completely as possible and on the ipsilateral (left) side all ventral and dorsal roots below L4 were cut intradurally and dissected at their exit points from the vertebral column. The exposed surgical areas were covered with warm liquid paraffin (37°–38 °C) by constructing a pool using skin flaps and the temperature was maintained by radiant heat. Rectal temperature was maintained at 36°±0.5 °C by using a heating pad through a temperature control unit. The experiments were started after return of the reflexes following spinalization, which usually took 2–4 h. Monosynaptic mass reflexes were elicited by supramaximal square wave stimulation (0.2 Hz; 0.1 ms duration) applied to the central cut end of the dorsal root L5 using a stimulator (SEN-3301, Nihon Kohden) and were recorded from the ipsilateral ventral root L5 using a bipolar Ag–$AgCl_2$ electrode. The recorded monosynaptic mass reflex reflects the direct excitability of α-motoneurones. The reflexes were amplified with a differential amplifier (AVB-10, Nihon Kohden) and displayed on an oscilloscope (VC-10, Nihon Kohden). A data averager (DAT-1100, Nihon Kohden) averaged eight consecutive responses for every minute and the analog output was recorded (RJG-4122, Nihon Kohden). Drug administration was initiated after the appearance of constant responses continuously for at least 30 min.

2.3. Drugs

(±)-8-Hydroxy dipropylaminotetraline hydrobromide (8-OH-DPAT) and (±)-2,5-Dimethoxy-4-iodoamphetamine hydrochloride (DOI) were purchased from Sigma RBI (St. Louis, MO, USA). Haloperidol and *d*-tubocurarine HCl was from Sigma (St. Louis, MO, USA). The concentrations of the all drug solutions are expressed in terms of their respective salts. All chemicals were dissolved in normal saline just before use.