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BRAIN RESEARCH

Research Report

Involvement of nitric oxide in 3-nitropropionic acid-induced striatal toxicity in rats

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ARTICLE INFO

Article history:
Accepted 10 June 2006
Available online 20 July 2006

Keywords:
Huntington's disease
Neurodegenerative disease
Nitric oxide synthase
Nitric oxide donor
Striatum
Astrocyte

ABSTRACT

The roles of nitric oxide (NO) in 3-nitropropionic acid (3-NPA)-induced toxicity were investigated using in vivo and in vitro models. Chronic 3-NPA administration (10 mg/kg) to rats produced selective striatal lesions that were associated with abnormal motor and EMG activities. In these animals, there was loss of glial fibrillary acidic protein (GFAP)-positive cells with extravasation of IgG in the lesion center, although microtubule-associated protein (MAP)-2-positive cells remained, indicating that astrocytes were involved. 3-NPA increased the NO_2^-/NO_3^- levels in microdialysates obtained from the striatum, thalamus and cerebellum. The basal NO_3^- level was much higher in the striatum than in the other areas. The NO_2^-/NO_3^- levels in the striatum were much higher in animals exhibiting abnormal muscular activity. Expression of endothelial NO synthase (eNOS), but not neuronal NOS (nNOS), was greatly increased in the striatum at 5 h after a second 3-NPA exposure, but not in other areas. In astrocyte cultures, the toxic effects of 3-NPA were associated with corresponding increases in the NO_2^- level, and this toxicity was attenuated by hemoglobin (Hb; 20 μM), which quenches NO. The NO_2^- generated by 3-NPA, even without cells, was also antagonized by Hb. 3-NPA, *S*-nitroso-*n*-acetyl-DL-penicillamine (SNAP) and sodium nitroprusside (SNP) all increased the NO current (detected by NO-sensitive electrodes) in concentration-dependent manners, and Hb significantly attenuated the NO generation induced by 3-NPA, SNAP or SNP. Taken together, these results suggest that 3-NPA generates NO both directly as a donor and indirectly by enhancing NOS expression to produce toxic effects on astrocytes and neuronal toxicity.

1. Introduction

3-Nitropropionic acid (3-NPA), a toxin from moldy sugarcane, irreversibly inhibits succinate dehydrogenase (SDH), an enzyme essential for complex II of the respiratory chain activity of Kreb's cycle, thereby leading to decreased cellular ATP levels (Alston et al., 1977). Rats intoxicated with 3-NPA exhibit selective striatal lesions and motor abnormalities,

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doi:10.1016/j.brainres.2006.06.032

resembling those of Huntington's disease (Beal et al., 1993; Nishino et al., 1995, 1997). Excitotoxicity involving glutamate and dopamine (DA), anatomical vulnerability of the lateral striatal artery, disruption of the blood–brain barrier and hormonal factors, among others, have been proposed as causes for the striatal lesions produced by 3-NPA (Beal et al., 1993; Nishino et al., 1997, 1998, 2000). 3-NPA increases the $[Ca^{2+}]_i$ in astrocytes and neurons, although the increase in neurons is less marked (Deshpande et al., 1997; Fukuda et al., 1998). The $[Ca^{2+}]_i$ increase in astrocytes is mediated via the Na^+–Ca^{2+} exchanger system, while that in neurons involves other mechanisms (Fukuda et al., 1998). Furthermore, astrocytes disintegrate earlier than neurons following 3-NPA administration, indicating that the neuronal loss is secondary to the primary astrocyte loss (Fukuda et al., 1998). However, the underlying mechanisms responsible for the selective striatal toxicity by 3-NPA remain obscure.

Recently, S-nitro-glutathione, an endogenous carrier of nitric oxide (NO), was reported to protect fetal rat striatal cultures against 3-NPA toxicity (Ju et al., 2004). In contrast to this protective action, NO can become toxic under neuropathological conditions (Lipton et al., 1993; Dawson and Dawson, 1998; Moro et al., 2004). NO in brain cells can be synthesized via three isoforms of nitric oxide synthase (NOS). Endothelial NOS (eNOS) and neuronal NOS (nNOS) are constitutively expressed in the respective cells (Knowles and Moncada, 1994; Murphy et al., 1993), whereas inducible NOS (iNOS) generates NO from astrocytes, microglial cells and macrophages in response to inflammatory reactions (Murphy et al., 1993). In addition to these commonly accepted cells, most brain cell types are also able to express other NOS isoforms (Moro et al., 2004). To date, iNOS-like immunoreactivity in the lateral striatal artery region has been identified in 3-NPA-intoxicated animals (Nishino et al., 1996; Schulz et al., 1997). However, previous reports regarding the involvement of nNOS activity in 3-NPA toxicity are not consistent. In one report, an nNOS inhibitor protected against the toxicity (Schulz et al., 1995), while in another report, the nNOS activity remained unaltered after 3-NPA administration (Hellweg et al., 2003). Thus, the role of NO in 3-NPA toxicity currently remains unclear. Furthermore, 3-NPA contains a nitro group, but its NO donor activity is unknown. Therefore, the aim of the present study was to identify the roles of NO in 3-NPA intoxication using both in vitro and in vivo models.

2. Results

2.1. 3-NPA produces lesions and motor abnormalities

Animals chronically intoxicated with 3-NPA exhibited motor abnormalities similar to those previously observed following acute intoxication (Nishino et al., 1997). These abnormalities were evident when the animals were allowed to climb a pole. Typical climbing patterns are shown in Figs. 1A and B. The intoxicated animals used only their forelimbs to climb the pole and their hindlimbs just hung in the air. The intoxicated animals were therefore clinging onto the pole with one of their forelimbs, in contrast to control animals that showed well-coordinated postural control.

EMG recordings of the gastrocnemius of control and 3-NPA-intoxicated animals are shown in Fig. 1C. The EMG recordings of control animals exhibited high frequency waves with low amplitudes (Fig. 1C1), whereas those of 3-NPA-intoxicated animals exhibited low-frequency waves with low amplitudes (Fig. 1C3–4). Occasionally, high frequency waves with high amplitudes were also seen in intoxicated animals (Fig. 1C2). Thus, the EMG patterns of intoxicated animals were distinctly different from those of control animals.

Hematoxylin–eosin staining of brain sections revealed a dark area in the lateral striatum, although other parts looked healthy (Fig. 1D). Microtubule-associated protein (MAP)-2 immunostaining was faintly detected in this region (Fig. 1D). Glial fibrillary acidic protein (GFAP) immunostaining revealed a loss of GFAP-positive cells in the lateral striatum, as indicated by empty spaces surrounded by gliosis (Fig. 1D). IgG immunostaining revealed dense staining at the centre of the lesions observed in the GFAP-stained sections (Fig. 1D). Taken together, these histological observations indicate loss of astrocytes in the lateral striatal area.

2.2. 3-NPA also induces astrocyte loss in vitro

In astrocyte cultures, 3-NPA produced concentration-dependent decreases in the cell number after 24 h. The cell number decreased by approximately 50% in the presence of 1.7 mM 3-NPA (Fig. 2B), accompanied by an increase in LDH activity (175% of the initial activity; Fig. 2A). The decreased cell number and increased LDH level indicate toxicity of 3-NPA toward astrocytes. Minimal toxicity was seen with 0.017 mM of the toxin. Concurrently, there were increases in the NO_2^- level in the supernatants from these wells (Fig. 2B). The increase was much greater (12 μM NO_2^-) in the presence of 1.7 mM 3-NPA. 3-NPA even produced NO_2^- in culture plates without astrocytes and the increase was greater than that detected in the presence of astrocytes (Fig. 2C). This may indicate that astrocytes have a NO-buffering action. NO_2^- levels as high as 40 μM (for 24 h) did not show any toxic effects on astrocytes (Fig. 2D).

In a separate series of experiments, we exposed astrocytes to 3-NPA (1.7 mM) in the presence of hemoglobin (Hb; 20 μM) to block the effects of NO (Fig. 3). 3-NPA decreased the astrocyte number and increased the NO_2^- level significantly. Although the presence of Hb per se did not have any effect on the astrocyte population, it greatly attenuated the 3-NPA-induced decrease in cell number ($P<0.05$; Student's *t*-test for unpaired observations). The NO_2^- level in these wells was also attenuated significantly in the presence of 20 μM Hb (Fig. 3; $P<0.05$, Student's *t*-test for unpaired observations).

2.3. 3-NPA acts as a NO donor

The concentration of NO was determined by a NO meter that measures a redox current between working electrode having NO selectivity and counter electrode. NO selectivity was made by three membranes (KCl, NO selective resin and silicon membrane) that did not allowed to the detection of NO_2 and NO_3 in this system. A standard NO donor, S-nitroso-*n*-acetyl-DL-penicillamine (SNAP), increased the NO-induced redox current in a concentration-dependent manner (Fig. 4B),