C57/BL6 mice were administered either 7.5 mg Fe²⁺/kg or vehicle (saline) postnatally on Days 10-12 after birth. From 64 days of age onwards for 24 days, groups of mice were administered either haloperidol (0.25 or 1 or 2 mg/kg, s.c.) or vehicle (Tween-80). Twenty-four hours after the final injection of either neuroleptic compound or vehicle, spontaneous motor activity was measured over a 60-min interval. Postnatal Fe²⁺-treatment (7.5 mg/kg, postnatally) reduced motor activity parameters during the initial 20-min periods (0-20 and 20-40 min) and then induced hyperactivity during the final 20-min period over all three parameters of activity, confirming previous observations. Subchronic administration of haloperidol, at the 1 and 2 mg/kg doses, and to a lesser extent the 0.25 mg/kg dose, increased the levels of activity in all three motor activity parameters in postnatal iron-treated mice: locomotion (1st and 2nd 20 min periods), rearing (1st and 2nd 20 min periods) and total activity (1st 20 min period). All three doses of haloperidol abolished the later hyperactivity in iron-treated mice, with the exception of the 0.25 mg/kg dose with regard to rearing behaviour. Apomorphine (1 mg/kg, s.c) -induced activity was elevated by postnatal iron administration and by subchronic administration of apomorphine at the higher dose levels. In the context of these and other observations, it is suggested that subchronic administration of haloperidol interacting with postnatal iron induces different expressions of dopamine neuron comorbidity underlying movement disorder.

Keywords: Iron administration; Postnatal; Fe²⁺; Vehicle; Haloperidol; Basal ganglia; Locomotion; Rearing; Total activity; C57/BL6 Mice; DA D₂ Receptor supersensitivity; Schizopsychotic; Movement disorder

INTRODUCTION

Several studies have indicated that the postnatal administration of iron (Fe²⁺, Ferromyn®), particularly during postnatal days 10-12, to neonatal mice induces marked functional alterations when the animals have reached adult ages. These functional alterations are evidenced in motor behaviour and instrumental learning deficits (Archer et al., 2003; Fredriksson et al., 2003; Fredriksson and Archer, 2003; 2004). The motor activity alterations of mice exposed to iron postnatally, typically following a dose of 7.5 mg/kg administered on postnatal days 10-12, are expressed by a marked reduction in all three parameters, locomotion, rearing and total activity, during the initial 20-30 min of testing in the test chambers. This initial hypoactivity is then followed, during the final stages of the total 60-min testing interval, by a marked elevation, or hyperactivity. The instrumental learning deficits by mice postnatally exposed to iron overload, e.g., 7.5 mg/kg on postnatal days 10-12, are expressed by drastic impairments in the number of correct arm choices and latency to acquire all eight food reward pellets in the eight-arm radial arm maze (Fredriksson et al., 1999; 2000; 2001; 2003). Schröder et al. (2001) found marked impairments in radial arm maze acquisition by rats that had been administered postnatal iron overload (cf., Archer et al., 2003). In each study, total iron content in the frontal cortex and basal ganglia was assayed. Postnatal iron overload induced significant elevations in the total iron content (146% to 172% of control values following the 7.5 mg/kg dose). Taken together, these studies confirmed the putative role of iron in the neuropathogenesis of movement disorders (Riederer et al., 1989; Ben-Shachar and Youdim, 1991; Janetzky et al., 1997; Youdim, 2001).
The putative role of iron in the etiopathology of Parkinson’s disease (PD) has been amply discussed over a number of years (Dexter et al., 1987; 1991; Youdim et al., 1991a; 1999; Gerlach et al., 1994). The effects of chronic or subchronic administration of neuroleptic compounds, particularly haloperidol (HPD), upon the mobilization of iron from peripheral stores with an eventual influence on expressions of neuroleptic-induced dopamine (DA) receptor hypersensitivity has been shown in several studies (Yehuda and Youdim, 1989; Ben-Shachar and Youdim, 1990; Ben-Shachar et al., 1991; 1993). Co-administration of FeCl₂ (5 mg/kg per day, over 21 days) with chlorpromazine (10 mg/kg per day, over 21 days) blocked dopamine (DA) D₂ receptor supersensitivity. Recently, Fredriksson et al. (2006) administered iron (Fe²⁺, 7.5 mg/kg, on days 10-12 postnatally) or saline to mouse pups, and when they had achieved adult age, treated them subchronically with either clozapine (1 or 5 mg/kg) or HPD (1 mg/kg) over 21 days. Later testing of spontaneous motor behaviour of 60-min test intervals indicated that the higher dose of clozapine and the single dose of HPD abolished or attenuated both the initial hypoactivity and the later hyperactivity induced by postnatal iron treatment.

The purpose of the present study was to ascertain: (1) the effects of subchronic administration of HPD at different doses upon the functional deficits induced by postnatal iron administration, and (2) the acute effects of apomorphine (1 mg/kg) upon motor activity following postnatal iron and subchronic HPD administration.

MATERIAL AND METHODS

Animals

Pregnant C57/BL6 mice were purchased from B&K, Sollentuna, Sweden. Each litter adjusted within 48 h to 8-10 mice and to contain offspring of either sex in about equal number, was kept together with its respective mother in a plastic cage in a room at temperature of 22 ± 1°C and a 12/12 h constant light/dark cycle (lights on between 06.00 and 18.00 h). Only male offspring were used in this study. At the age of 4 weeks the mice were weaned and the males were placed and raised in groups of 4 to 6 animals in a room maintained for male mice only.

Male mice, postnatal days 10-12, were administered Fe²⁺ (see below) or saline. At 64 days of age and onwards for 24 days, these mice, weighing 21-25 g, were administered either HPD (0.25, or 1 or 2 mg/kg, s.c.) or Vehicle (Tween-80, s.c.). Free access to food and water was maintained throughout. Mice were housed six per group, and tested only during the hours of light (08.00-15.00 h). Behavioural testing was initiated three weeks following the start of treatment with the neuroleptic compounds or vehicle. All testing was performed in a normally lighted room. This test room, in which all 12 ADEA activity test chambers, each identical to the home cage, were placed, was well-secluded and used only for this purpose. Each test chamber (i.e., motor activity test cage) was placed in a sound-proofed wooden box with 12 cm thick walls and front panels and a small double-glass window to allow observation; each box had dimmed lighting.

Experiments were carried out in accordance with the European Communities Council Directive of 24th November 1986 (86/609/EEC) after approval from the local ethical committee (Uppsala University and Agricultural Research Council), and by the Swedish Committee for Ethical Experiments on Laboratory Animals (license S93/92 and S77/94, Stockholm, Sweden).

Drugs

Ferromyn® (Iron succinate: 3.7 mg Fe²⁺/ml, AB Hässle, Göteborg, Sweden). Dosages, expressed as mg Fe²⁺/kg b. w., was administered orally via a metallic gastric tube in a volume of 10 ml/kg body weight. Saline was used as vehicle and to prepare the dose of Fe²⁺. Ferromyn S is applied to the treatment of anemia and as a prophylactic measure for blood donors and pregnant women. HPD (Leo, Hälsingborg, Sweden) was suspended in Tween-80 (vehicle). Apomorphine (Ferak Labora GmbH, Berlin, Germany) was dissolved in physiological saline (0.9% NaCl). All compounds were administered subcutaneously (s.c.).

Behavioural Measurements and Apparatus

Activity test chambers: An automated device, consisting of macrolon rodent test cages (40 x 25 x 15 cm) each placed within two series of infra-red beams (at two different heights, one low and one high, 2 and 8 cm, respectively, above the surface of the sawdust, 1 cm deep), was used to measure spontaneous motor activity (RAT-O-MATIC, ADEA Elektronic AB, Uppsala, Sweden). The distance between the infra-red beams was as follows: the low levels beams were 73 mm apart and the high level beams, placed only to the test chamber; the high level beams, placed only lengthwise and 58 mm apart breadthwise in relation to the test chamber, had dimmed lighting. REARING was registered when the mouse was in the horizontal plane, ambulating around the test-cage. front panels and a small double-glass window to allow observation; each box had dimmed lighting.

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