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ADIOL protects against 3-NP-induced neurotoxicity in rats: Possible impact of its anti-oxidant, anti-inflammatory and anti-apoptotic actions

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ABSTRACT

Huntington's disease (HD) is a progressive neurodegenerative disorder with a wide spectrum of cognitive, behavioral and motor abnormalities. The mitochondrial toxin 3-nitropropionic acid (3-NP) effectively induces specific behavioral changes and selective striatal lesions similar to that observed in HD. Some neurosteroids, synthesized in neurons and glial cells, previously showed neuroprotective abilities. 5-Androstene-3β-17β-diol (ADIOL) is a major metabolite of dehydroepiandrosterone (DHEA) with previously reported anti-inflammatory, anti-apoptotic and neuroprotective activities. The neuroprotective potential of ADIOL in HD was not previously investigated. Therefore, the present study investigated the neuroprotective effects of ADIOL against 3-NP-induced behavioral changes, oxidative stress, inflammation and apoptosis. Intraperitoneal administration of 3-NP (20 mg/kg) for 4 consecutive days in rats caused significant loss in body weight, reduced prepulse inhibition (PPI) of acoustic startle response, locomotor hypoactivity with altered cortical/striatal histological structure, increased cortical/striatal oxidative stress, inflammation and apoptosis. Administration of ADIOL (25 mg/kg, s.c.) for two days before 3-NP significantly attenuated the reduction in body weights and PPI, increased locomotor activity and restored cortical/striatal histological structure nearly to normal. Moreover, it displayed anti-oxidant, anti-inflammatory and anti-apoptotic activities as evidenced by the elevation of cortical and striatal reduced glutathione levels, reductions of cortical and striatal malondialdehyde, striatal tumor necrosis factor alpha and interleukin-6 levels. Only a small number of iNOS and caspase-3 positive cells were detected in sections from rats pretreated with ADIOL. This study suggests a potential neuroprotective role of ADIOL against 3-NP-induced Huntington's disease-like manifestations. Such neuroprotection can be attributed to its anti-oxidant, anti-inflammatory and anti-apoptotic activities.

1. Introduction

Huntington's disease (HD) is a devastating inherited neurodegenerative disorder with progressive widespread neuropathological deficits resulting in a triad of motor, cognitive and behavioral abnormalities (Rosenstock et al., 2009; Ryu et al., 2004). HD is characterized by the selective death of the striatal medium-sized spiny neurons and cerebral cortical atrophy (Vonsattel and DiFiglia, 1998; Vonsattel et al., 1985). The underlying pathogenesis of HD is the abnormal CAG repeat expansion in the first exon of a gene encoding the large abnormal protein, huntingtin (Ross, 2004; The Huntington's disease Collaborative Research Group, 1993). Evidence proved the link between this genetic defect and the impairment of energy metabolism (Jenkins et al., 1998) through the inhibition of electron transport chain enzymes and subsequent production of reactive oxygen species (Browne et al., 1997), causing oxidative stress and striatal excitotoxicity which thereby contribute to neuronal death (Beal, 1992). Neuroinflammation is an integral component of HD, where mutant huntingtin is found to be expressed in microglial cells, the central effectors of neuroinflammation. Moreover, huntingtin inclusions were believed to directly cause apoptosis in HD (Wellington et al., 1998).

3-Nitropropionic acid (3-NP) is a mitochondrial toxin that can effectively induce specific behavioral changes and selective striatal lesions in rats and non-human primates mimicking those in HD (Lee and Chang, 2004). 3-NP is an irreversible inhibitor of succinate dehydrogenase, an enzyme located in the inner mitochondrial membrane, thus inhibits both Krebs cycle and complex II and III activity of the electron transport chain (Ludolph et al., 1991). 3-NP-induced HD phenotype can be

Abbreviations: HD, Huntington's disease; 3-NP, 3-nitropropionic acid; ADIOL, 5-androstene-3β-17β-diol; DHEA, dehydroepiandrosterone; PPI, prepulse inhibition; iNOS, inducibe nitric oxide synthase; HPF, high power field; H&E, hematoxylin and eosin; DHSB, 3,5-dichloro-2-hydroxybenzene sulfonic acid; GSH, reduced glutathione; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde; TNF-α, tumor necrosis factor-alpha; IL-6, interlukin-6; ABC, avidin-biotin peroxidase complex; HRP, horseradish peroxidase; TMB, 3,3′,5,5′-tetramethylbenzidine; BSA, bovine serum albumin; TBS, tris buffered saline; ANOVA, analysis of variance; OD, optical density; A%, mean area%; ROS/RNS, reactive oxygen/nitrogen species; SDH, succinate dehydrogenase; COX-2, cyclooxygenase-2; O2−, superoxide anion; ONOO−, peroxynitrite anion

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manifested in experimental animals as locomotor hypoactivity, disruption of prepulse inhibition (PPI) of acoustic startle response, striatal histopathological lesions, reduced oxidative defense, increased inflammation and apoptosis (Ahuja et al., 2008; Bhateja et al., 2012; Kumar and Kumar, 2010; Kumar et al., 2007, 2011; Tadros et al., 2005).

Acoustic startle response is a fast involuntary contraction of facial and skeletal muscles evoked by sudden and intense auditory stimuli (Koch, 1999). The magnitude of the startle reflex aroused by a given signal is suppressed when a weak, non-startling sensory event precedes the startle-eliciting signal by 30–500 ms, a phenomenon termed as PPI (Davis et al., 1982). Such sensory gating mechanism protects the brain from stimulus-inundation, which could otherwise lead to cognitive fragmentation and disturbed thought (Braff and Geyer, 1990; Swerdlow et al., 1995).

The pivotal role of neuroinflammation in HD makes it a key target for therapeutic interventions. Licofelone, a competitive inhibitor of 5-lipoxygenase and cyclooxygenases 1 and 2 (Kumar et al., 2011), fenofibrate, a PPAR-α activator (Bhateja et al., 2012), and fumaric acid ester dimethyl fumarate, an immunomodulator (Ellrichmann et al., 2011) showed promising neuroprotective effects in 3-NP model by reducing neuroinflammation. Neurosteroids are those steroid hormones synthesized in the nervous system either *de novo* from cholesterol or by *in situ* metabolism of blood-borne precursors (Citraro, 2006). Neurosteroids and their metabolites, which act within the central nervous system, were shown to have neuroprotective, myelinating, anti-apoptotic and anti-inflammatory effects (Azcoitia et al., 2003; Charalampopoulos et al., 2004; He et al., 2004; Mayo et al., 2005; Pettus et al., 2005; Wang et al., 2010). 5-Androstene-3β, 17β-diol (ADIOL) is a member of 5-androstene family of endogenous steroids, a major natural metabolite of dehydroepiandrosterone (DHEA) (Parker et al., 1996; Regelson et al., 1994), and an intermediate in the synthesis of testosterone and estrogen. DHEA's poor potency, high metabolic potential and androgenic and estrogenic side effects, make it not suitable for use as a pharmaceutical in humans (Auci et al., 2005). ADIOL has approximately 1.4% the androgenicity of DHEA and 0.2% the androgenicity of testosterone (Coffey, 1988). ADIOL also showed greater protective effects than DHEA against lethal bacterial infections and endotoxic shock (Ben-Nathan et al., 1999). Previous studies showed that ADIOL has anti-inflammatory (Auci et al., 2005; Nicoletti et al., 2010; Suzuki et al., 2006; Szalay et al., 2006), anti-apoptotic (Kiang et al., 2007) and possible neuroprotective activities against experimental auto-immune encephalomyelitis, the model of multiple sclerosis (Saijo et al., 2011). The neuroprotective effects of neurosteroids in HD were previously demonstrated with progesterone that significantly attenuated 3-NP-induced behavioral alterations, oxidative stress and inflammation (Kumar et al., 2014).

Therefore, this study aimed to investigate the potential neuroprotective effects of ADIOL against 3-NP induced behavioral, neurochemical and histopathological changes.

2. Materials and methods

2.1. Animals

Male albino rats weighing 200–250 g were purchased from the Nile Company, El Amyria, Cairo, Egypt. They were housed in groups of five in plastic cages at constant temperature (21 ± 2 °C), with alternating 12 h light/dark cycle where animal chow and water were provided *ad libitum*. One week before the experiment, all animals were acclimatized to laboratory conditions. All animal treatments adhered strictly to institutional and international ethical guidelines of the care and use of laboratory animals. The experimental protocol was approved by Ain Shams University Faculty of Pharmacy Review Committee for the use of animal subjects.

2.2. Drugs and chemicals

In the present study, all chemicals and biochemical reagents of analytical grade and highest purity were used. ADIOL was purchased from Steraloids (Newport, USA). 3-NP and chemical reagents were all purchased from Sigma-Aldrich (St. Louis, MO, USA).

ADIOL was dissolved in absolute ethanol and saline (1:3) and administered subcutaneously (Ben-Nathan et al., 1999) in doses of (1, 5 and 25 mg/kg) for two consecutive days (Saijo et al., 2011). 3-NP was dissolved in saline (adjusted at pH 7.4) and administered intraperitoneally in a dose of 20 mg/kg daily for four consecutive days (Bhateja et al., 2012).

2.3. Experimental groups

A preliminary study was conducted in which rats were randomly divided into six groups, eight animals each. The first group, serving as a control group, received ADIOL vehicle (s.c.) for 2 days followed by 3-NP vehicle (i.p.) for the next 4 days. The second group received ADIOL vehicle (s.c.) for 2 days then 3-NP (20 mg/kg, i.p.) for 4 consecutive days. The third to fifth groups received s.c. injections of ADIOL (1, 5 and 25 mg/kg), respectively, for the first 2 days followed by daily i.p. injections of 3-NP (20 mg/kg) for 4 consecutive days. The sixth group received 25 mg/kg of ADIOL for 2 days followed by 4 daily saline injections. Rats were tested for changes in body weights, PPI and locomotor activity then decapitated following treatments on the last day of the experiment. Whole brains were excised and fixed in 10% formalin (pH 7.4) for the preparation of paraffin blocks. Paraffin sections (1.6 to 2.8 mm posterior to the bregma and 0 to 1.2 mm anterior to the bregma) were cut from each brain (Paxinos and Watson, 1986). The most effective ADIOL dose was selected for further investigations (Fig. 1).

In the next series of experiments, rats were randomly divided into four groups, six animals each. The first group received the respective vehicles. The second and fourth groups received vehicle then 3-NP or ADIOL-alone then saline, respectively, as before. The third group was subcutaneously injected with the selected ADIOL dose for 2 days followed by 3-NP (20 mg/kg, i.p.) for 4 days. Rats were decapitated, brains were excised, cortices and striata were dissected out and 10% homogenate in phosphate buffer (pH 7.4) was prepared (Fig. 1).

2.4. % change of body weight

Animal body weight was recorded on the first and last days of the experiment. The % change in body weight was calculated:

$$[\text{Body weight(1st day–last day)} \times 100]/[\text{1st day body weight}].$$

2.5. Behavioral tests

2.5.1. PPI response measurement

Startle responses were measured using Startle Responder X apparatus (Columbus, OH, USA) which consists of Plexiglas cages and force platforms that are equipped with precise load cells to be used as sensors. Animal movement on the platform develops a transient force, which can be transduced by an accelerometer into a voltage that is proportional to the displacement velocity which is measured at its peak (negative or positive). These signals were amplified, digested, and fed into a data-acquisition board in a computer for further analysis. For acoustic startle measurements, animal cages were housed in a sound-attenuating chamber with a high-frequency speaker located on the side of each cage. The high frequency speaker delivered the acoustic stimulus in a background noise level of 70 dB. After a 5 min acclimatization period, during which time there was no stimulus, each rat received 36 sessions of either pulse alone or prepulse/pulse sessions presented in a random order