Neuroscience Research 62 (2008) 206–209

Contents lists available at ScienceDirect

Neuroscience Research

journal homepage: www.elsevier.com/locate/neures

Rapid communication

Restorative effects of uridine plus docosahexaenoic acid in a rat model of Parkinson's disease

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ARTICLE INFO

Article history:
Received 3 June 2008
Received in revised form 10 July 2008
Accepted 25 July 2008
Available online 3 August 2008

Keywords:
Parkinson's disease
Uridine
Docosahexaenoic acid
Dopamine
Tyrosine hydroxylase activity
Synapse

ABSTRACT

Administering uridine-5′-monophosphate (UMP) and docosahexaenoic acid (DHA) increases synaptic membranes (as characterized by pre- and post-synaptic proteins) and dendritic spines in rodents. We examined their effects on rotational behavior and dopaminergic markers in rats with partial unilateral 6-hydroxydopamine (6-OHDA)-induced striatal lesions. Rats receiving UMP, DHA, both, or neither, daily, and intrastriatal 6-OHDA 3 days after treatment onset, were tested for *d*-amphetamine-induced rotational behavior and dopaminergic markers after 24 and 28 days, respectively. UMP/DHA treatment reduced ipsilateral rotations by 57% and significantly elevated striatal dopamine, tyrosine hydroxylase (TH) activity, TH protein and synapsin-1 on the lesioned side. Hence, giving uridine and DHA may partially restore dopaminergic neurotransmission in this model of Parkinson's disease.

Parkinson's disease (PD) is characterized by the progressive degeneration of dopaminergic nigrostriatal neurons, and reductions in striatal dopamine (DA) levels, dopaminergic synapses, and the density of dendritic spines on striatal medium spiny neurons. No treatment for PD currently available is thought to restore the numbers of dopaminergic nigrostriatal terminals or striatal dendritic spines.

We previously observed that chronic oral administration of two circulating phosphatide precursors, uridine (as UMP) and the omega-3 fatty acid DHA, along with dietary choline, can increase neuronal levels of the phosphatides and of specific proteins that characterize synaptic membranes (Wurtman et al., 2006), as well as the numbers of dendritic spines, in rodent brain (Sakamoto et al., 2007). The present report examines the effects of giving these precursors in a rat model of PD with unilateral neurotoxin-induced nigrostriatal damage and impaired DA neurotransmission. In this model 6-hydroxydopamine (6-OHDA), injected into one corpus striatum, causes ipsilateral decreases in DA synthesis and release, and characteristic turning behavior.

Male Sprague–Dawley rats (200–250 g) consumed the control diet (containing 16% protein and 0.1% choline) fortified with UMP (0.5%) and received by daily gavage (1 ml/kg) 300 mg/kg of DHA in 5% gum arabic solution; control rats were gavaged with DHA's vehicle. Three days after the start of UMP/DHA administration, rats were injected with 6-OHDA (8 μg in 2 μl of 0.3% ascorbic acid/0.9% saline) into two different sites within their right striata (Kirik et al., 1998). In preliminary experiments that used DA levels, TH levels, and TH activity as markers, we found that pretreatment with UMP and DHA did not diminish the initial toxic responses to the 6-OHDA. Hence, subsequent studies used animals killed 28 days after starting UMP/DHA, a period previously shown to reliably increase membrane phosphatides, synaptic proteins (Wurtman et al., 2006) and dendritic spines (Sakamoto et al., 2007).

Rotational behavior was induced by intraperitoneal injection of *d*-amphetamine (5 mg/kg) 3 weeks after animals received the 6-OHDA treatment (day 25), and ipsilateral rotations by the rats, videotaped between 15 and 45 min following the *d*-amphetamine injection, were counted by two blind observers. Animals were sacrificed 3 days after testing for rotational behavior. Striatal DA was measured using an HPLC assay (Wang et al., 2005), and TH activity was determined by a radiometric method (Ulus and Wurtman, 1976). Striatal phospholipids were extracted and individual phospholipid classes were separated and quantified

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0168-0102/$ – see front matter
doi:10.1016/j.neures.2008.07.005

by measuring their phosphorus contents (Cansev and Wurtman, 2007). TH, synapsin-1 and β-tubulin proteins were analyzed by Western blot (Cansev and Wurtman, 2007).

Data were analyzed using one-way analysis of variance (ANOVA) followed by *post hoc* Tukey tests. Comparisons between values obtained from intact and lesioned striata were made using Student's *t*-test. Data are presented as mean ± S.E.M.; *P* less than 0.05 was considered significant.

Animals that received UMP, DHA, or UMP plus DHA for 24 days exhibited significant reductions in the numbers of *d*-amphetamine-induced rotations, compared with those in control rats, by 48, 47, or 57% (all $P < 0.05$), respectively (Table 1). The numbers of rotations exhibited by individual animals in all of the experimental groups were inversely correlated with the dopamine contents ($r = -0.447$; $P < 0.05$) and TH activities ($r = -0.546$; $P < 0.01$) in the lesioned striata.

Among control rats DA levels and TH activity (Table 2), and TH protein (Fig. 1A) in the lesioned striata were 64, 65, and 35% lower (all $P < 0.001$) than those in the intact striata, respectively, and levels of the two DA metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were reduced by about 50%. UMP administration, alone or with DHA, restored DA levels [by 41% ($P < 0.01$) or by 37% ($P < 0.05$)], as well as TH activity [by 53% ($P < 0.05$) or 52% ($P < 0.05$)] in lesioned striata (Table 2). Reductions in TH protein levels in lesioned striata were partially restored, increasing by 21% ($P < 0.01$) or 22% ($P < 0.01$), following DHA supplementation alone or after UMP plus DHA (Fig. 1A). UMP plus DHA treatment also increased DOPAC levels from 0.18 ± 0.01 to 0.22 ± 0.01 nmol/mg protein ($P < 0.05$) and HVA levels from 0.15 ± 0.01 to 0.19 ± 0.04 nmol/mg protein ($P < 0.05$) in lesioned striata.

Levels of synapsin-1, reduced in lesioned striata by 15% ($P < 0.001$) (Fig. 1B), were increased significantly by UMP, DHA, or UMP plus DHA (by 17, 16, or 25%, respectively (Fig. 1B). In contrast, levels of β-tubulin, our loading control, were unaffected by either 6-OHDA injection or dietary treatments (Fig. 1). Since intrastriatal 6-OHDA is selectively neurotoxic to DA terminals, and since these terminals comprise only a small proportion of striatal structures, its administration failed to affect striatal phospholipid levels. Administration of UMP plus DHA did increase these levels (Table 3), as expected (Wurtman et al., 2006) in both the lesioned and the control sides, those of each of the individual phosphatides [i.e., phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI) and sphingomyelin (SM)] also increasing significantly (Table 3). This treatment is known to increase phosphatide levels significantly throughout the brain (Cansev and Wurtman, 2007), and probably affects most or all types of brain cells since all, apparently, utilize the same substrate-unsaturated Kennedy cycle enzymes (Wurtman et al., in press) for phosphatide synthesis.

These data show that chronic oral administration of uridine (as UMP) and/or DHA, which can stimulate brain phosphatide and

Table 1
Rotational behavior

Treatment	Ipsilateral rotations/30 min
Control diet + vehicle	151 ± 21
UMP diet + vehicle	79 ± 22*
Control diet + DHA	81 ± 12*
UMP diet + DHA	65 ± 18*

Rats receiving UMP, DHA, both, or neither, daily, and intrastriatal 6-OHDA 3 days after treatment onset, were tested for *d*-amphetamine-induced rotational behavior after 24 days. Ipsilateral rotations were recorded for 30 min, between 15 and 45 min, after i.p. injection of 5 mg/kg of *d*-amphetamine, $N = 6$–9 in each group. *$P < 0.05$; **$P < 0.025$ compared with control diet + vehicle group using one-way ANOVA followed by *post hoc* Tukey test.

Table 2
Dopamine (DA) levels and tyrosine hydroxylase (TH) activity

Treatments	Left (intact) striatum	Right (lesioned) striatum
Dopamine levels (nmol/mg protein)		
Control diet + vehicle	0.704 ± 0.024	0.252 ± 0.018[a]
UMP diet + vehicle	0.749 ± 0.022	0.355 ± 0.025*
Control diet + DHA	0.745 ± 0.021	0.311 ± 0.016
UMP diet + DHA	0.830 ± 0.022**	0.345 ± 0.017*
TH activity (nmol DOPA formed/h/mg protein)		
Control diet + vehicle	3.983 ± 0.26	1.405 ± 0.06[a]
UMP diet + vehicle	3.591 ± 0.20	2.144 ± 0.19*
Control diet + DHA	4.014 ± 0.12	1.906 ± 0.17
UMP diet + DHA	4.189 ± 0.24	2.131 ± 0.17*

Rats receiving the treatments for 28 days were sacrificed on day 29. Striata were assayed for DA levels (A) and TH activity (B), $N = 6$–9 in each group. *$P < 0.05$; **$P < 0.01$ compared with control diet + vehicle group within the same localization using one-way ANOVA followed by *post hoc* Tukey test.

[a] $P < 0.001$ compared with values from left striata of animals that received the control diet + vehicle using Student's *t*-test.

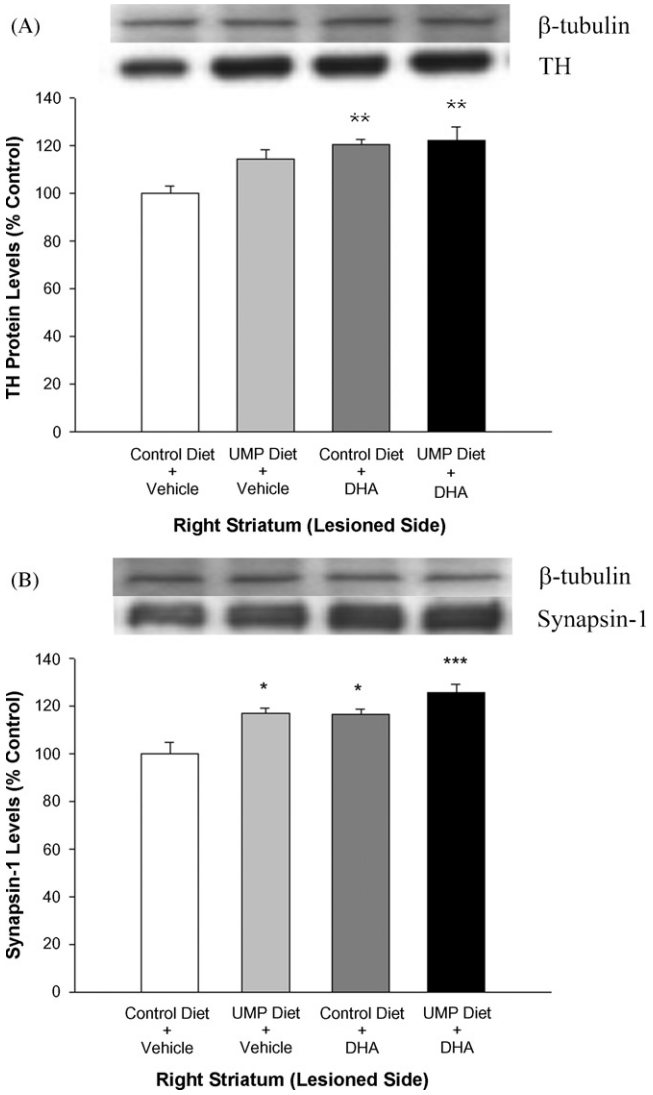

Fig. 1. TH protein and synapsin-1 levels. Homogenates of the lesioned striata were analyzed for TH protein (A) and synapsin-1 (B). Values obtained from rats treated with UMP, DHA, and UMP + DHA were expressed by reference to those (normalized to 100) obtained from rats that received the control diet + vehicle (control group). β-Tubulin was used as the loading control, $N = 6$ per group. *$P < 0.05$ and **$P < 0.01$ compared with control group using one-way ANOVA.