Antioxidant and Antiaging Activity of *N*-Acetylserotonin and Melatonin in the *in Vivo* Models

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ABSTRACT: It is generally accepted that antioxidant properties of melatonin significantly contribute to its antiaging effect. Antioxidant effects of *N*-acetylserotonin (NAS), a melatonin precursor and metabolite, might predict its antiaging action as well. The antiaging effect of NAS was studied in female retired breeders and male C3H mice. Both NAS and melatonin administered with drinking water prolonged life span in male animals by about 20% versus control animals ($p < 0.01$) but did not affect the life span of female mice. Antioxidative activity was evaluated by determining the malonaldehyde + 4-hydroxynonenal (MDA + 4-HNE) and cellular glutathion peroxidase (GPx) levels in male, 11-month-old, C57Bl/6J mice with very limited (if any) capacity to convert pineal NAS into melatonin. NAS increased the antioxidant capacity of kidney. Both NAS and melatonin (four weeks daily i.p. injections) increased the antioxidant capacity of brain as demonstrated by decreased MDA + 4-HNE and increased GPx levels. NAS-treated C57Bl/6J mice experienced a weight loss of 9%, whereas the saline and melatonin groups only 3%. NAS- and melatonin-treated animals had healthy and luxuriant fur coats with some gray fur in the melatonin group; animals in the saline group had large areas of baldness. This study demonstrates, for the first time, the antiaging effect of NAS. This effect needs to be confirmed in animals with impaired capacity to convert NAS into melatonin.

KEYWORDS: Antioxidant activity; Antiaging activity; *N*-acetylserotonin; Melatonin.

INTRODUCTION

According to the free radical theory of aging, reactive oxygen species initiate degradative processes that contribute to the development of aging.[1] The increased vulnerability of aging organisms to oxidative stress implies that antioxidants might exert antiaging effects. In this vein, the antioxidative properties of melatonin suggest that it might exhibit an antiaging effect.[2] Indeed, melatonin prolongs the life span of rodents.[3] However, the antiaging effect of melatonin was found to vary with strain,

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gender, age at the initiation of melatonin administration, and other factors. Thus, melatonin increases the life span of female BALB and C57Bl mice of both genders, whereas it shortens the life span of female C3H mice.[4] We are not aware of any studies assessing the melatonin effect on the life span of male C3H mice. Therefore, the first aim of this study was to compare the effects of melatonin on the life span of female and male C3H mice.

Apart from melatonin, several related pineal constituents also poses antioxidant properties.[5–8] One of them is *N*-acetylserotonin (NAS), the melatonin precursor and metabolite.[9,10] Although the antioxidant capacities of NAS suggest that it may show antiaging effects, there are no reports evaluating NAS effect on longevity. Thus, the second aim of the study reported here was to evaluate NAS effect on the life span of male and female C3H mice.

The antioxidant effects of melatonin and, especially, NAS were tested either against prooxidant agents or in *in vitro* models. The third aim of this study was to evaluate the antioxidant effects of melatonin and NAS in the *in vivo* models under naturally occurring prooxidant conditions, that is, aged mice.

METHODS AND PROCEDURE

Experiment #1: Effects of Melatonin and NAS on the Life Span of C3H Mice

Mice of C3H strain were purchased from Harlan Sprague-Dawley, Inc. (Indianapolis, IN). Male mice were four-weeks of age, female mice were retired breeders (approximately eight-months old). Animals were housed five per cage under a 12/12 light/dark cycle with lights on/off at 0600/1800. NAS and melatonin were administered with drinking water at 2.5 mg/kg/day. Drinking solutions were prepared from melatonin and NAS stock solutions of 10 mg/ml in 1% Tween-20. Melatonin and NAS content in drinking water were adjusted according to body weight and amount of water intake. Control animals were given the solvent solution. Each group consisted of 20 animals.

Experiment #2: Effects of Melatonin and NAS on the Antioxidant Capacities of Aged C57Bl/6J Mice

To differentiate between the effects of melatonin and NAS, we used mice of C57Bl/6J strain, since this strain does not have enzymes for pineal melatonin biosynthesis from serotonin.[11,12] Therefore, the effects of NAS in C57Bl/6J mice could be studied without interference from melatonin converted from NAS and/or from the endogenously produced NAS. We used 11-month old mice since, having passed the midpoint of their life span, these animals were at a stage that is known to show age-associated decline in their antioxidant capacities.[13]

Male C57Bl/6J mice (Harlan Sprague-Dawley, Inc., Indianapolis, IN) were housed five per cage under 12/12 light/dark cycle with lights on/off at 0600/1800. NAS (20 mg/kg) and melatonin (1 mg/kg) were dissolved in 1% Tween-20 saline solution and administered daily, i.p., at 1200H for four weeks. Control animals were injected with the solvent solution. Each group consisted of eight animals.

Antioxidant activity was evaluated by measuring the malonaldehyde and 4-hydroxynonenal (MDA + 4-HNE) levels in tissues, expressed in micromol/mg protein, and from the cellular glutathion peroxidase (GPx) level expressed as mU/mg

protein, using the Bioxytech LPO-586 kit and Biotech GPX-340 kit, respectively, (Oxis International, Portland, OR). Protein was determined by the Lowry method (Sigma Chem. Co., St. Louis, MO). Twenty-four hours after the last injection, animals were decapitated; brains, kidneys and livers were immediately removed, frozen in dry ice, and stored at −70°C until assayed. In addition, changes in body weight and quality of fur were noted as supplemental outcome measures. The results were obtained as mean values ± SEM and analyzed using one-way ANOVA and the Student's *t*-test. The level of significance was $p < 0.05$.

RESULTS

Experiment #1

Neither NAS nor melatonin affected the life span of female C3H mice (see FIGURE 1). However, melatonin did not cause premature death as is described elsewhere.[4] On the other hand, both NAS and melatonin prolonged the lifespan in male C3H mice by approximately 20% when compared to control animals ($p < 0.01$) (see FIGURES 2 and 3).

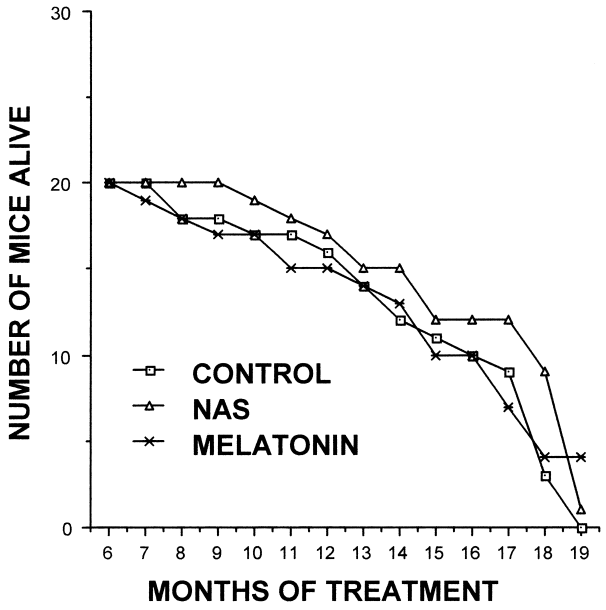

FIGURE 1. Effect of *N*-acetylserotonin and melatonin on life span in female C3H mice. NAS and melatonin were administered with drinking water at 2.5 mg/kg/day to male (starting at four weeks of age) and female (starting at about eight months of age) mice. Control animals were given the solvent solution. Each group consisted of 20 animals.

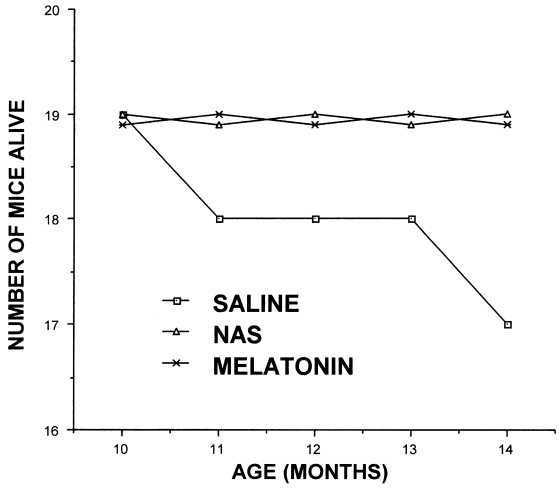

FIGURE 2. Effect of *N*-acetylserotonin and melatonin on life span in male C3H mice. NAS and melatonin were administered with drinking water at 2.5 mg/kg/day to male mice (starting at four weeks of age). Control animals were given the solvent solution. Each group consisted of 20 animals.

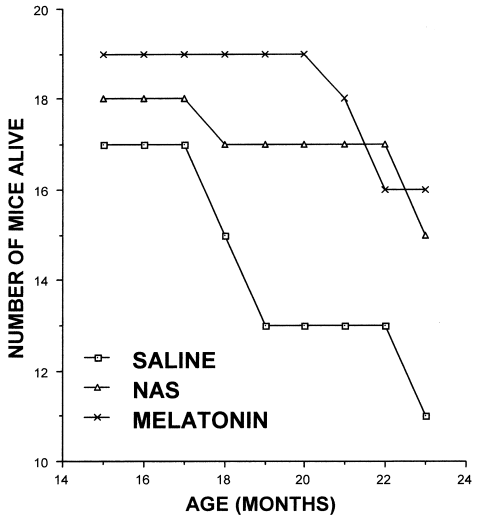

FIGURE 3. Effect of *N*-acetylserotonin and melatonin on life span in male C3H mice. NAS and melatonin were administered with drinking water at 2.5 mg/kg/day to male mice (starting at four weeks of age). Control animals were given the solvent solution. Each group consisted of 20 animals.

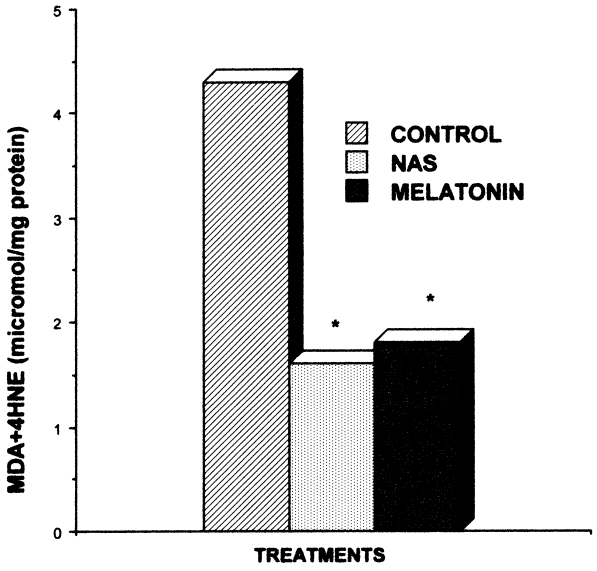

FIGURE 4. Effect of *N*-acetylserotonin and melatonin on malonaldehyde and 4-hydroxynonenal (MDA + 4-HNE) levels in C57BL/6J mouse brain tissue. NAS (20 mg/kg) and melatonin (1 mg/kg) were administered daily, i.p., at 1200 h for four weeks. Control animals were injected with the solvent solution. Each group consisted of eight animals, $*p < 0.01$ (ANOVA and Student's *t*-test).

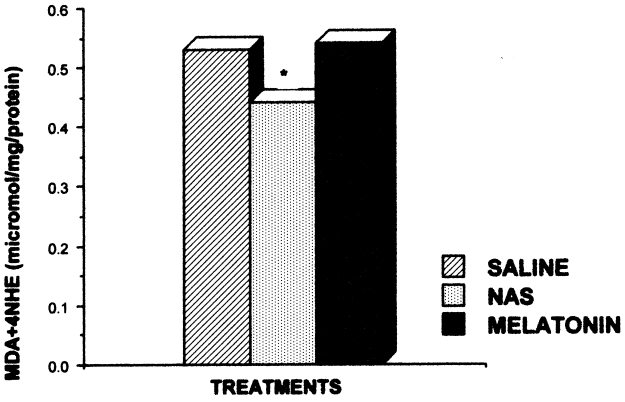

FIGURE 5. Effect of *N*-acetylserotonin and melatonin on malonaldehyde and 4-hydroxynonenal (MDA + 4-HNE) levels in C57BL/6J male mouse kidney. NAS (20 mg/kg) and melatonin (1 mg/kg) were administered daily, i.p., at 1200 h for four weeks starting at 11 months of age. Control animals were injected with the solvent solution. Each group consisted of eight animals, $*p < 0.03$ (ANOVA and Student's *t*-test).

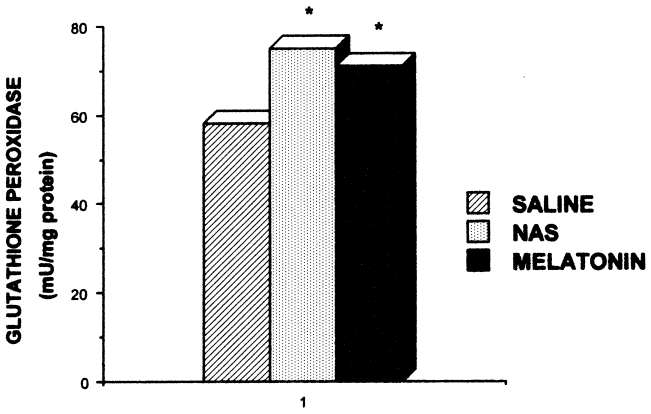

FIGURE 6. Effect of *N*-acetylserotonin and melatonin on cellular glutathion peroxidase levels in brain tissues of C57BL/6J male mice. NAS (20 mg/kg) and melatonin (1 mg/kg) were administered daily, i.p., at 1200 h for four weeks starting at 11 months of age. Control animals were injected with the solvent solution. Each group consisted of eight animals, $*p < 0.01$ (ANOVA and Student's *t*-test).

Experiment #2

There was an almost three-fold reduction in the MDA + 4-HNE formation in the brains of both NAS and melatonin treated mice, in comparison with the control group ($p < 0.01$), showing increased antioxidant capacity in the brain of NAS and melatonin treated animals (see FIGURE 4). Only the NAS treated animals showed

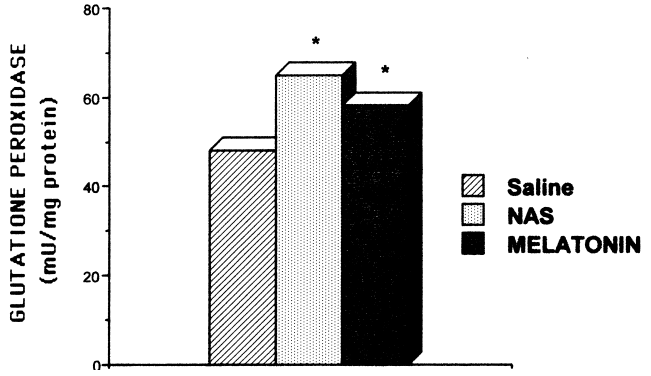

FIGURE 7. Effect of *N*-acetylserotonin and melatonin on cellular glutathion peroxidase levels in kidney tissues of vC57BL/6J male mice. NAS (20 mg/kg) and melatonin (1 mg/kg) were administered daily, i.p., at 1200 H for four weeks starting at 11 months of age. Control animals were injected with the solvent solution. Each group consisted of eight animals, $*p < 0.01$ (ANOVA and Student's *t*-test).

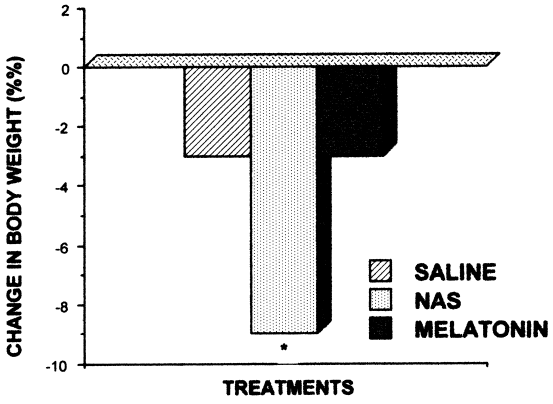

FIGURE 8. Effect of *N*-acetylserotonin and melatonin on body weight of C57BL/6J male mice. NAS (20 mg/kg) and melatonin (1 mg/kg) were administered daily, i.p., at 1200 h for four weeks starting at 11 months of age. Control animals were injected with the solvent solution. Data show the difference between body weights at the beginning and the end of a four-week drug administration period. Each group consisted of eight animals, $*p < 0.03$ (ANOVA and Student's *t*-test).

decrease in the MDA + 4HNE levels in the kidneys ($p < 0.03$) (see FIGURE 5). NAS and melatonin treated mice showed increased levels of cellular GRx in both brains and kidneys ($p < 0.01$) (see FIGURES 6 and 7). No difference between the groups studied was found in the liver (data not shown).

The NAS-treated animals revealed a weight loss of 9% ($p < 0.01$) from their respective baselines whereas the control and melatonin-treated groups had weight loss of only 3% (non-significant) (see FIGURE 8). Additional outcome measures noted: NAS-treated animals had the healthiest and most luxuriant fur coat among the groups; the animals in the control group had large areas of baldness; and, although the melatonin-treated animals did not have areas of baldness, the fur had more graying compared to the NAS-treated animals (see FIGURE 9).

DISCUSSION

The study we report shows, for the first time the antiaging effect of melatonin in male C3H mice, confirming previous similar data on BALB and C57Bl/6J strains.[3,4] The lack of the antiaging effect of melatonin (and NAS) in the female C3H mice may suggest gender differences in response to antiaging medication. However, the earlier commencement of the administration of pineal hormones in male (at one month of age) than in female (about eight months of age) mice might be also accountable for the difference.

The present study is the first demonstration of the antiaging effect of NAS. Considering that NAS is a biosynthetic precursor of melatonin, one might suggest that the antiaging effect of NAS depends on its conversion to melatonin. The pineals of

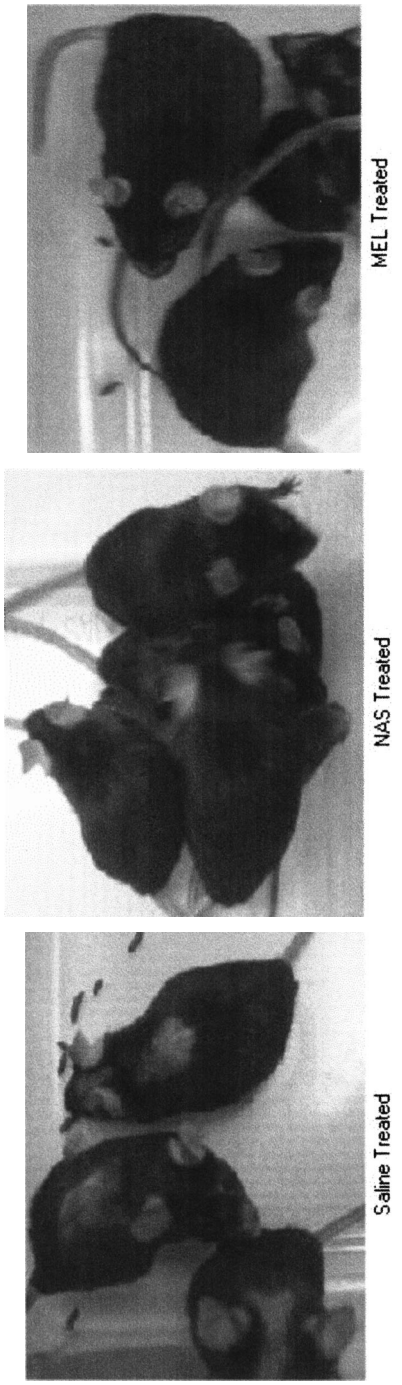

FIGURE 9. Effect of four weeks' administration of *N*-acetylserotonin and melatonin on C57BL/6J male mice. Note: NAS-treated animals with the healthiest and most luxuriant fur coat; the large areas of baldness in the control group; the coat of melatonin-treated mice had more graying fur than the NAS-treated animals.

the C3H mice do capable of converting NAS to melatonin.[14] However, this capacity is very limited in comparison to rat pineals. Efforts in our laboratory to induce the biosynthesis of melatonin in the pineals of C3H mice have been unsuccessful. The administration of NAS and selective monoamine oxidase inhibitor, clorgyline, agents known to increase the biosynthesis of melatonin in the rat pineals,[15,16] failed to induce melatonin biosynthesis in the pineals of C3H mice. NAS (30 mg/kg) administered to mice at the beginning of the dark cycle did not increase the melatonin content in the pineal (0.12 ± 0.02 ng/pineal for NAS group versus 0.09 ± 0.02 ng/pineal for control, 30 minutes after injection). Clorgyline (2.5 mg/kg) administered to light-primed mice also failed to induce melatonin biosynthesis in the pineal (0.05 ± 0.01 ng/pineal for clorgyline group versus 0.05 ± 0.01 for control group, 90 minutes after injection) (unpublished results). In agreement with the previous reports on antioxidant effects of NAS in the *in vitro* models[6–8] our study demonstrated the antioxidant effects of NAS proper in the brain and kidney tissues of the aged C57Bl/6J mice. Since the antioxidant effect might contribute to the antiaging action, one might suggest that NAS indeed has its own antiaging effect. Further evaluation of the NAS effect on life span conducted in mice unable to convert NAS into melatonin (i.e., C57Bl/6J strain) could help to differentiate between NAS proper and melatonin-mediated effects of NAS on aging.

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