Original article

Oral reduced B-nicotinamide adenine dinucleotide (NADH) affects blood pressure, lipid peroxidation, and lipid profile in hypertensive rats (SHR)

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Abstract

A gradual increase in blood pressure (BP), often attaining hypertensive levels, is common during aging – "age-related hypertension." Therefore, means to prevent or ameliorate this elevated BP safely are important. Although oral B-nicotinamide adenine dinucleotide (NADH), a natural coenzyme, is used principally to treat various neurologic disorders, we wished to investigate whether this agent had the same potential to lower BP and benefit the cardiovascular system as does coenzyme Q_{10}, a similar-type agent. As a first approximation, spontaneously hypertensive rats (SHR) were used to determine effects of oral NADH. In a blinded, placebo-controlled study, ten rats received placebo; and ten, NADH for ten weeks. Systolic BP was measured by tail plethysmography. Blood was collected terminally, and chemistries were performed by routine methodologies. Thiobarbituric acid reactive species (TBARS) (an estimate of lipid peroxidation / free radical formation) was measured in renal and hepatic tissues. The following was noted: water and food intake were comparable, and the steady weight gain of young SHR were similar in the placebo and NADH groups. Although systolic BP did not differ between the two groups over the first month, it decreased and stayed markedly lower for the remainder of study in SHR receiving oral NADH. At the end of 60 days, SBP in NADH-treated SHR was 184 mm Hg $\pm$ 2.8 (SEM) compared to 201 mm Hg $\pm$ 2.1 (SEM) in control SHR (p < 0.001). No significant differences were seen in blood levels of glucose, insulin, triglyceride, and HDL levels but NADH intake lowered total cholesterol (p < 0.002) and LDL (p < 0.02). Renal TBARS were also significantly lower in SHR receiving NADH (P < 0.001). Accordingly, supplementation with the natural coenzyme NADH theoretically could prove to be useful in preventing age-related increases in BP and, thus, various cardiovascular maladies.

Introduction

B-nicotinamide adenine dinucleotide (NADH), a naturally occurring coenzyme, plays a key role in energy production of cells [1] and affects metabolism of neurotransmitters [2]. In studies performed in Europe, supplemental NADH given orally has shown promise in alleviating the symptoms of depression [3], Alzheimer's Disease [4], and Parkinson's Disease [5]. Since this agent has antioxidant potential

and some antioxidants are known to decrease blood pressure (BP) significantly [6], we hypothesized that NADH might influence BP. For example, coenzyme Q_{10}, another natural factor in the electron transport chain known to have antioxidant properties, has been shown to have a significant antihypertensive effect [7]. Accordingly, our main purpose was to establish whether BP-lowering effects from supplemental NADH exist and to discern potential mechanisms behind any lowering of BP in spontaneously hyper-
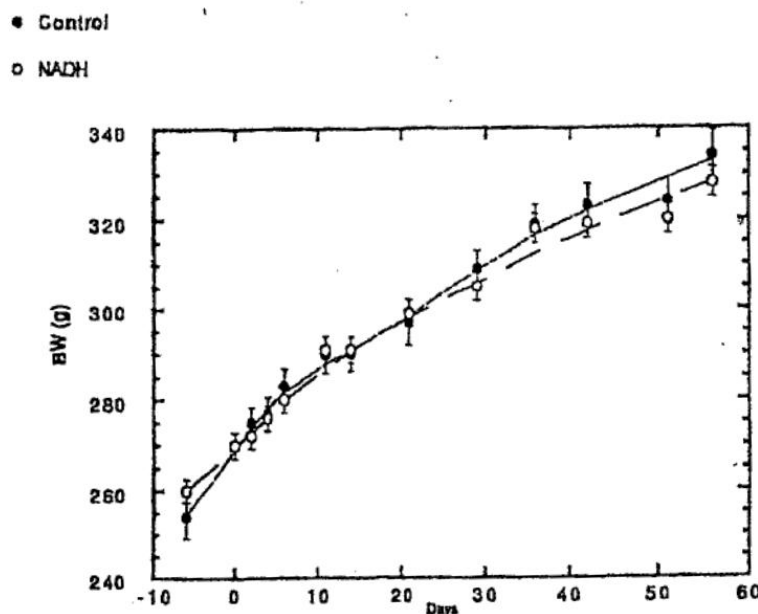
Figure 1. Body weights of the placebo (closed circles) and NADH (open circles) groups of SHR over the course of study.

tensive rats (SHR). In addition, we examined NADH effects on other cardiovascular risk factors.

Material and methods

Male SHR of the Okamoto strain [8], weighing approximately 250 g, were purchased from Taconic Farms, Germantown, NY. Their diet, obtained from Teklad, Inc., Madison, WI, derived 52% of calories from sucrose [9]. Minerals and vitamins were added at AIN levels. A blinded, placebo-controlled study was designed where only the supplier and study sponsor (Labor Birkmayer, Vienna, Austria) knew which pills were placebo and NADH. Ten rats received placebo; and ten NADH for ten weeks. Identically appearing pills containing 5 mg NADH (verum) or inactive filler (placebo), given as a single tablet daily, were provided by the study sponsor. These pills were administered with a special dispenser that allowed the technician to place the pills deep in the throat. Enteric-coated pills were used in order to avoid breakdown of coenzyme in stomach acid. The code pertaining to identification of placebo and active supplement was broken after completion of the study when all samples had been analyzed.

Systolic BP was estimated by tail plethysmography in unanesthetized rats after a five minute warming period [10, 11]. Readings were taken 30–60 seconds apart. To be accepted, SBP measurements had to be virtually stable for three consecutive readings. Measurements were made two times per week.

Blood was obtained at the end of the experiment (two months of study) after removal of the food for 4 hours. Following blood drawing, rats were sacrificed by inhalation of CO_2. Chemical analyses were performed by routine clinical procedures. Glucose was determined by the glucose oxidase method [12]. Immunoreactive insulin was determined by radioimmunoassay [13] and glycosylated hemoglobin (HBA1C) by routine clinical analysis, i.e., column chromatography.

Malondialdehyde (MDA) formed from the breakdown of polyunsaturated fatty acids serves as a convenient index for determining extent of lipid peroxidation [14]. Lipid peroxidation products were quantified by their reaction with thiobarbituric acid [15]. A 1.0 mL aliquot of hepatic and renal homogenates, precipitated with 0.15 mL of 76% trichloroacetic acid (TCA), was added to 0.35 mL of 1.07% thiobarbituric acid and incubated at 80°C for 30 min. A 0.5 mL volume of cold 90% TCA was added, and the absorbance read at 532 nm. 1,1,3,3 tetramethoxypropane served as the standard. Formation of thiobarbituric acid reacting species (TBARS) estimated the amount of lipid peroxidation products.

Table 1. Blood chemistries

Parameter	Placebo	NADH	p
Insulin (ulU/mL)	60.0 ± 38.5	45.3 ± 30.8	<0.4
Glucose	199.3 ± 33.8	221.0 ± 32.1	NS
Cholesterol	146.4 ± 6.8	113.3 ± 6.4	<0.002
HDL	36.5 ± 1.1	35.6 ± 1.4	NS
LDL	54.3 ± 3.2	32.1 ± 8.2	<0.02
Triglycerides	277.6 ± 30.3	227.6 ± 28.8	<0.3

Statistical differences were analyzed by the unpaired Student's t test comparing the placebo and verum situations. Statistical significance was set at $p < 0.05$.

Results

Body Weight (Figure 1). Over 12 weeks, there was a steady gain of body weight which did not differ significantly between placebo and NADH groups. Although not shown here, water and food intake were comparable when measured.

Systolic BP (Figure 2). A decided difference in response of systolic BP between groups was evident by the end of study. At week 4, systolic BP was similar between the control and NADH groups, i.e., 198 mm Hg $\pm$ 2.2 (SEM) in control compared to 198 mm Hg $\pm$ 2.0 (SEM) in SHR receiving NADH. For the remainder of the study, systolic BP was consistently lower in the NADH group. At the end of 60 days, systolic BP in SHR receiving oral NADH was 184 mm Hg $\pm$ 2.8 (SEM) compared to 201 mm Hg $\pm$ 2.1 (SEM) in control SHR ($p < 0.001$).

Blood Chemistries (Table 1). Significant differences were not seen in circulating glucose and HDL levels. However, NADH significantly lowered cholesterol [146 $\pm$ 7 (SEM) vs. 113 $\pm$ 6 (SEM) mg/dL ($p < 0.002$)] and LDL [54.3 $\pm$ 3.2 (SEM) vs. 32.1 $\pm$ 8.2 (SEM) mg/dL ($p < 0.02$)]. Although mean triglyceride and insulin levels were lower in SHR receiving NADH, these differences were not significant (278 $\pm$ 30 (SEM) vs. 228 $\pm$ 29 (SEM) mg/dL ($p < 0.3$) for triglycerides and 60.0 $\pm$ 38.5 vs. 45.3 $\pm$ 30.8 uLU/mL ($p < 0.4$) for insulin.

TBARS (Figure 3). Renal TBARS were significantly lower in NADH SHR [3.5 $\pm$ 0.2 (SEM) vs. 1.9 $\pm$ 0.2 (SEM) nmol/MDA/100mg, ($P < 0.001$)], but no significant changes were apparent in liver TBARS.

Discussion

Coenzyme Q_{10} given orally has many beneficial effects on the cardiovascular system and is a powerful antioxidant [7]. Recently, another agent similar to coenzyme Q_{10} in many respects, i.e. B-nicotinamide adenine dinucleotide (NADH) given orally, has been used extensively to treat various neurological diseases [3–5]. It had not been established whether NADH like coenzyme Q_{10}, both natural constituents of the electron transport system, provides similar beneficial effects on the cardiovascular system, particularly the ability to lower elevated BP.

In this blinded study, systolic BP of SHR decreased significantly after 30 days in rats ingesting NADH pills without obvious evidence of toxicity. In addition, NADH was shown to influence the lipid profile (lower total cholesterol and LDL), and affect lipid peroxidation in renal tissue. The latter was determined by measuring TBARS formation in renal tissue. It is not clear why changes in TBARS formation were not seen in hepatic tissue. This may relate to the known different biochemical and physiological effects among antioxidants. Nevertheless, finding decreased renal TBARS verifies that supplemental NADH has antioxidant properties. Thus, we found that oral NADH had effects similar to coenzyme Q_{10}, i.e., the ability to lower BP and act as an antioxidant. The common antioxidant properties would seem to be the most logical explanation for the similar "pressure effects" of NADH and coenzyme Q_{10} – two substances with dissimilar structures.

Many antioxidants have been shown to possess a BP lowering effect. [16–20]. Vitamin C beneficially affects BP, and lowers fasting plasma free radicals, insulin concentrations, total cholesterol, and LDL-cholesterol, and triglycerides [21–24] – trends similar to those seen in the present study. Deficiency of selenium, another antioxidant, may be a secondary factor in causation of hypertension. In the Kuopio Ischaemic Heart Disease Risk Factor Study, plasma selenium concentrations had a moderate, independent adverse association with mean resting BP in 722 Eastern Finnish men [25]. In a recent double-blind, placebo-controlled, crossover study of 21 hyperten-
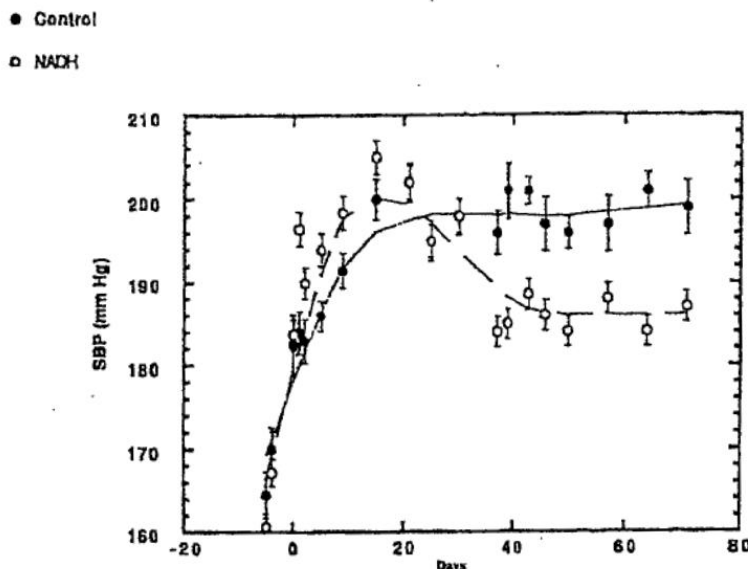
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Figure 2. Systolic blood pressures of the placebo (closed circles) and NADH (open circles) groups of SHR over the course of study.

sive patients, a combination of antioxidants (ZnSO₄-200 mg, vitamin C-500 mg, α tocopherol-600 mg, B carotene-30 mg) was given [26]. The hypertensive patients were allowed to stay on their same antihypertensive medications. Although virtually no change in average SBP from baseline was seen following placebo (168.7 mm Hg), a significant decrease was seen in hypertensive patients receiving the combination of antioxidants (159.7 mm Hg) (p < 0.010). No significant changes in diastolic BP were seen. Accordingly, the antioxidant properties of NADH, corroborated here by a lowering of TBARS formation in renal tissue, may explain, at least in part, the ability of NADH to decrease the elevated SBP of SHR.

How could antioxidants lower BP? There are at least three good hypotheses. First, the nitric oxide (NO) system may be involved [27–31], since an imbalance in NO contributes to the development of arterial hypertension [32]. Oxygen-derived free radicals are responsible for a faster degradation of nitric oxide. Accordingly, the antihypertensive effects of antioxidants may occur through protection of NO, a powerful vasodilator [33]. Second, some antioxidants favorably influence the insulin system, and perturbations in the insulin system are associated with elevated BP and lipid disturbances [34–38]. That vitamin C lowers BP may be due to its ability to overcome some manifestations of diabetes [21, 39]. Although insulin concentrations tended to be lower in SHR receiv-

ing NADH supplementation in the present study, the results did not reach significance possibly due to the small number of rats studied. A perturbed insulin system also affects the nitric oxide pathway [40]. Finally, some flavonoids have been shown to be angiotensin converting enzyme (ACE) inhibitors [41, 42]. None of these potential mechanisms related to antioxidants is mutually exclusive.

Although the mechanisms behind the blood pressure and lipid effects of oral NADH are not clear, these studies lead to interesting speculations concerning certain clinical entities. The physician should be aware of the potential BP-lowering effects of NADH when treating patients with depression [3] and Alzheimer's [4] or Parkinson's [5] Diseases, especially those with age-related hypertension. NADH could prove to be a useful agent to lower BP and improve the lipid profile in older patients undergoing therapy for these neurologic disorders.

From the present studies, we conclude that:

1. Supplemental NADH has a significant SBP lowering effect in SHR. This is the first study to definitively show this.

2. Supplemental NADH has antioxidant properties, at least in renal tissue. These antioxidant properties may play a role in lowering BP and favorably influencing the lipid profile.

3. Oral NADH lowers serum cholesterol and LDL.
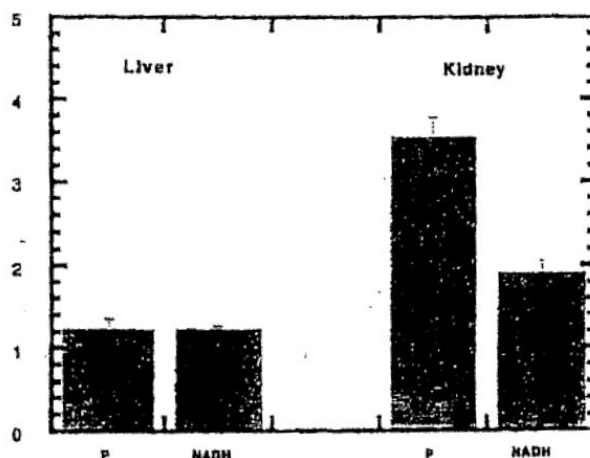
TBARS (mmol MDA/ 100 mg tissue)

Figure 3. Depiction of TBARS formation expressed as nmol malonyldialdehyde equivalents per 100 mg of tissue. Hepatic and renal tissue were assayed. Mean ± SEM of 10 SHR is shown for each group. Differences in kidney tissue were statistically significant.

4. Since NADH is a natural compound with no known toxic effects, it may turn out to be a useful agent to prevent and treat cardiovascular risk factors common in aging.

Acknowledgment

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