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by

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INTRODUCTION

The mammalian hormone melatonin is secreted by the pineal gland mainly during the dark phase of the circadian sleep-wake cycle. Its effects have been examined by many authors (1,2) for many years in order to determine the role melatonin plays in reproduction and related processes. Recent experiments involving mice have shown that nightly administration of melatonin delays the aging process and preserves aspects of their youthful state (3). One of the main effects melatonin has in the body is to suppress the gonadal function acting at different levels on the hypothalamo-pituitary-gonadal system (4).

Transgenic mice carrying the ectopic gene of bGH or hGH have a shortened life spans and have significantly higher elevated corticosterone levels in serum as compared to normal mice(5). It has therefore been hypothesized that cortocosterone, among other steroids, may contribute to the accelerated aging process in these transgenic mice. With the above information in mind, we will determine the effects, if any, melatonin has on the aging process of both normal and transgenic lines of mice by administering exogenous melatonin during the dark phase of the circadian sleep-wake cycle and then measuring testosterone and corticosterone blood plasma levels upon completion of the study. We feel that this experiment will lead to a better understanding of the role melatonin plays in the aging process.

MATERIALS AND METHODS

Transgenic (PEPCK- bGH, PEPCK- hGH) and normal lines of male mice were fed standard mouse chow and were housed 4-7 per cage. The housing quarters followed a light/dark cycle of 12:12 with darkness lasting from 6 p.m. to 6 a.m. All mice were randomly distributed into either a control or a melatonin administration group. Littermates were kept together to avoid fighting among different litters. For a two month period we administered nightly, either melatonin (stock solution: 1 mg/ml ethanol stored at -70 C; working solution: diluted to 20%) or a control (20% ethanol and 80% salt water) using the subcutaneous route of injection using a 24 c.c. needle. In order to assure that the mice would receive adequate amounts of melatonin, two daily doses were given, one at 5 p.m. and the other at 10 p.m. from June 22, 1995 to August 15, 1995. The melatonin treatment group received 20 μg of melatonin in 20% ethanol, while controls were injected with 20% ethanol in a saline solution. Upon completion of the experiment the animals were decapitated in total silence(to avoid elevated levels of ACTH which would cause corticosterone levels to rise) and blood was collected from each animal in order to obtain the blood plasma. Measurement of testosterone and corticosterone levels in each of the plasma samples were found by radioimmuno assay (commercial kit; DPC, Los Angeles, CA) .

RESULTS

Throughout the experiment we lost many mice in the melatonin group to fighting that occurred within the cages, because of this we did not have enough of a sample size to adequately measure testosterone or corticosterone levels in both the normal melatonin administered group and the PEPCK-hGH melatonin and control administered groups; therefore; their values were not included in the results. However, there was enough of a sample in the normal control and PEPCK-bGH (control and melatonin) groups for analysis (Fig. 1). We found that testosterone was lower in the PEPCK-bGH line (control and melatonin) groups as compared to the normal control group. Corticosterone levels were higher in the PEPCK-bGH (control and melatonin) groups than in the normal control group. Despite melatonin treatment

corticosterone and testosterone levels remained constant between both groups of PEPCK-bGH (control and melatonin) mice.

MELATONIN GROUP

PEPCK-hGH	DATE OF BIRTH	TESTOSTERONE	CORTICOSTERONE
T	01/11/95	4.06	128.4
T	01/19/95	1.59	173.4
N	01/19/95	.414	410.5
N	01/19/95	3.29	68.83
PEPCK-bGH	**DATE OF BIRTH**	**TESTOSTERONE**	**CORTICOSTERONE**
T	12/20/94	1.56	180.1
T	12/20/94	1.49	365.1
T	02/14/95	1.65	192.8
T	2/14/95	6.08	211.6
N	2/14/95	.252	136.8
T	02/22/95	.317	300.7
T	02/22/95	.306	175.9
T	02/22/95	3.32	190.0

T = Transgenic
N = Normal

CONTROL GROUP

PEPCK-hGH	DATE OF BIRTH	TESTOSTERONE	CORTICOSTERONE
T	01/11/95	.581	20.4
T	10/24/94	.555	49.8
T	09/15/94	1.41	280.0
T	09/15/94	6.56	159.1
T	02/15/95	9.82	103.0
T	02/15/95	3.9	216.7
T	02/15/95	4.26	129.6
N	1/11/95	.852	98
N	1/11/95	10	193.9
N	1/11/95	.486	43.4
N	10/24/94	.555	330.6
N	9/15/94	.454	52.6
N	2/15/95	8.3	32
N	2/15/95	.52	50.34
PEPCK-bGH	**DATE OF BIRTH**	**TESTOSTERONE**	**CORTICOSTERONE**
T	01/03/95	2.31	148.8
T	01/03/95	.42	183.1

T	01/02/95	2.09	225.4
T	01/02/95	1.33	132.5
T	02/18/95	.364	112.6
T	02/18/95	1.62	295.7
T	02/18/95	1.55	110.0
T	01/25/95	.227	580.5
T	01/05/95	4.1	347.0
T	01/05/95	6.12	224.0
N	01/03/95	.69	20.9
N	01/02/95	18.3	40.0
N	01/05/95	20.7	25.1
N	01/25/95	12.03	38.2
N	01/25/95	12.36	67.0
N	01/25/95	8.91	113.8
N	01/25/95	5.33	93.0
N	01/05/95	1.03	91.5

T = Transgenic
N = Normal

Effects Of Melatonin Treatment On Corticosterone And Testosterone Levels In Plasma Of Normal and PEPCK-bGH Mice

(Fig. 1)

	Corticosterone					Testosterone			
	Control	Std Error	Melatonin	Std Error		Control	Std Error	Melatonin	Std Error
Normal	61.18 (8)	± 12.48	---	---		8.84 (8)	±2.54	---	±
PEPCK-bGH	236 (10)	± 45.6	230.9 (8)	±27.7		2.01 (10)	±0.58	2.1 (8)	±0.7

( ) = Sample size

Students "t" test

PEPCK-bGH Corticosterone (Control and Melatonin groups) = .293
PEPCK-bGH Testosterone (Control and Melatonin groups) = .297
PEPCK-bGH and Normal Corticosterone (Control Groups) = 12.65 *
PEPCK-bGH and Normal Testosterone (control Groups) = 9.31 *
* Statistically Significant (greater then 2.1)
As one can see by the "t" tests performed, the only significant numbers occurred between the control populations. Meaning that the mice tested naturally have different levels of testosterone and corticosterone.

DISCUSSION

The daily injections of melatonin at 5 p.m. and again at 10 p.m. seemed to have little effect on the mice being tested. Since melatonin is known to decrease levels of testosterone, we would expect to find testosterone levels in the PEPCK-bGH control group to be higher than those in the PEPCK-bGH melatonin administered group. This was not the case, instead testosterone levels were slightly higher in

the PEPCK-bGH melatonin administered group than in PEPCK-bGH controls, which is a good indication that the experiment was not successful in determining the role melatonin plays in the aging process. In measuring corticosterone levels there was no differences in the plasma concentrations between PEPCK-bGH control or PEPCK-bGH melatonin groups. The conclusion that corticosterone levels are unaffected by melatonin should not be assumed, since the first half of our experiment was inconclusive. A few areas which should be addressed in future experiments include; 1.) Determining the ideal melatonin dosage. Perhaps higher doses greater then 20 g that was used in this experiment is needed. 2.) Longer experimentation time. Since our experiment lasted roughly two months, it may not have been enough time for tissue change or for differences in testosterone/cortocosterone levels to occur. 3.) The route of melatonin administration that we selected may not have been the ideal route. Perhaps placing melatonin in the drinking water during the dark phase of the circadian sleep/wake cycle instead of subcutaneous injections would have been more effective. It is clear that under the experimental situation and parameters that we used to study the effects of melatonin on the aging process, it was inconclusive.

We started out with 25 transgenic mice PEPCK-hGH(7), PEPCK-bGH (18) and 23 normal mice. At the end of the experiment we lost (5) PEPCK-hGH from the melatonin group, (9) normal from the melatonin group. For lack of numbers the normal melatonin group and the PEPCK-hGH (melatonin and control) groups were discarded from being analyzed. With our experiment being inconclusive, additional research is needed to find out the true effect melatonin has on the aging process in mice

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