Treatment with reduced nicotinamide adenine dinucleotide (NADH) improves water maze performance in old Wistar rats

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Abstract

Age-associated cognitive impairment and related neurodegenerative disorders are an increasing major public health problem. Nicotinamide adenine dinucleotide (NADH), a co-substrate for energy transfer in the mitochondrial respiratory chain, is speculated to induce positive effects in some of these diseases. Studies showed diminished mitochondrial function in patients with M. Alzheimer. In a preliminary clinical trial NADH given peripherally improved cognitive function in Alzheimer disease.

Previous own experiments revealed an increased NADH level in the rat brain following peripheral application of NADH (10–100 mg/kg, i.p. + i.v.). Therefore, we wanted to know, whether or not NADH has an effect on cognitive function in animals. We analysed the effect of repeated i.p. injection of NADH on the performance of 3-month-old and 22-month-old Wistar rats in the Morris water maze and in the rota-rod test of motor coordination. The rats were injected for 10 days once daily with the doses of NADH used in the bioavailability study (10–100 mg/kg) or vehicle 20 min before the behavioural tests. The repeated administration of NADH improved the performance of old rats in the acquisition phase (place version) and the spatial probe of the Morris water maze compared to vehicle-treated controls. The effect of NADH on learning-related processes is supported by the lack of effects on motor performance on the rota-rod. In summary, our results suggest cognitive enhancing properties of NADH in learning impaired old rats.

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1. Introduction

Age-associated cognitive impairment and related neurodegenerative disorders are an enormous public health and socio-economic burden and therefore a major problem for health systems. The full pathological process is not fully understood yet.

Therapeutic interventions aimed at either finding a cure or prevention of progression of age-related or pathologic decline in cognitive function cannot be overstated.

Mitochondrial dysfunction could be a factor for predisposition to neurodegeneration, and may accelerate cell dysfunction and loss. While probably not having a primary role in pathoetiology, respiratory chain dysfunction may still be an important risk factor for development of late life neurodegenerative disorders and an important effector mechanism in neuronal dysfunction [5]. A recent study showed alterations of mitochondrial function in patients with M. Alzheimer [4].

Furthermore, there is increasing evidence for diminished cerebral mitochondrial metabolism in Alzheimer patients [6].

Considering the relatively small safety margin between energy supply and neuronal function [1] and the known age-related changes in mitochondrial function, it has been thought that an exogenous supply with reducing equivalents for the energy metabolism could counteract age-related deficits in cognitive function.

Nicotinamide adenine dinucleotide (NADH), a co-substrate for energy transfer in the mitochondrial respiratory chain, is speculated to induce positive effects in some degenerative disorders of the central nervous system. Clinical studies demonstrated positive effects of peripherally given NADH on serious disorders such as Parkinson’s disease and chronic fatigue syndrome [3,10].

Effects of oral NADH treatment in patients with cognitive impairment are ambivalent. While one study showed promising results [2] another study was unable to replicate the NADH-induced effects [26].

A criterion for a possible central action of a drug is the cerebral bioavailability. Previous own experiments in rats...
revealed an increased NADH level in the CNS following peripheral application of NADH [27], which raises the question whether NADH may induce an effect in an behavioural test of cognitive function.

The Morris water maze is the most common animal test in behavioural neuroscience to investigate spatial learning in laboratory animals [7,16,23]. Compared to the radial arm maze and often used passive avoidance procedures the water maze test has advantages [22] since it needs neither appetitive stimuli nor additional punishment which may interfere with memory processes, although the animals behaviour is partly aversively motivated.

However, changes in motor abilities can affect the performance of the animals in the Morris water maze. The rota-rod provides a relatively easy way to test the motor function in rodents. Central nervous system damage, drug effects on motor coordination or fatigue can be assessed by measuring the time during which the animal remains walking on a rotation drum [8,19].

The aim of the present study was the assessment whether or not NADH could counteract age-related cognitive performance deficits. Thus, young adult and old rats received for 10 days once a day intraperitoneal injections of NADH before testing in the Morris water maze and on the rota-rod motor coordination task [15,33].

2. Material and methods

2.1. Animals

Male Wistar (Shoe: Wist, Tierzucht Schönwalde GmbH, Germany) rats 3 months old (250 ± 22 g, young adult) and 22 months old (404 ± 5 g, old) were used. They were group-housed, five per cage (45 cm × 60 cm × 25 cm), at room temperature (22 ± 2 °C) and under a 12 h-light/12 h-dark cycle (light on at 06:00 h). Standard pellet food (Altromin 1326) and water were freely available. All rats appeared healthy before testing.

2.2. Drugs and treatment regimen

NADH (10–100 mg/kg, Gerbu, Germany) as well as the vehicle (NaHCO3-buffer, pH 8.0) were administered intraperitoneally with an injection volume of 1 ml/kg. All animals were treated with verum or vehicle once daily 20 min pretrial.

2.3. Apparatus and experimental protocol

The Morris water maze was a circular tank (200 cm) with 60 cm high walls filled to a depth of 42 cm with water at 22 ± 1 °C. Young adult and old animals were habituated to the water maze by lowering each rat into the apparatus for 90s with no opportunity to escape. Commencing on the second day, rats were tested in the hidden-platform version of the task on seven consecutive days (three trials per day). For each animal, the submerged platform (transparent perspex: 16 cm × 16 cm) was placed in the centre of one quadrant of the maze at 1.5 cm below water level. The location of the platform was constant for each rat and was counterbalanced within groups. Rats were placed gently in the water maze at a start point in the middle of the rim of a quadrant, which did not contain the platform. The starting points for the three trials of a rat varied randomly during the day so that the same start point was not used twice in a rat during a session. After reaching the platform, the rats could stay on it for 30 s; the next trial started 1 min following this 30-s period. If an animal failed to find the platform in 90 s, it was placed on the platform for 30 s. Parameters measured were: time needed to reach the platform, swim distance and swim speed. On the ninth day, the platform was removed and the animals were tested as described for the habituation session. The parameter assessed during this 90-s spatial probe was the time spent in each quadrant. On day 10, the animals were observed for three additional trials in the visible-platform version of the task. Rats were tested as described for the hidden-platform task, with the exception that the platform was made clearly visible and positioned 1.5 cm above water level in the quadrant opposite to its original location. During the different versions of the maze, the animals were tracked and the behaviour analysed with a computer aided videotracking system (VideoMot, TSE, Germany).

2.4. Rota-rod test

In a separate experiment the motor coordination was evaluated with an accelerating rota-rod for rats (TSE, Germany). The treadmill consisted of four rotating drums (7 cm diameter, 24 cm above ground), divided by flanges. The first day, rats were familiarized with the apparatus; they were placed for three 2-min runs on the constantly revolving drum (speed 4 min⁻¹). If a rat fell off during the trials, it was immediately placed back onto the drum. On the next day, the rats received either NADH or vehicle. Twenty minutes later the rats were placed on the accelerating rotating drums (speed 4–32 min⁻¹) for maximal 5 min. The latency until the rat fell off the drum was registered.

2.5. Statistics

The data were analysed using two-way ANOVA or one-way ANOVA for multiple comparisons and the Holm-Sidak method for the post hoc comparisons. Differences of the means (P < 0.05) were considered significant.

3. Results

3.1. Morris water maze

Vehicle-treated old animals showed an impairment in learning to navigate the maze compared with young adult
controls, as indicated by increased time to find the hidden platform over days [factor age: F(1, 78) = 54.523, P < 0.0001]. There was also a significant interaction between age and day [F(1, 78) = 51.2, P < 0.001], since the performance of the young adult, but not of the old animals, improved across daily sessions. Significant differences between the two groups were evident from the second day onward (Fig. 1B).

The repeated treatment with NADH improved the navigation performance of the old animals in the hidden-platform task [F(3, 279) = 3.347, P = 0.020].

Within-group comparisons revealed significantly shorter escape latencies from the sixth day onward (P-values < 0.05 versus old controls). On the last testing day (day 8), the escape latency of the old vehicle-treated rats was prolonged compared to the NADH-treated old rats and all young adult rats [F(7, 77) = 5.817, P < 0.001]. Based on the present statistical methods, there was no significant difference between old NADH-treated rats and young adult rats [F(6, 67) = 2.003, P = 0.079]. Nevertheless, there was a trend for superior performance in the young rats. The young adult controls showed a higher swim speed relative to vehicle-treated old animals [factor age: F(1, 29) = 5.6, P = 0.025] The swim speed decreased in the young adult animals over the days starting at day 6 [factor day: F(6, 279) = 5.267, P < 0.001]. The treatment with NADH did not affect the swim speed neither in the young adult rats nor in the old rats [factor group: F(3, 279) = 2.226, P = 0.102] (Table 1).

During the free swim trials on the ninth day, the vehicle-treated old controls spent less time in the quadrant of the maze where the escape platform was located in the visible-platform task compared to vehicle-injected young adult controls [factor age: F(1, 78) = 11.125, P < 0.001].

Treatment with NADH increased the time spent in the former platform quadrant [F(3, 78) = 2.826, P = 0.045] in the old rats relative to old controls (Fig. 2A).

In the visible-platform task, the time it took the old vehicle-injected animals to escape onto the platform was significantly increased compared with young adult controls (factor age: F(1, 79) = 16.392, P < 0.001); pretreatment with NADH had no impact on the escape latencies [F(3, 79) = 1.873, P = 0.159] when the platform was visible (Fig. 2B).

### 3.2. Rota-rod test

In the rota-rod test the vehicle-injected old animals showed a shorter latency to fall off the revolving drum.

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### Table 1

<table>
<thead>
<tr>
<th>Day of testing</th>
<th>Young adult rats</th>
<th>Old rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>NADH (10mg/kg)</td>
</tr>
<tr>
<td>Day 2</td>
<td>23.9 ± 1.9</td>
<td>24.7 ± 3.1</td>
</tr>
<tr>
<td>Day 3</td>
<td>23.5 ± 1.5</td>
<td>24.0 ± 0.8</td>
</tr>
<tr>
<td>Day 4</td>
<td>20.2 ± 1.6</td>
<td>21.6 ± 1.4</td>
</tr>
<tr>
<td>Day 5</td>
<td>21.3 ± 1.3</td>
<td>20.9 ± 1.3</td>
</tr>
<tr>
<td>Day 6</td>
<td>18.4 ± 1.2*</td>
<td>18.6 ± 1.1*</td>
</tr>
<tr>
<td>Day 7</td>
<td>19.7 ± 1.9*</td>
<td>18.1 ± 1.0</td>
</tr>
<tr>
<td>Day 8</td>
<td>16.9 ± 0.9*</td>
<td>17.9 ± 1.2*</td>
</tr>
</tbody>
</table>

Note: Day 10: 169 ± 24, 169 ± 19, 171 ± 13, 127 ± 6*, 103 ± 17*, 99 ± 12, 103 ± 16, 92 ± 10

Data are presented as mean ± S.E.M (n = 10–12).
indicating a higher spatial accuracy. The specificity of the quadrant (target quadrant) than the vehicle-treated old rats, NADH-treated rats spent more time in the former platform of the maze. Additionally, in the spatial probe session the ing of the escape response in the hidden-platform version navigation deficits of old rats, shown by the delayed learn-
no effect in young adult rats, it counteracted the age-related be shown. While the repeated treatment with NADH had effects on the age-related learning deficits in the Morris water maze could involve with cognition, e.g. the hippocampus[14]. The treatment with NADH had no effects on the age-related cognitive function, since in vitro study showed that NADH stimulates dopamine synthesis via an increased activity of the tyrosine hydroxylase [31]. It is widely accepted that dopamine neurons play an important role in the regulation of cognitive functions [21,24]. A clinical microdialysis study demonstrated a sustained activation of the mesolimbic dopaminergic system with an increased release of dopamine during performance of cognitive tasks [12]. More specifically, transgenic mice lacking dopamine-1A recep-
tors, showed an impaired performance in the Morris wa-
ter maze without swimming disabilities [28]. The dopamine antagonist haloperidol impair also learning [25] while ap-
plication of the dopamine D1 receptor agonists SKF 38393 and SKF 81297 enhanced performance in memory-impaired aged rats [17].

Secondly, the functions of the central nervous system are closely linked to the energy metabolism in the neurons [1]. Mitochondrial defects are described in a wide spectrum of human illnesses, including degenerative diseases of the central nervous system and aging [32]. For example, the defi-
ciency in cytochrome c oxidase is the most invariable defect in mitochondrial electron transport enzymes in Alzheimers disease [6].

NADH is an essential substrate for the energy transfer in the mitochondrial respiratory chain [9]. Targets of NADH include NAD+/NADH dependent enzymes as the mitochon-
drial cytochrome c oxidase [34] or the NADH oxidoreduc-
tase, which is coupled to the mitochondrial respiratory chain [18]. Additional supply could restore function of these mi-
tochondrial enzymes.

In conclusion, our data indicate that NADH may count-
teract learning deficits occurring in the course of brain aging, since a memory-enhancing effect of repeated NADH treatment was evident in old, but not young adult rats. 

4. Discussion

The present study confirms earlier findings that old rats show a definitive cognitive decline in the water maze and have impaired motor functions [13,15,30]. It is well-known that the performance in the Morris water maze is declining with increasing age of the animals [11]. The inferior perfor-
mance could be induced partly by changes in motor abilities, but aged rats also show, like humans, alterations in cogni-
tive performance [29]. Changes in the water maze behaviour seem to occur at the same time as changes in brain regions involved with cognition, e.g. the hippocampus [14].

In the present study a positive action of NADH on age-related learning deficits in the Morris water maze could be shown. While the repeated treatment with NADH had no effect in young adult rats, it counteracted the age-related navigation deficits of old rats, shown by the delayed learn-
ing of the escape response in the hidden-platform version of the maze. Additionally, in the spatial probe session the NADH-treated rats spent more time in the former platform quadrant (target quadrant) than the vehicle-treated old rats, indicating a higher spatial accuracy. The specificity of the NADH-effect on processes related to spatial learning and memory processes is supported by the lack of effect on motor performance, as measured by the swimming speed, and on visually guided behaviour, determined in the Morris water maze cued version using a visible platform. Com-
pared to old vehicle-treated animals the swim speed of the rats treated with NADH was similar and the escape latency of old rats in the visible-platform task was not dependent on the treatment.

Furthermore, the rats were also evaluated in the rota-rod test to investigate possible alterations in motor activity and/or performance, respectively. The rota-rod test is one of the tests commonly used in the context of motor function, endurance and balance [8,19]. In the rota-rod test, perfor-
mance of the old rats was significantly impaired, compared to the young rats. However, NADH did not alter the rat’s performance in the rota-rod test.

The mechanisms underlying the actions of NADH in the brain are not known precisely yet. Apparently, two possible mechanisms of action might contribute to the mnemotropic effect of NADH in the old rats.

One possible mechanism of action could include a stim-
ulation of dopamine function [20], since an in vitro study showed that NADH stimulates dopamine synthesis via an increased activity of the tyrosin hydroxylase [31]. It is widely accepted that dopamine neurons play an important role in the regulation of cognitive functions [21,24]. A clinical mi-
crodialysis study demonstrated a sustained activation of the mesolimbic dopaminergic system with an increased release of dopamine during performance of cognitive tasks [12]. More specifically, transgenic mice lacking dopamine-1A recep-
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ter maze without swimming disabilities [28]. The dopamine antagonist haloperidol impair also learning [25] while ap-
plication of the dopamine D1 receptor agonists SKF 38393 and SKF 81297 enhanced performance in memory-impaired aged rats [17].
The difference was not only visible in the hidden-platform version but also in the water maze spatial probe trial, indicating that NADH also improved retention of the learned task. However, we have yet to address the underlying neurobiological mechanisms causing the enhanced cognitive performance of the NADH-treated rats.

References