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Hypercholesterolemia causes psychomotor abnormalities in mice and alterations in cortico-striatal biogenic amine neurotransmitters: Relevance to Parkinson's disease

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ABSTRACT

The symptoms of Parkinson's disease (PD) include motor behavioral abnormalities, which appear as a result of the extensive loss of the striatal biogenic amine, dopamine. Various endogenous molecules, including cholesterol, have been put forward as putative contributors in the pathogenesis of PD. Earlier reports have provided a strong link between the elevated level of plasma cholesterol (hypercholesterolemia) and onset of PD. However, the role of hypercholesterolemia on brain functions in terms of neurotransmitter metabolism and associated behavioral manifestations remain elusive. We tested in Swiss albino mice whether hypercholesterolemia induced by high-cholesterol diet would affect dopamine and serotonin metabolism in discrete brain regions that would precipitate in psychomotor behavioral manifestations. High-cholesterol diet for 12 weeks caused a significant increase in blood total cholesterol level, which validated the model as hypercholesterolemic. Tests for akinesia, catalepsy, swimming ability and gait pattern (increased stride length) have revealed that hypercholesterolemic mice develop motor behavioral abnormalities, which are similar to the behavioral phenotypes of PD. Moreover, hypercholesterolemia caused depressive-like behavior in mice, as indicated by the increased immobility time in the forced swim test. We found a significant depletion of dopamine in striatum and serotonin in cortex of hypercholesterolemic mice. The significant decrease in tyrosine hydroxylase immunoreactivity in striatum supports the observed depleted level dopamine in striatum, which is relevant to the pathophysiology of PD. In conclusion, hypercholesterolemia-induced depleted levels of cortical and striatal biogenic amines reported hereby are similar to the PD pathology, which might be associated with the observed psychomotor behavioral abnormalities.

1. Introduction

The altered cholesterol metabolism with elevated level of plasma cholesterol (hypercholesterolemia) have been strongly associated with the pathophysiology of Alzheimer's disease as it not only causes cognitive impairment (Thirumangalakudi et al., 2008; Ullrich et al., 2010; Wood et al., 2014) but also influences the formation of the hallmark protein of the disease, the amyloid-β (Refolo et al., 2000; Thirumangalakudi et al., 2008; Xue-Shan et al., 2016). Studies of the last decade have provided a positive correlation between dietary factors, including cholesterol, and the occurrence of Parkinson's disease (PD) (Johnson et al., 1999; Hu et al., 2006, 2008; Miyake et al., 2010), however, some studies offered a contradictory inference (Huang et al., 2015; Tan et al., 2016). We have recently reviewed all the neuropathological implications

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caused by elevated cholesterol in the light of its putative contributions to the onset of PD pathology (Paul et al., 2015). *In vitro* studies have shown that excess cholesterol or its oxidation product (oxysterol) influences the aggregation of α-synuclein protein (Bar-On et al., 2008; Rantham Prabhakara et al., 2008; Marwarha et al., 2011), the hallmark pathology of PD (Di Maio et al., 2016). Rodents subjected to high-fat diet have been demonstrated to contribute towards the loss of striatal dopamine and tyrosine hydroxylase level in rodent models of PD (Choi et al., 2005; Bousquet et al., 2012). Moreover, treatment with cholesterol-lowering drugs (statins) has been reported to ameliorate the motor symptoms of PD as well as decrease the aggregation of α-synuclein protein (Bar-On et al., 2008; Roy and Pahan, 2011; Undela et al., 2013). In rodent models of hypercholesterolemia, the activity of mitochondrial complexes and antioxidant enzymes, as well as levels of antioxidant molecule have been reported to be reduced in non-dopaminergic regions of brain, such as cortex and hippocampus, which are indicative of oxidative stress (de Oliveira et al., 2011, 2013; Prasanthi et al., 2010; Otunola et al., 2014). However, the potential evidence that links cholesterol to brain functions, in terms of neurotransmitter metabolism and associated behavioral abnormalities, remains elusive. The present study investigated the effect of hypercholesterolemia on psychomotor behavior and neurochemical status in dopamine-rich region (striatum) and other regions of the brain of mice.

2. Materials and methods

2.1. Animals

Eight weeks old male Swiss albino mice (21–22 g) used in the present study were purchased from Pasteur Institute, Shillong, Meghalaya, India. The mice were housed under standard laboratory conditions of temperature (24 ± 2 °C) and humidity (60 ± 5%) and were provided with food and water *ad libitum*. An acclimatization time of 5 days was given prior to start of the experiment. The experimental protocols were in accordance with the National guidelines and the Institutional Animal Ethics Committee guidelines.

2.2. Chemicals and consumables

Cholesterol, acetonitrile, Cresyl violet, and paraformaldehyde were obtained from SISCO Research Laboratories (Mumbai, India). Dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), norepinephrine hydrochloride, ethylenediaminetetraacetic acid disodium salt (EDTA), heptane sulfonic acid, triethylamine, orthophosphoric acid, chloral hydrate, hydrogen peroxide (H_2O_2), poly-L-lysine, Triton X-100 and 3,3-diaminobenzidine (DAB) liquid substrate system (D3939) kit were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Primary antibodies such as rabbit polyclonal anti-tyrosine hydroxylase (TH; ab112) and rabbit polyclonal anti-Glial fibrillary acidic protein (GFAP; ab7260) were purchased from Abcam (Cambridge, UK). Anti-rabbit goat secondary antibody tagged with horseradish peroxidase (HRP; ap307p) was purchased from Millipore Co. (USA). Plasma cholesterol estimation kit (CHOL, Autopak) was obtained from Siemens Ltd. (India).

2.3. Experimental design

Swiss albino mice used in the study were randomly divided into two experimental groups: Control group (CS) fed with standard diet (normal rodent chow), and the high-cholesterol diet group (HCD) fed with standard diet mixed with 5% cholesterol (Refolo et al., 2000) for 12 weeks (84 days). Body weight was monitored after every 2 weeks. Motor behavioral tests, such as akinesia and catalepsy were performed in these animals in an interval of two weeks from the start of treatment till 12th week (0^{th}, 14^{th}, 28^{th}, 42^{nd}, 56^{th}, 70^{th} and 84^{th} day). The behavioral parameters, such as elevated plus maze, forced swim test, gait, and swim test were performed on 80^{th}, 81^{st}, 82^{nd} and 83^{rd} day respectively. Scoring of the behavioral tests was performed by trained experimenters who were blind to the treatment paradigm. Serum total cholesterol level was analyzed after the 12^{th} week (84^{th} day). The animals were sacrificed and/or perfused (4% paraformaldehyde) after 12 weeks of treatment (84^{th} day) for analysis of neurotransmitters and immunoreactivity study from discrete brain regions.

2.4. Analysis of behavioral parameters

2.4.1. Akinesia

The latency in moving all the four limbs were tested for 180 s (Bhattacharjee et al., 2016a). The animals were placed on a wooden platform (40 cm × 40 cm × 30 cm) for 5 min, and then the latency was recorded.

2.4.2. Catalepsy

Catalepsy is the inability of an animal to correct an externally imposed posture (Bhattacharjee et al., 2016a). Mice were placed on a flat surface with both hind limbs placed on a wooden block of 3 cm height. The time taken by the animals in moving both hind limbs to the flat surface was counted.

2.4.3. Swim test

Swimming ability test was carried out in tubs with 12 cm high water, maintained at 27 ± 2° C. Animals were placed in water and the swimming ability for a period of 10 min was scored every min as: 3-continuous swimming, 2-swimming with occasional floating, 1- more floating with occasional swimming with hind limbs, and 0-hind part sinks with only the head floating (Haobam et al., 2005).

2.4.4. Walk test

To determine the gait abnormalities of mice, we performed footprint analyses as described earlier (Klapdor et al., 1997). Mice were acclimatized to walk on an inclining gangway lined with a white sheet leading to a dark chamber/platform. The fore- and hind-paws of the animals were coloured respectively with red and green non-toxic colour to get the foot impression. The footprints were analyzed for stride length, stride width, and footprint length, manually.

2.4.5. Forced swim test

Forced Swim test (FST) was carried out according to Porsolt et al. (1997) with slight modifications. The animals were individually forced to swim in a transparent glass vessel containing 12 cm high water, maintained at 27 ± 2 °C, for a period of 6 min. The duration of immobility or floating occurring during the last 4 min was counted. A mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only movements necessary to keep its head above water.

2.4.6. Elevated plus maze test

This test is the most popular test of anxiety, and was carried out according to Walf and Frye (2007) with slight modification. The apparatus is a plus-shaped maze, with two open and two closed arms opposite to each other interconnected by a central platform. Animal was placed on the central platform, facing the open arm, and allowed to explore the plus maze for 5 min. Time spent in the