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Antidepressant-like effect of nicotinamide adenine dinucleotide in the forced swim test in rats

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Abstract

Nicotinamide adenine dinucleotide (NADH), a cosubstrate for energy transfer in the oxidative phosphorylation, has supposedly beneficial effects on central nervous system (CNS)-related diseases, e.g., shown in an open study with depressive patients. To our knowledge there are no data concerning the efficacy of NADH in animal tests.

Acute effects of NADH and the precursor nicotinamide, compared to controls and the antidepressants desipramine and fluoxetine, were examined in the forced swim test (FST) in Wistar rats. NADH, but not nicotinamide, reduced immobility and increased swimming behaviour in the FST, with a minimum effective dose of 5 mg/kg. NADH-induced behavioural profile was similar to fluoxetine, but different from desipramine.

Since NADH did not induce hyperlocomotion but even decreased motor activity in the open field test, the antidepressant-like effect cannot be attributed to an increase in motor activity. These data support an antidepressant potential of NADH.

Keywords: NADH; Antidepressant; Rat; Forced swim test; Behaviour

1. Introduction

Depressive disorders, including major depression, are serious and disabling. It is thought that every fifth individual is suffering by a mood disorder in his or her lifetime. The World Health Organization estimates that major depression is the fourth most important cause worldwide of loss in disability-adjusted life years.

Selective serotonin reuptake inhibitors (SSRIs) have improved safety and tolerability of antidepressant treatment. However, compliance is often hampered by adverse drug effects, mainly during the initial phases of treatment. The antidepressant efficacy of the SSRIs, particularly in severely depressed patients, is not superior to that of tricyclic antidepressants (about 30% of the patients show no improvement) (Anderson and Tomenson, 1994; Burke and Preskorn, 1995). For this reason, there is considerable interest in new therapeutic approaches in the treatment of depression.

Mitochondrial oxidative phosphorylation is the major energy-producing pathway, which supplies more than 95% of the total energy requirement in the cells (Erecinska and Wilson, 1982). In most cells, nicotinamide adenine dinucleotide (NADH), a cosubstrate for energy transfer, is accepted as the main source of reducing equivalents, and NADH is therefore an important factor in cellular metabolism. The vitamin B3 nicotinamide is the main precursor for the synthesis of NADH molecules.

There is some evidence that NADH has positive effects in diseases of the central nervous system (CNS). Clinical studies demonstrated positive effects of peripherally given NADH on serious diseases such as Parkinson's disease (open study) and chronic fatigue syndrome (double-blind study) (Forsyth et al., 1999; Birkmayer et al., 1993). A preliminary clinical open-label study suggested that NADH may also be beneficial in major depression. NADH was found to be superior to placebo and effectively reduced depression symptoms (Birkmayer and Birkmayer, 1991).

To our knowledge there are no data available on the activity of NADH in animal tests for antidepressive activity. Even if a drug shows an effect in man, further behavioural experiments are still advantageous for the following reasons: The animal test seems to be a more objective tool to assess the potential of a drug. More important, the mechanisms underlying the actions of

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NADH in the brain are not yet known precisely and the neurobiological mechanisms of NADH induced effects can be determined in more detail in animals. The use of animals could also lead to the detection of new pharmacological targets for future antidepressive treatment.

Approximately a dozen animal tests for antidepressant agents are commonly used (Cryan et al., 2002). The forced swim test (FST) or ''Porsolt test'' is mostly used. The forced swim test, was described and validated by Porsolt et al. (1977) for use in rats and in mice (Porsolt, 2000). The forced swim test exploits a behavioural approach in which the animal is forced to swim (about 15 min). Following a period of forceful activity at the beginning, the rodent quickly adopts a typically immobile posture, moving only minimally to keep floating (Porsolt et al., 1977). The validity of the forced swim test (Porsolt test) and its relationship to depression have been reviewed extensively (Borsini and Meli, 1988; Willner, 1984) and it subsequently became a screening test for antidepressant agents by pharmaceutical companies. Although different laboratories have made technical modifications in the apparatus, the fundamentals of the test remained the same. Unfortunately, the detection of SSRIs, although clinically effective, is unreliable in the conventional forced swim test, shown by sometimes contradictory results (Lucki, 1997; Cryan et al., 2002). A modification of the forced swim test, based on a more detailed analysis of the behaviour, allows the detection and discrimination of serotonergic and noradrenergic antidepressants (Detke et al., 1995; Lucki, 1997).

In connection with the clinical study suggesting positive effects in major depression, the aim of our study was to determine whether peripherally given NADH might change the behaviour of rats in the modified forced swim test.

2. Material and methods

2.1. Animals

Male Wistar rats (Shoe: Wist, Tierzucht Schönwalde, Germany) of 170–230 g body weight were used. They were group housed, five per cage (45 × 60 × 25 cm), at room temperature (22 ± 2 °C) and with a 12-h light–dark cycle (light on at 0600 h) illuminated with 170 lx. Standard pellet food (Altromin 1326) and water were freely available. To ensure adaptation to the new environment the rats were housed in the departmental animal unit for 2 weeks before testing. The rats were assigned randomly to the treatment groups on arrival. The tests were performed in a soundproof, brightly illuminated room between 1400 and 1700 h.

2.2. Drugs and treatment regimen

The following drugs were used: NADH (1–100 mg/kg, Gerbu, Germany) and the precursor nicotinamide (10–100 mg/kg, Sigma-Aldrich, Germany). The established antidepressants, the SSRI fluoxetine (10, 20 mg/kg) and the noradrenaline reuptake inhibitor desipramine (10, 20 mg/kg) as well as the respective vehicle-treated controls ($NaHCO_3$ buffer for NADH and nicotinamide and saline for the antidepressants) are included to validate the experimental procedures.

All animals were treated with verum or vehicle three times: shortly after the habituation session (23 h) and 5 and 1 h prior to testing. All drugs were administered intraperitoneally with an injection volume of 1 ml/kg.

2.3. Apparatus and experimental protocol

The animals were tested in a glass tank (23 × 30 cm, height 40 cm) filled to a depth of 28 cm with water at 22 °C (the animals could not touch the bottom). The glass tank was illuminated indirectly and was surrounded by dark brown shading walls (distance from the tank 20 cm) to screen the view from the experimenter. The experiments were performed between 1400 and 1700 h and the method was in general performed as described (Porsolt et al., 1979; Lucki, 1997).

On the first experimental day, rats were gently placed in the water for a 15-min period of habituation. On removal from the water, they were placed in a standard Plexiglas box with the floor covered with paper towels, under an infrared heater for 30 min to dry. The next day, they were once more placed gently in the glass tank and observed for 5 min. The behaviour of the animals was videotaped.

At the end of the 5-min period, the rats were transferred to the infrared heated box and allowed to dry.

Following the experiment the videotapes were analysed manually and the duration of the following behaviours was recorded: immobility: floating and making only those movements necessary to keep the nose above the water; swimming: when an animal exhibits active motions, i.e., moving around the tank including diving; climbing: when rats strongly move their forepaws in and out of the water, usually against the walls.

2.4. Open field test

To detect a probable influence of the drugs on locomotor activity, we studied the effect of NADH, nicotinamide, desipramine and fluoxetine in the open field test. The experiments were performed in a soundproof and moderately illuminated (≈ 50 lx) cubic observation chamber (2 × 2 × 2 m) between 1400 and 1700 h, using a white wooden open field (100 × 100 cm, walls 40 cm high). The naïve animals received their drugs 23, 5 and 1 h prior to testing and were not habituated to the open field. The animals were placed in the centre of the open field for 5 min and the distance travelled was assessed by a video tracking software (CPL Systems, U.K.).

2.5. Statistics

Data are presented as means ± S.E.M. and the group size was 10 to 12 rats. Comparisons between groups were carried out with a one-way ANOVA followed by intergroup comparisons using the Holm–Sidak method. Results were considered as significant for values of $P < .05$. All statistical procedures were carried out using SigmaStat version 3.0.

3. Results

NADH (10 and 50 mg/kg) decreased the time spent immobile during the Forced swim test ($F = 3.278$, $df = 5$, 64; $P = .008$) and increased the duration of swimming in doses of 5–100 mg/kg ip ($F = 5.229$, $df = 5$, 64; $P < .001$), while the climbing behaviour was not changed (Fig. 1).

The established antidepressants, desipramine and fluoxetine induced overall modifications of immobility time [$F(4,45) = 12.89$, $P < .001$] and time spent climbing [$F(4,45) = 13,23$, $P < .001$]. Pairwise comparisons to control performance indicate that desipramine significantly reduced the time spent immobile and increased the climbing time at 10 and 20 mg/kg, and increased the time spent swimming at the 20 mg/kg dose. Fluoxetine reduced the time spent immobile and increased the swimming time at 10 and 20 mg/kg and decreased the time spent climbing at the 20 mg/kg dose (Fig. 2).

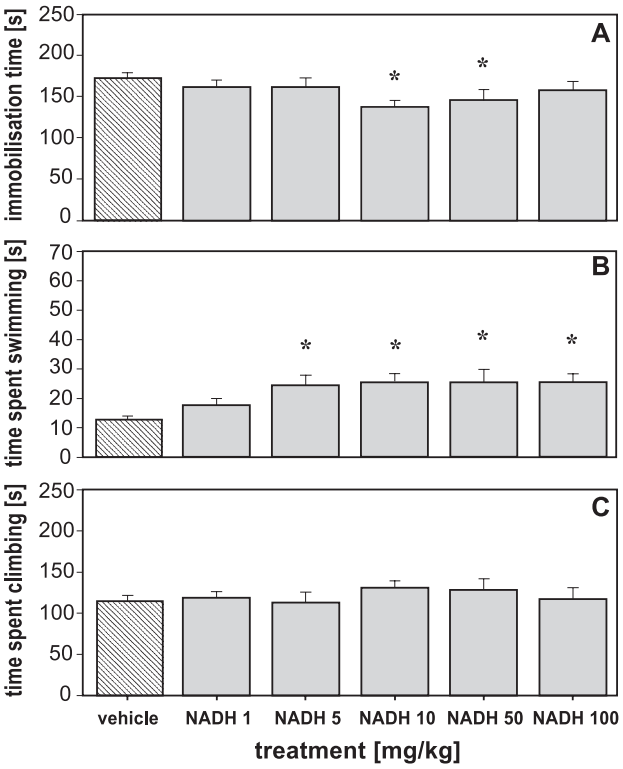

Fig. 1. Effects of NADH (1.0–100 mg/kg) on the time spent immobile (A), the duration of swimming (B) and the time span the animals tried to climb out (C) in the rat forced swim test compared to vehicle-treated controls. * $P < .05$, one-way ANOVA followed by the Holm–Sidak method. Data are presented as mean ± S.E.M. ($n = 10–12$).

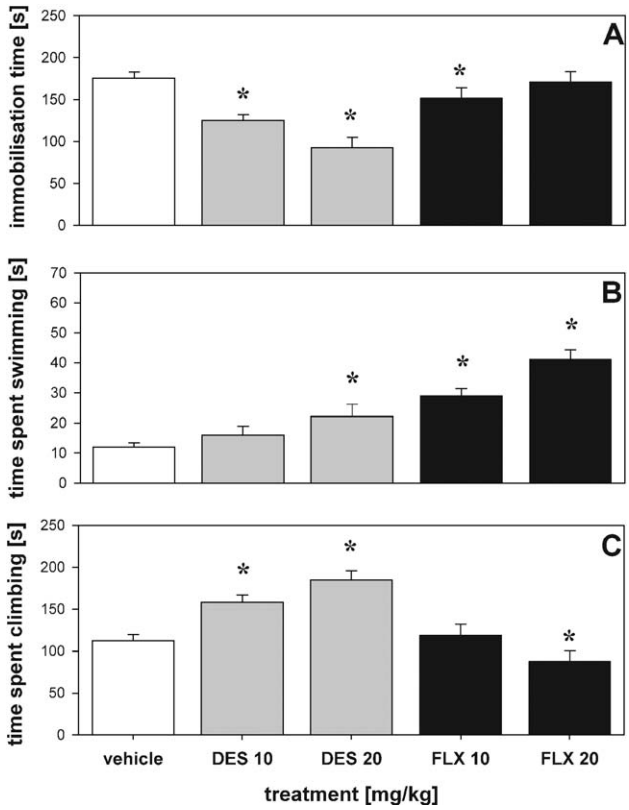

Fig. 2. Effects of desipramine (DES, 10 + 20 mg/kg) and fluoxetine (FLX, 10 + 20 mg/kg) on the time spent immobile (A), the duration of swimming (B) and the time span the animals tried to climb out (C) in the rat forced swim test compared to vehicle-treated controls. * $P < .05$, one-way ANOVA followed by the Holm–Sidak method. Data are presented as mean ± S.E.M. ($n = 10–12$).

Nicotinamide, the precursor of NADH (10–100 mg/kg) failed to modify the immobility and the climbing behaviour

Table 1
Effects of nicotinamide (10–100 mg/kg) on the time spent immobile, the duration of swimming and the time span the animals tried to climb out in the rat forced swim test and on the distance travelled in the open field compared to vehicle-treated controls

Parameter	Treatment			
	Vehicle	Nicotinamide, 10 mg/kg	Nicotinamide, 50 mg/kg	Nicotinamide, 100 mg/kg
Immobilisation time (s)	175.4 ± 6.9	166.4 ± 39.0	177.6 ± 13.9	188.9 ± 11.2
Time spent swimming (s)	12.7 ± 1.3	18.6 ± 1.9 *	16.3 ± 1.4	15.7 ± 1.0
Time spent climbing (s)	112.5 ± 6.9	113.0 ± 13.6	106.2 ± 13.6	94.8 ± 11.4
Distance travelled in the open field (m)	16.9 ± 1.7	15.4 ± 1.8	14.4 ± 1.9	16.4 ± 1.6

One-way ANOVA followed by the Holm–Sidak method. Data are presented as mean ± S.E.M. ($n = 10–12$).
* $P < .05$.

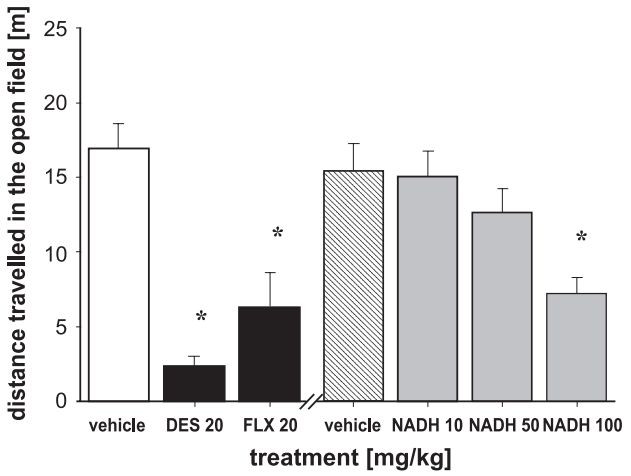

Fig. 3. Impact of the drugs, found effective in the forced swim test, on the distance travelled in the open field compared to the respective vehicle-treated controls: $NaHCO_3$ buffer [striped bar] for NADH or saline [white bar] for desipramine (DES) and fluoxetine (FLX). $*P<.05$, one-way ANOVA followed by the Holm–Sidak method. Data are presented as mean ± S.E.M. ($n=10-12$).

in the forced swim test, while the duration of swimming was increased slightly at a dose of 10 mg/kg ip ($F=4.573$, $df=3, 46$, $P=.007$) (Table 1).

In the open field test, desipramine (20 mg/kg), fluoxetine (20 mg/kg) and NADH (100 mg/kg) caused a decrease in the distance travelled compared to the controls ($F=9.523$, $df=8, 91$, $P<.001$). No significant differences were found between animals treated with other doses of NADH or nicotinamide and the control group (Fig. 3, Table 1).

4. Discussion

There are a few animal tests for the investigation of "antidepressant" effects of substances used (Cryan et al., 2002; Willner, 1990). Among them, the forced swim test is a well-established animal test, causing a status of behavioural despair by comprehensible stimuli and is the most widely used model to assess antidepressant effects in small rodents (Willner, 1984; Porsolt et al., 1979). Most of the clinically active antidepressants are active in the forced swim test and neuroleptics and anxiolytics are discriminated (Porsolt et al., 1979). False-positives for the forced swim test as stimulants can be eliminated by their effects on locomotion (Borsini and Meli, 1988).

In the conventional version of the animal model, an antidepressant effect is evaluated by a decrease in immobility during exposure to the unescapable water tank. Clinically effective SSRIs (e.g., fluoxetine) induced quite different effects in the classical version of the forced swim test. For example, some authors found an antidepressant-like activity, while others could not (Lucki, 1997). A possible reason could be the use of different rat strains (Lopez-Rubalcava and Lucki, 2000). Further behavioural registration of the time spent swimming in the tank or climbing/trashing (attempted vertical movement) allows the detection of the SSRIs and the discrimination between drugs affecting primarily the serotonergic or noradrenergic neurotransmitter systems (Lucki, 1997; Page et al., 1999; Reneric, 2001). While drugs stimulating the serotonergic system, as SSRIs, preferentially stimulate active swimming in the water tank, drugs primarily blocking noradrenaline uptake preferentially increase climbing behaviour.

In our study, NADH induced a modest antidepressant-like effect, decreasing the time spent immobile floating in the forced swim test. The NADH precursor nicotinamide showed no antidepressant-like effects in the test, since the nicotinamide-treated rats did not differ from the vehicle-treated rats in the time spent immobile in the tank.

Since NADH did not induce a hyperlocomotion but even decreased motor activity in the open field test, the antidepressant-like effect cannot be attributed to an increase in motor activity.

Based on the hypothesis that mitochondrial dysfunction might serve as a component of the pathophysiological processes in diseases of the CNS (Krieger and Duchen, 2002), the potential therapeutic use of NADH has been evaluated for approximately 10 years. For example, studies showing that NADH stimulates dopamine biosynthesis in vitro have led to its experimental use in Parkinson's disease (Vrecko et al., 1997). Some favourable results have been reported in case studies and open-label trials using both intravenous and oral NADH (Birkmayer et al., 1993; Kuhn et al., 1996).

While some clinical studies showed an effect of NADH in CNS-related diseases, data on the cerebral bioavailability of NADH are rare (Rainer et al., 2000). In a study preceding the behavioural experiments, it could be shown that NADH given peripherally increased the central concentration of NADH (Rex et al., 2002). Based on these results, it is conceivable that the administration of exogenous NADH may have any direct or indirect effect on the brain.

The neurobiological mechanisms by which NADH induces the antidepressant-like effects in the forced swim test are unknown. An in vitro study could show that NADH is able to increase the activity of the tyrosine hydroxylase, the first and major rate-limiting enzyme in catecholamine biosynthesis (Vrecko et al., 1993).

To obtain some rough information about the mechanism behind NADH's antidepressant-like effects, the behaviour during the forced swim test was analysed in the more complex method allowing the differentiation between various classes of antidepressant agents (Lucki, 1997; Page et al., 1999). Consistent with previous studies in which behavioural sampling was used (Detke et al., 1995; Lucki, 1997; Lopez-Rubalcava and Lucki, 2000), the standard norepinephrine uptake inhibitor desipramine preferentially reduced the duration of immobility and increased climbing behaviour and lengthened the swimming time only in the

high dose. The SSRI fluoxetine boosted swimming behaviour without increasing the climbing. Because of the observation that the effects of NADH on the measured parameters in the forced swim test are similar to those of fluoxetine (increased swimming) instead of those of desipramine (reduced immobility, enhanced climbing) it is imaginable that the action of NADH may involve the serotonergic system. Decreased serotonergic neurotransmission has been proposed to play an important role in the aetiology of depression. This has been established mainly by the clinical efficacy of the third-generation antidepressants, the SSRIs, which enhance serotonergic transmission (Beique et al., 2000; Blier, 2001).

Our results are supported by a case report showing associations between depression and mitochondrial dysfunction. Significant decreases in mitochondrial ATP production were found in a depressive patient compared to healthy controls (Gardner et al., 2003).

In summary, despite the limitations of the animal model used, our results suggest antidepressant properties of NADH. For the determination of the putative neurobiological mechanisms of NADH further pharmacological studies modulating serotonergic function and in vivo microdialysis experiments are favourable.

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