Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 78 (2004) 179–183
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Early administration of nicotinamide prevents learning and memory impairment in mice induced by 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine

Jun Yang[a,b], Lina He[b], Jing Wang[b], James D. Adams Jr.[a,*]

[a]School of Pharmacy, University of Southern California, 1985 Zonal Avenue, Los Angeles, CA 90089, USA
[b]Anhui Biological and Medicinal Key Laboratory, 15 Yonghong Road, Hefei, Anhui, 230061, PR China

Received 20 October 2003; received in revised form 14 January 2004; accepted 11 March 2004

Available online 17 April 2004

Abstract

Background and purpose: NAD has been reported to improve the dementia of the Alzheimer type or sensory register, short- and long-term memory loss in the aged. Although nicotinamide has been confirmed to decrease infarct volumes and neurological deficit findings in several animal stroke models, it is not clear whether its neuroprotective effects can prevent memory damage sequelae. *Methods*: We have addressed this topic by designing two behavioral paradigms. A memory impairment and cognitive change model was used in mice following 1-methyl-4-phenyl-l, 2, 3, 6-tetrahydropyridine (MPTP) exposure. Step-down and step-through tests were performed to examine the effects of nicotinamide on learning and memory impairment. *Results*: It was found that the early administration of nicotinamide (2 h after the injection of MPTP) could decrease error numbers, lessen stimulation time and prolong residence duration on the safety platform in the step-down test. Delayed administration of nicotinamide resulted in decreased effects. Similar results were found in the step-through test. Nicotinamide administrated 12 h after the induction of a memory-impairment model still exerted its effects on memory dysfunction. *Conclusions*: The injection of MPTP can cause a loss of brain functions including learning and memory. Learning and memory dysfunction probably occurs secondary to damage to arterioles and dopaminergic neurons by MPTP. By inhibiting oxidative stress, increasing NAD synthesis and ATP production and inhibiting poly (ADP-ribose) polymerase, nicotinamide is known to rescue the still viable, but injured, cells. This rescue process may partially restore learning and memory.

Keywords: Nicotinamide; 1-methyl-4-phenyl-l, 2, 3, 6-tetrahydropyridine; MPTP; Vascular dementia; Learning; Memory

1. Introduction

Vascular dementia (VaD) is the second most common cause of dementia in the elderly, after Alzheimer's disease (AD). VaD is defined as a loss of cognitive function resulting from ischemic or hemorrhagic brain lesions due to cerebrovascular disease. Diagnosis requires the following criteria: cognitive loss, often predominantly subcortical; vascular brain lesions, demonstrated by imaging; a temporal link between stroke and dementia; and exclusion of other causes of dementia. Poststroke VaD may be caused by large-vessel disease caused by multiple strokes (multi-infarct dementia) or by a single stroke (strategic stroke VaD). A common form is subcortical ischemic VaD caused by small-vessel occlusions with multiple lacunas and by hypoperfusive lesions resulting from stenosis of medullary arterioles, as in Binswanger's disease (Roman, 2003). An animal VaD model has been reported in rats following the embolization of the right middle cerebral artery and subsequent reperfusion (Nagasawa and Kogure, 1990). In the current study, MPTP has been used to model vascular damage in the brain. MPTP is known to damage arterioles in the brain (Adams et al., 1989, 1991). Of course, MPTP also damages dopaminergic neurons in the midbrain (Adams et al., 1989).

Risk factors possibly associated with dementia after strokes include advancing age, previous cerebral or myocardial infarction, an atherothrombotic stroke mechanism and cerebral atrophy (Kase, 1991). In recent years, interest in the vascular causes of dementia has increased. It has been proposed that VaD may be more common than previously

* Corresponding author. Tel.: +1-323-442-1362; fax: +1-323-442-1681.
E-mail address: jadams@hsc.usc.edu (J.D. Adams).

0091-3057/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2004.03.007

supposed. This may have important implications because VaD, at present, may be more amenable to prevention and treatment than AD is (Skoog, 1994). Memory deficits in AD have been reported to improve following NAD administration (Birkmayer, 1996).

Nicotinamide does not enhance learning and memory in normal rodents (Koppen et al., 1996). However, nicotinamide has been confirmed to inhibit neuronal necrosis and apoptosis effectively by the inhibition of DNA fragmentation and the rapid repair of DNA damage. As an essential precursor of NAD^+ and a PARP inhibitor, nicotinamide prevents the depletion of NAD^+ and protects against the decreased production of ATP (Klaidman et al., 1996). Delayed treatment with nicotinamide improved neurological outcome and reduced infarct volume after transient focal ischemia in Wistar rats, even when administered up to 2 h after the onset of stroke, and improved both the anatomic and functional indices of brain damage (Mokudai et al., 2000). Posttreatment with nicotinamide reduced infarct volume following permanent focal cerebral ischemia in female Sprague–Dawley or Wistar rats. The neuroprotection observed was more robust when administered as an intravenous bolus compared with intraperitoneal administration (Ayoub et al., 1999; Sakakibara et al., 2000).

To observe whether the neuroprotection of nicotinamide against cerebral ischemia and reperfusion is closely related to the prevention of VaD, we used a rodent memory impairment and cognitive change model induced by MPTP (Miyoshi et al., 2002). Mice treated with MPTP have been reported to be comparable with controls in T-maze delayed alternation, with fixed delays, but were impaired when trials with mixed 20- and 120-s delays were presented, indicative of a spatial working memory impairment. The impairment in spatial working memory after MPTP exposure in mice parallels the findings in MPTP-treated monkeys (Fernandez-Ruiz et al., 1995; Tanila et al., 1998). Male mice treated with MPTP have been reported to suffer from severely disrupted habituation–dishabituation response profiles and significantly reduced concentrations of norepinephrine within the olfactory bulb and hippocampus. These results suggest that in addition to its putative effects upon the nigrostriatal dopaminergic system and motor behavior, MPTP is also exerting substantial effects upon other systems, in particular, the noradrenergic system, which may be involved in memory/recognition processes (Dluzen and Kreutzberg, 1993).

In this study, MPTP-treated mice were tested in step-down and step-through paradigms. Both passive and active avoidance responses were observed and automatically recorded. A possible improvement induced by nicotinamide on learning impairment and memory loss was investigated.

2. Materials and methods

Male Kuming mice (Grade II, Certificate No. WSD-002), aged 8 weeks and weighing 22–26 g, were supplied by the Department of Experimental Animals, Anhui Institute of Medical Sciences. The animals were housed in plastic cages, with free access to food and water, except during behavioral experiments, and kept in a regulated environment, 20 ± 1 °C under a 12-h light/dark cycle. The animal experiments conformed to the current "Regulations on the Use and Care of Laboratory Animals" and were approved by the Animal Care and Use Committee in Anhui Institute of Medical Sciences.

2.1. Induction of learning and memory dysfunction by injection of MPTP

MPTP was dissolved in sterile saline at a concentration of 1 mg/ml and stored at 4 °C. Nicotinamide was dissolved in sterile distilled water at a concentration of 20 mg/ml and stored at 4 °C. After the first-day of training, the mice in the control group were injected twice with saline. The mice in the other groups were injected twice with MPTP 25 mg/kg ip at an interval of 2 h. The mice were tested 24 h later in learning and memory paradigms.

2.2. Administration of nicotinamide

Nicotinamide was administrated intraperitoneally to mice in doses of 500 mg/kg. Nicotinamide was given to mice at 0, 2, 6 or 12 h after the second injection of MPTP. Vehicle solution in the same volume was given to the control group.

2.3. Step-down passive avoidance test

The apparatus consisted of an acrylic box with a stainless steel grid floor. A platform was fixed at the end of the box. Electric shocks (36 V) were delivered to the grid floor for 3 s with an isolated pulse stimulator. At the beginning of training, the mice were placed on the platform to adapt for 2 min. When the mouse stepped down and placed all its paws on the grid floor, it would jump to the platform as a shock was delivered. Step-down latency (time of staying on the platform), the number of errors and the total stimulation time were recorded for 5 min and repeated 24 h after training. Following the first training session, the mice were injected with MPTP. A possible alteration of passive avoidance was examined 24 h later.

2.4. Step-through active avoidance test

The apparatus consisted of a standard rectangular shuttle box with plexiglass walls, the floor of which was a stainless steel grid floor. The box was divided into two compartments with a plate. There was a hole at the bottom of the plate, by which mice could shuttle freely between the compartments. Two lamps, as signals in the ceiling of each compartment, were set up to alternate between 10 s of light followed by 20 s of electric shocks (36 V) delivered to the grid floor. For training, each mouse was placed in one of the compart-

ments. It would go to the other compartment as it was shocked by an isolated pulse stimulator. The active escape latency (escape following a lamp signal), the accumulated stimulation time, the active escape numbers and the passive avoidance numbers (to avoid an electric shock) were recorded automatically during 30 shuttles. After the first training session, the mice were injected with MPTP. After 24 and 48 h, the mice were tested to examine their active avoidance ability.

2.5. Statistical analysis

The group size for the assessment of learning and memory ability from the various nicotinamide treatment groups and the group treated with vehicle was 12 mice, at least. The data were expressed as means ± S.E.M. ANOVA and Newman Keul's test were used. P values less than .05 were considered significant.

3. Results

3.1. Step-down passive avoidance test

This test was used as an evaluation of passive learning and is a standard test used in learning and memory research. All mice developed a remarkable loss of learning and memory 24 h after the injection of MPTP (Table 1). Compared with the control group, stimulation time and error numbers in the MPTP model group were increased significantly, but latency was decreased significantly. After treatment with nicotinamide, all indices in the MPTP-treated groups were obviously improved. Following the administration of 500 mg/kg of nicotinamide, 0 or 2 h after the injection of MPTP, latency was increased significantly ($P<.05$ or .01). Both stimulation time and error numbers were reduced significantly ($P<.01$).

Table 1
Nicotinamide (NAM) amelioration of learning and memory dysfunction in MPTP-treated mice (25 mg/kg ip × 2) 24 h after training, as assessed by the step-down test

Group	Dose (mg/kg)	Latency (s)	Stimulation time (s)	Error numbers
Control	–	270 ± 29	29 ± 24	3 ± 3
MPTP model	25	186 ± 55[a]	123 ± 57[a]	14 ± 5[a]
NAM (0 h)	500	234 ± 30[b]	66 ± 28[c]	7 ± 4[c]
NAM (2 h)	500	242 ± 23[c]	58 ± 26[c]	6 ± 4[c]
NAM (6 h)	500	207 ± 33	92 ± 29	10 ± 5
NAM (12 h)	500	210 ± 41	89 ± 39	10 ± 6

Nicotinamide (500 mg/kg) was given at different times after the second injection of MPTP.
Values are means ± S.E.M. There were 12 mice in each group. One mouse died after the injection of MPTP in a nicotinamide (12 h) group. Compared with the values in the control group, the early administration of nicotinamide (not more than 2 h after the onset of brain oxidative stress) exerted significant improvements in learning and memory dysfunction.
[a] $P<.01$ vs. the control group.
[b] $P<.05$ vs. the MPTP model group, by ANOVA and Newman-Keul's test.
[c] $P<.01$ vs. the MPTP model group, by ANOVA and Newman-Keul's test.

Table 2
Nicotinamide (NAM) amelioration of learning and memory dysfunction in MPTP-treated mice (25 mg/kg ip × 2) 24 h after training, as assessed by the shuttle-box test

Group	Dose (mg/kg)	Latency (s)	Stimulation time (s)	Number of escapes	Avoidance numbers
Control	–	4.2 ± 2.2	4.5 ± 2.4	1.2 ± 1.2	12.8 ± 3.5
MPTP model	25	2.1 ± 1.6[a]	8.1 ± 2.6[a]	1.4 ± 1.1	14.6 ± 2.5
NAM (0 h)	500	3.2 ± 1.8	5.5 ± 2.3[c]	1.3 ± 1.0	13.2 ± 3.0
NAM (2 h)	500	2.9 ± 1.3	6.9 ± 1.2	1.4 ± 1.3	13.1 ± 1.9
NAM (6 h)	500	3.0 ± 1.4	8.0 ± 2.5	1.0 ± 1.2	14.4 ± 2.9
NAM (12 h)	500	2.5 ± 1.5	7.8 ± 2.9	1.2 ± 1.3	15.1 ± 3.0

Nicotinamide (500 mg/kg) was given at different times after the injection of MPTP.
Values are means ± S.E.M. There were 12 mice in each group. One mouse died after the injection of MPTP in a nicotinamide (12 h) group. Compared with the values in the control group, the early administration of nicotinamide (0 h after the onset of brain oxidative stress) exerted a significant reduction in stimulation time.
[a] $P<.01$ vs. the control group.
[b] $P<.01$ vs. the MPTP model group, by ANOVA and Newman-Keul's test.
[c] $P<.01$ vs. the control group.

3.2. Step-through active avoidance test after 24 h

This test was used as an evaluation of active learning processes and is a standard test used in learning and memory research. All animals were tested in the step-through box 24 h after the first day of training (Table 2). It was found that mice in the MPTP model group showed slow responses to electrical stimulation. There were significant reductions in

Table 3
Nicotinamide (NAM) amelioration of learning and memory dysfunction in mice treated with MPTP (25 mg/kg ip × 2) 48 h after training, as assessed by the shuttle-box test

Group	Dose (mg/kg)	Latency (s)	Stimulation time (s)	Number of escapes	Avoidance numbers
Control	–	4.0 ± 1.6	4.3 ± 2.6	1.1 ± 0.9	11.4 ± 3.7
MPTP	25	2.3 ± 1.7[a]	7.9 ± 1.8[b]	1.3 ± 1.2	15.1 ± 2.1[b]
NAM (0 h)	500	3.6 ± 1.9	4.8 ± 2.1[c]	1.2 ± 1.1	13.0 ± 2.4
NAM (2 h)	500	3.5 ± 1.8	5.2 ± 2.0[c]	1.4 ± 1.1	13.7 ± 1.8
NAM (6 h)	500	3.6 ± 1.5	6.0 ± 1.9[d]	0.9 ± 1.3	13.9 ± 2.7
NAM (12 h)	500	2.6 ± 1.4	5.8 ± 2.3[d]	1.0 ± 1.3	14.6 ± 2.9

Nicotinamide (500 mg/kg) was given at different times after the injection of MPTP.
Values are means ± S.E.M. There were 12 mice in each group. One mouse died after the injection of MPTP in a nicotinamide (12 h) group. Compared with the values in the control group, the early administration of nicotinamide (0 h after onset of brain oxidative stress) exerted a significant reduction in stimulation time.
[a] $P<.01$ vs. the control group.
[b] $P<.01$ vs. the control group.
[c] $P<.01$ vs. the MPTP model group, by ANOVA and Newman-Keul's test.
[d] $P<.05$ vs. the MPTP model group, by ANOVA and Newman-Keul's test.

active escape latency and increases in stimulation time. Although, to some extent, nicotinamide treatment at different time intervals after the onset of oxidative stress provided improvement in learning and memory dysfunction, significant reductions in stimulation time were only observed in one group treated with nicotinamide (0 h).

3.3. Step-through active avoidance test after 48 h

All animals were tested in the step-through box, again, 48 h after the first day of training (Table 3). Besides slow responses in escape latency and stimulation time, significant increases in avoidance numbers were found in the MPTP model group. This implies that learning and memory damage were aggravated at 48 h. In groups treated with nicotinamide at different time intervals (0–12 h after the injection of MPTP), the stimulation time that the mice received was significantly reduced. Although the administration of nicotinamide led to improvements in escape latency and avoidance numbers, no significant differences were observed in those groups.

4. Discussion

Nicotinamide is a PARP inhibitor and can be used to maintain cellular energetics. It prevents ATP depletion by preventing NAD depletion (Yang et al., 2002b). This helps prevent necrosis and apoptosis in the brain. Nicotinamide protects brain function following focal ischemia and reperfusion, as assessed by neurological deficit (Yang et al., 2002a). However, nicotinamide, at doses of 500 mg/kg and below, has been shown to not increase learning and memory in normal rats (Koppen et al., 1996). At a very high dose, 1000 mg/kg, nicotinamide impaired learning and memory due to a sedative effect (Koppen et al., 1996). A PARP inhibitor, 5-iodo-6-amino-1,2-benzopyrone, was not found to increase learning and memory in normal animals but did increase learning and memory when used as a neuroprotective agent in traumatic brain damage (Satchell et al., 2003). Nicotinamide appears to work in a similar manner. It is neuroprotective and can prevent the loss of learning and memory in our model of vascular damage. However, it has no ability to increase learning and memory in the normal brain.

The latest research from our laboratory indicates that the early administration of nicotinamide can effectively inhibit the development of stroke and protect the viable, but injured, neurons (Yang et al., 2002a). In a rat transient focal cerebral ischemia model, induced by middle cerebral artery occlusion for 90 min, systemic dose-response and time-effect studies were designed. It was found that nicotinamide injected during the first 6 h of reperfusion could effectively inhibit the development of brain damage. The optimal dose of nicotinamide was 500 mg/kg and gave a maximal response. The administration of nicotinamide at a suitable dosage significantly prevents necrotic and apoptotic brain injury after focal ischemia reperfusion (Yang et al., 2002a). Similar research on the therapeutic window of nicotinamide was conducted in a model of stroke (Ayoub and Maynard, 2002). At 2, 4 or 6 h after the onset of transient (2 h) focal cerebral ischemia, Wistar rats received either saline or nicotinamide (500 mg/kg). Sensory and motor behavioral scores were obtained before surgery, 2 h, and 3 and 7 days after the stroke onset. Cerebral infarct volumes were measured on Day 7 after the stroke. Nicotinamide given 4 or 6 h after stroke onset significantly ($P < .05$) reduced cerebral infarction and improved behavioral scores, respectively, compared with saline-injected animals.

It is very clear that the amelioration by nicotinamide of memory dysfunction induced by MPTP is closely related to its mechanism of neuroprotection. We have previously shown that nicotinamide prevents necrosis and apoptosis in the brains of MPTP-treated mice (Mukherjee et al., 1997). In addition to improvements in the passive avoidance test, the effects of nicotinamide in active avoidance tests indicate that it promotes learning and memory. The protective effects of nicotinamide were greater in the active avoidance test because nicotinamide partially prevents apoptosis that is maximal at 24–48 h. Recovery for 48 h may give the brain time to recover from the apoptosis process. Nicotinamide appears to rescue the still viable, but injured, nerve cells and partially restores learning and memory.

References

Adams JD, Kalivas PW, Miller CA. The acute histopathology of MPTP in the mouse CNS. Brain Res Bull 1989;23:1–18.

Adams JD, Klaidman LK, Odunze IN, Johannessen JN. Effects of MPTP on the cerebrovasculature. Int J Dev Neurosci 1991;9:155–9.

Ayoub IA, Maynard KI. Therapeutic window for nicotinamide following transient focal cerebral ischemia. NeuroReport 2002;13(2):213–6.

Ayoub IA, Lee EJ, Ogilvy CS, Beal MF, Maynard KI. Nicotinamide reduces infarction up to two hours after the onset of permanent focal cerebral ischemia in Wistar rats. Neurosci Lett 1999;259(1):21–4.

Birkmayer JG. Coenzyme nicotinamide adenine dinucleotide: new therapeutic approach for improving dementia of the Alzheimer type. Ann Clin Lab Sci 1996;26(1):1–9.

Dluzen DE, Kreutzberg JD. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) disrupts social memory/recognition processes in the male mouse. Brain Res 1993;609(1–2):98–102 [Apr 23].

Fernandez-Ruiz J, Doudet DJ, Aigner TG. Long-term cognitive impairment in MPTP-treated rhesus monkeys. NeuroReport 1995;7(1):102–4.

Kase CS. Epidemiology of multi-infarct dementia. Alzheimer Dis Assoc Disord 1991;5(2):71–6.

Klaidman LK, Mukherjee SK, Hutchin TP, Adams JD. Nicotinamide as a precursor for NAD+ prevents apoptosis in the mouse brain induced by tertiary-butylhydroperoxide. Neurosci Lett 1996;206(1):5–8.

Koppen A, Klein J, Schmidt BH, Van Der Staay FJ, Loffelholz K. Effects of nicotinamide on central cholinergic transmission and on spatial learning in rats. Pharmacol Biochem Behav 1996;53:783–90.

Miyoshi E, Wietzikoski S, Camplessei M, Silveira R, Takahashi R, Da Cunha C. Impaired learning in a spatial working memory version and in a cued version of the water maze in rats with MPTP induced mesencephalic dopaminergic lesions. Brain Res Bull 2002;58:41–7.

Mokudai T, Ayoub IA, Sakakibara Y, Lee EJ, Ogilvy CS, Maynard KI. Delayed treatment with nicotinamide (Vitamin B3) improves neurolog-

ical outcome and reduces infarct volume after transient focal cerebral ischemia in Wistar rats. Stroke 2000;31(7):1679–85.

Mukherjee SK, Klaidman LK, Yasharel R, Adams JD. Increased brain NAD prevents neuronal apoptosis in vivo. Eur J Pharmacol 1997; 330(1):27–34.

Nagasawa H, Kogure K. Multi-focal delayed neuronal damage after transient regional ischemia-mechanism of vascular dementia. Rinsho Shinkeigaku 1990;30(4):396–401.

Roman GC. Vascular dementia: distinguishing characteristics, treatment, and prevention. J Am Geriatr Soc 2003;51(5 Suppl Dementia):S296–304.

Sakakibara Y, Mitha AP, Ogilvy CS, Maynard KI. Post-treatment with nicotinamide (Vitamin B3) reduces the infarct volume following permanent focal cerebral ischemia in female Sprague–Dawley and Wistar rats. Neurosci Lett 2000;281(2–3):111–4.

Satchell MA, Zhang X, Kochanek PM, Dixon CE, Jenkins LW, Melick J, et al. A dual role for poly-ADP-ribosylation in spatial memory acquisition after traumatic brain injury in mice involving NAD depletion and ribosylation of 14-3-3 gamma. J Neurochem 2003;85:697–708.

Skoog I. Risk factors for vascular dementia: a review. Dementia 1994; 5(3–4):137–44.

Tanila H, Bjorklund M, Riekkinen Jr P. Cognitive changes in mice following moderate MPTP exposure. Brain Res Bull 1998;45(6):577–82.

Yang J, Klaidman LK, Chang ML, Kem S, Sugawara T, Chan P, et al. Nicotinamide therapy protects against both necrosis and apoptosis in a stroke model. Pharmacol Biochem Behav 2002a;73(4):901–10.

Yang J, Klaidman LK, Nalbandian A, Oliver J, Chang ML, Chan PH, et al. The effects of nicotinamide on energy metabolism following transient focal ischemia in Wistar rats. Neurosci Lett 2002b;333:91–4.