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Pharmacological benefits of agomelatine and vanillin in experimental model of Huntington's disease

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ABSTRACT

Huntington's disease (HD), a devastating neurodegenerative disorder, is characterized by progressive motor dysfunction, emotional disturbances, dementia, weight loss, depression. Melatonin receptors are widely expressed in the central nervous system. Vanilloids are also valuable as pharmacological tools for investigating neurobiology. This study investigates the utility of agomelatine, a dual agonist of MT_1 and MT_2 melatonin receptor as well as vanillin, a selective agonist of $TRPV_1$ (vanilloid receptor) in 3-nitropropionic acid (3-NPA) induced experimental HD in rats. Locomotor activity (Actophotometer), motor coordination (Rota rod) and learning-memory (Morris water maze) were assessed. Brain striatum oxidative stress (lipid peroxidation-MDA, glutathione-GSH, superoxide dismutase-SOD and catalase-CAT), nitrosative stress (nitrite/nitrate) and mitochondrial enzyme complexes (I, II and IV) were also assessed. 3-NPA has induced weight loss, impaired locomotion, motor coordination as well as learning and memory. It has induced brain striatum oxidative as well as nitrosative stress, cholinergic dysfunction and impaired mitochondrial enzyme complexes (I, II and IV). Tetrabenazine (TBZ) was used as positive control. Treatment with agomelatine and vanillin and TBZ has significantly attenuated 3-NPA induced weight loss, impaired locomotion, motor coordination and learning-memory as well as biochemical impairments. Thus, agomelatine and vanillin exhibit protective effects against 3-NPA induced HD. It may be concluded that agomelatine and vanillin may provide benefits in HD.

1. Introduction

Huntington's disease (HD), a neurodegenerative disorder with an autosomal dominant expression pattern is caused by the expansion of a CAG (cytosine adenine guanine) repeat in the huntingtin gene. Expanded polyglutamine facilitates formation of huntingtin protein aggregates, eventually leading to deposition of cytoplasmic and intranuclear inclusion bodies containing huntingtin protein. It is a movement disorder with a heterogeneous phenotype characterized by involuntary dance-like gait, motor impairment, and cognitive, psychiatric deficits and bioenergetic deficits (Bhateja et al., 2012).

3-nitropropionic acid (3-NPA) is an irreversible inhibitor of mitochondrial complex II (Brouillet et al., 1993) that inhibits the activity of succinate dehydrogenase, a key enzyme of oxidative energy production and characteristically provokes neurodegeneration in the striatum, resembling HD symptoms. 3-NPA-induced neurodegeneration has been widely used as an HD animal model because of its symptom similarity with HD (Bhateja et al., 2012; Pouladi et al., 2013).

Melatonin receptors are widely expressed in the central nervous system (Tardito et al., 2012). They exert their actions through the activation of G protein-coupled receptors (GPCRs), named MT_1 and MT_2 (Pala et al., 2013). Strong reductions of circulating melatonin are observed in numerous disorders and diseases, including Alzheimer's disease, various other neurological and stressful conditions, pain and metabolic disorders, in particular diabetes mellitus (Hardeland, 2012). CNS effects of melatonin comprise anti-excitatory, anxiolytic, anti-inflammatory, antioxidant and other neuroprotective actions (Hardeland and Poeggeler, 2012). Agomelatine is a selective melatonergic MT_1/MT_2 dual agonist (Chenu et al., 2013). Agomelatine has been reported in different depressive symptom clusters (core depression symptoms, sleep symptoms, anxiety, retardation, somatic symptoms, and work and activities) (Demyttenaere, 2011). Agomelatine enhances adult hippocampal neurogenesis and increases expression of several neuroplasticity-associated molecules (Dagyte et al., 2011).

It has been reported that expression of melatonin-MT_1 receptor is reduced pathologically, which increases the vulnerability of neurons to cell death in HD. Therefore, therapies restoring high levels of melatonin-MT_1 receptor complexes will provide benefits in HD (Wang et al., 2011). Neuroprotective effect due to activation of cerebral melatonin MT_2 receptor has already been reported in cerebral ischemic damage (Lee et al., 2010) and in case of glutamate toxicity (Das et al.,

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2010). Reduced expression of MT_1 and MT_2 receptors has been reported in neurodegenerative diseases such as Parkinson's disease (Srinivasan et al., 2005). In the present study, we hypothesized that melatonin receptor modulation may exert a protective effect on oxidative stress, mitochondrial oxidative damage, apoptosis and neurodegeneration. So modulation of melatonin receptors may provide benefits in HD. Thus, melatonin receptor modulation deserves investigations for its potential in HD.

Transient receptor potential vanilloid subtype 1 ($TRPV_1$) is a pain-sensing, ligand-gated, non-selective cation channel, which is mainly expressed in peripheral sensory neurons (Pecze et al., 2012). $TRPV_1$ channels are involved in the complex physiological functions of the cannabinoid system that includes motor coordination, memory procession, control of appetite, pain modulation and neuroprotection (Grotenhermen, 2004). $TRPV_1$ channels are present in various regions of the brain (Ray et al., 2003) that are highly susceptible to neurodegenerative insults, suggesting that this ion channel might contribute to the cellular processes involved in neuronal death (Ray et al., 2003). Vanillin (2-hydroxy-3-methoxybenzaldehyde), a compound widely used in foods, beverages, cosmetics and drugs, has been reported to exhibit multifunctional effects such as anti-mutagenic, and anti-angiogenetic effects (Tai et al., 2011). Vanillin reduces the expressions of proinflammatory cytokines [interleukin (IL)-1beta, IL-6, interferon-gamma, and tumor necrosis factor-alpha] and stimulates the expression of anti-inflammatory cytokine (IL-4) in tissues (Wu et al., 2009).

It has been reported that $TRPV_1$ activation is beneficial in the alleviation of specific motor symptoms such as choreic movements in HD (Fernández-Ruiz and Gonzáles, 2005). Modulation of $TRPV_1$ has been reported to influence synaptic plasticity (Bennion et al., 2011). We hypothesized that $TRPV_1$ receptors may be beneficial in motor dysfunction, memory process and neurodegeneration. The role of the modulation of vanilloid receptors is still unknown in HD. Therefore, modulation of vanilloid receptor deserves investigations for their potential in HD.

It has been reported that cholinergic neurons participate in the regulation of memory processes (Agrawal et al., 2008). Melatonin receptor activation has been documented to decrease cholinergic degeneration (Corrales et al., 2014). $TRPV_1$ activation has also been reported to be beneficial for disorders of striatal cholinergic neurotransmission (Musella et al., 2010). TRPV1 agonists have been reported to increase melatonin secretion (Reuss et al., 2010), which may be due to melatonin receptor activation. Therefore, $TRPV_1$ and melatonin receptor activation may be beneficial in cholinergic neurodegeneration.

Tetrabenazine (TBZ), an approved drug by USFDA (Chen et al., 2012), has been used for the management of various movement disorders, including Huntington chorea (Gros and Schuldiner, 2010). It is reported to offer symptomatic relief without disease modifying therapy. TBZ selectively depletes central monoamines by reversibly binding to the type-2 vesicular monoamine transporter ($VMAT_2$) (Frank, 2009), more selectively depletes dopamine than norepinephrine. The highest binding density for TBZ is in the caudate nucleus, putamen, and nucleus accumbens, areas known to bear the brunt of pathology in HD (Frank, 2009). We have used TBZ as treatment control in the present study.

In light of the above, the present study has been undertaken to investigate the potential of agomelatine (melatonin-MT_1 and MT_2 receptor agonist) and vanillin ($TRPV_1$ agonist) in 3-NPA induced experimental HD condition.

2. Material and methods

2.1. Animals

Albino Wistar rats have been widely used for the induction of HD symptoms by 3-nitropropionic acid (Shivasharan et al., 2013; Sandhir et al., 2012). Adult albino Wistar rats (3–5 months old), of either sex, weighing 200–250 g (purchased from Indian Veterinary Research Institute, Izatnagar, India), were employed in the present study and were housed in an animal house with free access to water and standard laboratory pellet chow diet (Kisan Feeds Ltd., Mumbai, India). The animals were exposed to natural light (sunlight) and dark cycle. The experiments were conducted between 9.00 and 18.00 h in a semi-soundproof laboratory. The animals were acclimatized to the laboratory condition five days prior to behavioral study and were maintained in the laboratory until the completion of the study. The protocol of the study was duly approved by the Institutional Animal Ethics Committee (IAEC) and the care of the animals followed the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India (Approval number: BIT/SOP/IAEC/2013/pharmacol./03; Reg. No. 25/230/2011/AWD/CPCSEA).

2.2. Drugs and reagents

Agomelatine was obtained from Abbott Healthcare Pvt. Ltd., India. Vanillin was obtained from CDH Laboratories, India. Tetrabenazine was obtained from Sun Pharma Pvt. Ltd., India. 3-nitropropionic acid (3-NPA), Lowry's reagent, 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), Folin-Ciocalteu reagent, bovine serum albumin (BSA) and N-naphthylethylenediamine were purchased from Sigma Aldrich, India. 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), ethylene glycol tetra acetic acid (EGTA), mannitol, glycyl glycine buffer, nicotinamide adenine dinucleotide (NADH), nitrazobluetetrazolium (NBT) and cytochrome-C were purchased from SISCO Research Laboratory Pvt. Limited, Mumbai, India.

2.3. 3-Nitropropionic acid (3-NPA) experimental model

3-NPA was dissolved in 0.9% saline solution neutralized to pH 7.4 using sodium hydroxide and was administered to rats alternatively for 28 days at a dose of 10 mg kg^{-1} through the intraperitoneal route in a volume of 0.5 ml 100 g^{-1} animal body weight (Gopinath and Sudhandiran, 2012; Pandey et al., 2008). When we administered 3-NPA (10 mg kg^{-1}) once a day for two weeks to rats, we observed a greater mortality rate in animals due to excessive weight loss, but when we administered 3-NPA (10–20 mg kg^{-1} intraperitoneally) for 4 days to rats, we observed no significant changes in behavior and biochemical estimations. By using alternate day schedule we have found significant differences in behavior and biochemistry. Weight, locomotor activity and motor function were measured before the initiation of 3-NPA treatment (day 1) and at the end of the study (day 28).

2.4. Drug administration

All drug solutions were freshly prepared before use. Selection of doses and the dosing schedule were based on previously published reports from other labs. It has been reported that systemic administration of 3-NPA (10 mg kg^{-1} for 4 days) caused significant body weight reduction, impaired motor function (locomotor activity, movement pattern) and striatal lesions mimicking symptoms of HD (Pandey et al., 2008). After 4 doses 3-NPA exhibited its effect on behavioral and biochemical parameters. Hence, we selected the administration of treatment drugs from the 10th day onwards i.e. after 4 doses of 3-NPA in rats. All the drug treatments were started from the 10th day onwards (from the 10th day to 28th day) daily.

Saline; vehicle of 3-NPA was administered to rats from 1st day to 28th day (on alternate days). CMC; vehicle of treatment drugs, agomelatine (2 and 4 mg kg^{-1}), vanillin (75 and 150 mg kg^{-1}) and tetrabenazine (3 mg kg^{-1}) per se were administered once a day using oral canula to rats for total 19 days. Agomelatine, vanillin and tetrabenazine were suspended in 0.5% CMC. Agomelatine (2 and 4 mg kg^{-1} orally) (Monti et al., 2001), Vanillin (75 and 150 mg kg^{-1} orally) (Makni et al., 2012) and tetrabenazine (3 mg kg^{-1}orally) (Meyer et al., 2011) were administered to rats orally once a day, as