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Mechanisms of Ageing and Development 124 (2003) 721–731

mechanisms of ageing and development

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Effect of delta-sleep inducing peptide-containing preparation Deltaran on biomarkers of aging, life span and spontaneous tumor incidence in female SHR mice

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Received 28 October 2002; received in revised form 24 January 2003; accepted 25 February 2003

Abstract

From the age of 3 months until their natural deaths, female Swiss-derived SHR mice were subcutaneously injected 5 consecutive days every month with 0.1 ml of normal saline (control) or with 2.5 μg/mouse (~ 100 μg/kg) of delta-sleep inducing peptide (DSIP, Trp–Ala–Gly–Gly–Asp–Ala–Ser–Gly–Glu) as the preparation Deltaran® solved in 0.1 ml of saline. There were 54 mice in each group. The results of this study show that the treatment with Deltaran did not influence food consumption, but decreased the body weight of mice; it slowed down the age-related switching-off of estrous function; it decreased by 22.6% the frequency of chromosome aberrations in bone marrow cells; it did not influence mean life span; and it increased by 17.1% life span of the last 10% of the survivors and by 24.1% maximum life span in comparison with the control group. We also found that treatment with Deltaran significantly decreased total spontaneous tumor incidence (by 2.6-fold), mainly mammary carcinomas and leukemias in mice as compared with the control group. This is the first report on geroprotector and anticarcinogenic effect of DSIP-containing preparation Deltaran.

Keywords: Deltaran; DSIP; Aging; Longevity; Biomarkers of aging; Spontaneous tumors; Mice

1. Introduction

The search for effective and safe means of prevention of premature aging has high priority in gerontology (Anisimov, 2001; Butler et al., 2002; De Grey et al., 2002). During the last decade, a number of reports appeared on the role of the pineal gland in aging (Armstrong and Redman, 1991; Reiter, 1995; Reppert and Weaver, 1995; Pierpaoli, 1998; Reiter et al., 2002). Thus, a modulating effect of the pineal gland on the neuroendocrine and the immune system was shown to change during aging (Arendt, 1995). Pinealectomized rats showed a reduced life span (Malm et al., 1959; Reiter et al., 1999) whereas the administration of the pineal hormone melatonin to rodents of syngeneic transplantation of pineal glands from young donors into the thymus or in situ of old mice prolonged the life span of the recipients (Pierpaoli et al., 1991; Pierpaoli and Regelson, 1994; Lesnikov and Pierpaoli, 1994; Anisimov et al., 2001b, 2003b; Oxenkrug et al., 2001). Most investigators invoked melatonin as a primary

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0047-6374/03/$ - see front matter © 2003 Published by Elsevier Science Ireland Ltd.
doi:10.1016/S0047-6374(03)00082-4

mediator of the endocrine capabilities of the pineal gland. However, some of the effects of the pineal gland might have obviously resulted from pineal peptide secretion (Benson, 1977; Bartsch et al., 1992; Yuwiler and Brammer, 1993; Arendt, 1995). Some crude peptide extracts or purified peptides isolated from pineal glands were shown to have antigonadotropic, metabolic and antitumor activity (Lapin and Ebels, 1976; Noteborn et al., 1988b; Bartsch et al., 1992; Anisimov et al., 1994). Among the peptides identified in the pineal gland there are vasoactive intestinal peptide, neuropeptide Y, secretoneurin, histidine–isoleucine, somatostatin, substance P, delta-sleep inducing peptide (DSIP), releasing hormones and other (Noteborn et al., 1988a; Yuwiler and Brammer, 1993; Arendt, 1995; Moller, 1997). It is worth noting, that the majority of these peptides are not synthesised in the pineal gland. They are present in the pineal gland because they are neurotransmitter/neuroimmunomodulators present in pineal afferent nerves (Pevet, 1983; Simonneaux et al., 1997a,b, 1999).

The nonapeptide DSIP (Trp–Ala–Gly–Gly–Asp–Ala–Ser–Gly–Glu) (Schoenenberger and Monnier, 1977; Graf and Kastin, 1986; Pollard and Pomfrett, 2001) is immunohistochemically demonstrated to reside not only in pineal gland, but also in the hypothalamus, septum, hippocampus and other limbic regions of rat brain (Skagerberg et al., 1991). It also may be present in pineal nerves (Ouichou and Pevet, 1992). Several studies indicate that biological activity of DSIP is not restricted to sleep effects. DSIP affects the neuroimmune system (Yehuda and Carasso, 1988; Kruger and Karnovsy, 1995), modifies thermoregulation, locomotion, heart rate and blood pressure (Yeguda et al., 1980, 1988) and increases monoamine oxidase A activity and serotonin levels in the brain (Khvatova et al., 1995; Ouichou et al., 1992). Sudakov (1991) considers DSIP as a regulatory oligopeptide that modulates emotional stress, preventing stress-induced disturbances in the organism. DSIP also inhibits pituitary secretion of ACTH and stress-induced corticosterone secretion (Chiodera et al., 1994) mediated by inhibition of stress-induced expression of *c-fos* gene (Sudakov et al., 2001). The inhibitory effects of DSIP on free radical production have been manifested in the reducted accumulation of lipid peroxidation products (conjugated dienes, malonic dialdehyde, Schiff bases), in stimulation of activity of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, myeloperoxidase and in accumulation of antioxidants (reduced glutathione in particular) in rat tissues (Rikhireva et al., 1993; Bondarenko et al., 1999; Lysenko et al., 1999; Shustanova et al., 2001), in stabilization of cellular membranes (Bondarenko et al., 1998) and stimulation of protein biosynthesis (Rikhireva et al., 1995) in rodent tissues. These observations allow us to suggest a possible positive effect of DSIP on life span of animals. In this paper we show that long-term administration of DSIP-containing preparation Deltaran® slows down the aging rate, increases maximum life span and inhibits spontaneous tumor development in mice. To our knowledge the effects of Deltaran as well as of DSIP on aging, life span and tumor development have not been studied before.

2. Material and methods

2.1. Animals

One hundred eight female Swiss-derived SHR 2-month-old mice were purchased from the Rappolovo Animal Farm of the Russian Academy of Medical Sciences (St. Petersburg). The mice were kept in polypropylene cages (30 × 21 × 9 cm), five mice in a cage at a temperature of 22 ± 2 °C. A regimen of 12 h of light and 12 h of dark was followed. The animals received sterilized standard laboratory chow (Anisimov et al., 2003a) and tap water ad libitum. Mice were checked daily by animal care personnel and weekly by a veterinarian. The study was carried out in accordance with the regulations for ensuring the humane treatment of animals under the approval of the Committee on Animal Research of the N.N. Petrov Research Institute of Oncology.

2.2. Reagents

The preparation Deltaran® was designed at M.M. Shemyakin and Yu.A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences and has been received from the Research Center 'COMCON' (St. Petersburg). Deltaran® is a mixture of synthetic DSIP (Trp–Ala–Gly–Gly–Asp–Ala–Ser–Gly–Glu) and glycine (w/w as 1:10). It is purchased in glass ampoules containing lyophilized powder of DSIP + glycine. Each glass contains 0.3 mg of DSIP and 3 mg glycine. Normal saline (0.9% NaCl) was from Phoenix Pharmaceuticals Ltd, UK.

2.3. Experiment

At the age of 3 months, the mice were randomly divided into 2 groups, 54 animals in each. Each animal was individually marked. Mice of the control group were subcutaneously injected 0.1 ml of normal saline 5 consecutive days monthly until their natural deaths. Mice of another group were injected under the same schedule 0.1 ml of the solution of Deltaran in normal saline. The dose of DSIP was 2.5 μg of DSIP per mouse, i.e. 100 μg/kg of body weight. Solution was prepared ex tempore.

To evaluate the initial level of chromosome aberrations four intact female SHR mice were euthanized at the age of 3 months. Additionally four mice from each group were euthanized at the age of 12 months for a cytogenetic study of chromosome aberrations in hone marrow cells (see below). Once in every 3 months, simultaneously with weighting, the amount of food consumed was measured. Thirty grams of food were given in each cage after cleaning. After 24 h the food which was not consumed was collected from each cage and weighed. The mean amount of food (grams) consumed per mouse during this day was calculated for each group.

Once every 3 months, vaginal smears taken daily for 2 weeks from the animals were cytologically examined to estimate the phases of their estrous functions. In the same period, rectal body temperatures of the mice were measured with an electronic thermometer, TPEM (KMIZ, Russia). Animals were observed until natural death. The date of each death was registered, and mean life span, the age by which 90% of the animals died, and maximal life span estimated.

2.4. Cytogenetic study

Chromosomal aberrations in bone marrow cells were studied by the modified Ford's method described by Rosenfeld et al. (2001). Mice were sacrificed with ether anaesthesia. Both femurs of each mouse were dissected and bone marrow cells flushed gently with 0.56% KCl solution into a centrifuge tube. Cells were treated for 20 min with hypotonic solution and fixed with ethanol:acetic acid mixture (3:1). Slides were stained with 4% acetoorseine. Twenty to thirty well spread anaphases were analyzed for each animal and cells with chromosome breaks, acentric fragments, and other aberrations were evaluated on 1000 × magnification with a light microscope (Leitz, Germany).

2.5. Pathomorphological examination

All animals that died, or were sacrificed when moribund, were autopsied. At autopsy their skin and internal organs were examined. Neoplasia were classified according to the recommendations of the International Agency of Research on Cancer (IARC) as 'fatal' (i.e., those, that directly caused the death of the animal) or 'incidental' (for cases where the animal died of a different cause) (Gart et al., 1986). All tumors, as well as tissues and organs with suspected tumors, were excised and fixed in 10% neutral formalin. After routine histological processing, tissues were embedded in paraffin. Thin, 5–7 μm, histological sections were stained with hematoxylin–eosins and microscopically examined. The experimental group to which the mouse belonged was blinded. Tumors were classified according to IARC recommendations (Turusov and Mohr, 1994).

2.6. Statistics

Experimental results were statistically processed by methods of variation statistics (Goubler, 1978). The significance of discrepancies was defined according to Student's t-criterion, Fischer's exact method, χ^2-analysis, and the non-parametric criterion of Wilkokson–Mann–Whitney (Goubler, 1978). For discrepancies in neoplasm incidence to be estimated, an IARC method of combined contingency tables calculated individually for the fatal and incidental tumors (Gart et al., 1986). For survival analysis, Cox's method (Cox and Oakes, 1996) was used. All reported test values used in survival analyses of data are two sided.

2.7. Mathematical models and estimations

The mathematical model used to describe survival is the Gompertz model with the survival function

$$S(x) = \exp\left\{-\frac{\beta}{\alpha}[\exp(\alpha x) - 1]\right\}$$

where parameters α and β are associated with the population rate of aging, and initial mortality rate, respectively. Parameter α is often characterized by the value of mortality rate doubling time (MRDT), calculated as $\ln(2)/\alpha$. Parameters for the model were estimated from data using the maximum likelihood method implemented in the Gauss Statistical System (Gauss System and Graphic Manual, 1994). Confidence intervals for the aging rate parameter estimates were calculated using log-likelihood functions (Cox and Oakes, 1996).

3. Results

3.1. Age-related body weight dynamics

Mean values of body weight for mice at different ages in the control and treated with Deltaran groups are displayed in Table 1. The body weight of the mice in both groups increased with age, exceeding by 16 months the body weight of 3-month-old animals by 37.7% in the control group ($P < 0.001$), and by 22.41% in the group given Deltaran ($P < 0.01$). The mean body weight of mice exposed to Deltaran was decreased from the age of 7 to 13 months in comparison with those in the control group.

Table 1
Body weight gain dynamics in female SHR mice treated with saline or Deltaran

Group	Body weight (g)							
	3 mo	5 mo	7 mo	9 mo	11 mo	13 mo	16 mo	17 mo
Saline	24.7±0.29	27.8±0.63	30.9±0.85	32.4±1.30	34.3±1.24	35.7±1.67	34.0±1.73	32.4±1.50
Deltaran	25.0±0.34	27.9±0.58	28.3±0.54*	30.2±0.62	30.0±0.87*	30.8±1.25*	30.6±1.77	30.1±1.12

* The difference from the saline group is significant, $P < 0.05$ (Student's t-test).

3.2. Age-related dynamics of food consumption

Measurements have shown that the amount of food consumed by the mice in the control (saline) group and treated with Deltaran groups were practically the same during the entire period of observation. Only at the age of 18 months animals treated with DSIP eat less than that in the saline-treated group (Table 2). The obtained data indicated that the decrease in the body weight of mice treated with Deltaran is not caused by the drug impact on food consumption by the animals.

3.3. Age-related dynamics of estrous function in mice

Investigations of the estrous function in the animals of both age groups were performed every 3 months, starting with 3 month old mice. The following parameters of estrous function were estimated: the length of the estrous, the relative rate of estrous cycle phases (in percent); and the relative number of short (<5 days) and long (>5 days) estrous cycles. The relative number of animals with regular and irregular cycles (persistent estrus and anestrus) were calculated as well. Judging by the data presented in Table 3, the length of estrous cycle in the control mice increases with the advance in age ($P < 0.05$; Student t test). Thus, no essential age-related alterations in the rate of estrous cycle phases were observed. However, the relative number of short estrous cycles significantly decreased with age (37.1% at the age of 3 months, 9.4% at the age of 12 months ($P < 0.05$; Fischer exact test) and zero at the age of 15 months, whereas the number of long cycles rose (5.1% at the age of 6 months and 36% at the age of 15 months, $P < 0.05$; Fischer exact test).

In the group of mice exposed to Deltaran the length of estrous cycles did not change with the age of the animals and shortened in comparison with the age-matched controls during the entire period of observation. There was no age-related decrease in the number of short cycles or increase in the number of long cycles. The number of mice with short estrous cycles was much higher in mice exposed to Deltaran as compared with the control groups practically during the whole period of observation (from the age of 3 to 15 months). The number of mice with regular cycles did not change significantly with the age in both groups (Table 3).

3.4. Age-related dynamics of body temperature in mice

Data on body temperature alterations in the mice exposed or not exposed to Deltaran are presented in Table 4. The control mice revealed significant decrease in body temperature with age, both on the whole (irrespective of the estrous cycle phases) and in any of the phases. No cyclic alterations in rectal body temperature during the estrus cycle were observed in mice of the control group, but the temperature at diestrus was significantly higher than that in estrus in mice treated with Deltaran at the entire period of observation. It should be noted that the average body temperature in the mice treated with Deltaran at the age of 7 months was lower, and at the age of 19 months was higher than that in the corresponding controls during the phases of estrus and diestrus of the estrus cycle (Table 4). There was no age-related decrease in the temperature in Deltaran-treated mice.

3.5. Chromosome aberrations in mouse bone marrow cells

The incidence of chromosome aberrations in hone marrow cells of 3-month-old female, SHR mice was 2.1±0–29%. At the age of 12 months this parameter

Table 2
Food consumption dynamics in female SHR mice treated with saline or Deltaran

Group	Daily food consumption (g/mous)							
	3 mo	5 mo	7 mo	9 mo	11 mo	13 mo	16 mo	18 mo
Saline	4.6±0.03	2.8±0.05	3.3±0.02	2.8±0.03	3.1±0.01	3.4±0.03	3.8±0.06	5.3±0.15
Deltaran	4.4±0.14	3.4±0.07	3.2±0.34	3.3±0.21	3.4±0.14	3.6±0.23	3.9±0.23	4.03±0.24*

* The difference from the saline group is significant, $P < 0.01$. (Student's t-test).

Table 3
Age-related dynamics of estrous functional parameters in SHR mice treated with saline or Deltaran

Age (mo)	No. of mice	Length of estrous cycle (days)	Rate of separate phases of estrous cycle (%)			Rate of estrous cycles of various fraction length (%)			Fraction of mice with regular cycles (%)	Fraction of mice with irregular cycles (%)
			E	D	P+M	< 5 days	5–7 days	> 7 days		
Saline										
3	28	5.68 ±0.19	42.4	54.7	2.9	31.7	55.0	13.3	88.0	12.0
6	22	5.38 ±0.19	45.4	50.5	4.1	30.8	64.1	5.1	95.5	4.5
9	22	5.77 ±0.30	34.2	62.4	3.4	25.6	59.0	15.4	95.5	4.5
12	22	6.25 ±0.26*	47.1	51.3	1.6	9.4**	81.2	9.4	95.5	4.5
15	17	6.84 ±0.26*	34.2	65.1	0.7	0**	64.0	36.0**	88.2	11.8
Deltaran										
3	30	4.59 ±0.21a	44.4	52.6	3.0	55.1[b]	40.8	4.1	96.7	3.3
6	24	4.23 ±0.22a	48.5	47.0	4.5	64.1[c]	33.3[c]	2.6	100	0
9	24	3.90 ±0.25[a]	39.7	58.3	2.0	75.0[d]	21.9[d]	3.1	87.5	12.5
12	19	4.09 ±0.21[a]	41.8	57.1	1.1	81.0[d]	19.0[d]	0	89.5	10.5
15	10	3.75 ±0.35[a]	42.9	56.0	1.1	75.0	25.0[b]	0	93.4	6.6

Note: E, estrus; D, diestrus; P, proestrus; M, metaestrus; the difference from the parameters at the age of 6 months in the same group: $^{*}P < 0.05$ (Student t-test); $^{**}P < 0.05$ (Fischer's exact test). The difference with the corresponding age in the control group: [a] $P < 0.01$ (Student t-test); [b] $P < 0.05$;[c] $P < 0.01$; [d] $P < 0.001$ (Fischer's exact test).

rose to 8.4 ±0.38% ($P < 0.001$; Wilkokson–Mann–Whitney test) in the group injected with saline. In mice treated from the age of 3 months with DSIP the incidence of chromosome aberrations at the age of 12 months was 6.5 ±0.29 (i.e. 22.65% lower than in the control; $P < 0.05$; Wilkokson–Mann–Whitney test).

3.6. Survival and longevity of female SHR mice

Survival dynamics in the mice treated with either saline or Deltaran are demonstrated in Table 5 and Fig. 1. The survival dynamics were in general similar in all groups up to the age of 2 years. However after this age the number of survivors in Deltaran-treated groups was considerably higher than in saline-treated groups.

The last mouse in the control group died at the age of 739 days (24.3 months), whereas in the groups treated with Deltaran this age survived 12% of mice, and the maximum life span was 917 days (30.2 months, + 24.1%). The mean life span of mice treated with DSIP was not significantly increased as compared with controls. However, the life span in the last 10% of the Deltaran-treated mice increased by 4.4 months (+ 17.1%, $P < 0.01$; Student t-test) (Table 6).

3.7. Spontaneous tumor development in female SHR mice

The total tumor incidence in the control female mice was 36%. Mammary carcinomas and leukemias developed most frequently, which corresponded to the oncological characteristics of female SHR mice (Anisimov, 1987). The treatment with Deltaran was followed by 2.6-fold decrease in total and by 3.0-fold decrease in malignant tumor incidence in comparison with that of the control group ($P < 0.01$; Fischer exact test) (Table 7). The incidence of mammary carcinomas under the treatment with Deltaran decreased fivefold ($P < 0.01$; Fischer exact test) and the incidence of lung metastases of mammary carcinomas decreased sevenfold. In the saline-treated group, six cases of leukemia have been detected whereas in the Deltaran-treated group no eases of leukemia have been observed. There was no any significant difference in the incidence of other tumors between the group of mice treated with Deltaran and saline (Table 7). The treatment with Deltaran significantly shifted to elder age the survival of fatal tumor-bearing mice (Fig. 2) as well as total tumor yield curve as compared with the control group (Fig. 3).

3.8. Mathematical model and estimations of survival of tumor-free and tumor-bearing mice

A mathematical analysis of the survival data of the mice from the control and melatonin-treated groups has been carried out separately for three different contexts: (1) for all animals in each group (total cases); (2) for

Table 4
Body temperature dynamics in SHR mice treated with saline or Deltaran

Age (mo)	Number of mice	Total cycle (without phase sub-division)	Mean body temperature (°C)		
			Estrus	Diestrus	Metacstrus+proestrus
Saline					
7	22	39.95±0.14	39.80±0.30	40.00±0.20	39.40±0.10
12	22	38.83±0.18[a]	38.73±0.30[a]	38.90±0.24[a]	–
15	17	38.73±0.17[a]	38.68±0.30[a]	38.75±0.20[a]	–
17	12	38.05±0.24[a]	37.40	38.10±0.30[a]	–
19	10	37.70±0.11[a]	37.60±0.10[a]	37.70±0.14 [a]	–
Deltaran					
7	15	38.40±0.15*	38.20±0.05**	38.50±0.01**	38.60±0.30*
12	23	38.81±0.14	38.30±0.16	38.90±0.17[b]	–
15	18	38.60±0.16	38.20±0.10	39.00±0.19[b]	38.40
17	11	38.00±0.30	37.80±0.15[a]	38.20±0.54[b]	38.00
19	6	38.26±0.16*	38.00	38.30±0.30	–

The difference from the corresponding parameter in the saline group is significant, *$P < 0.01$; **$P < 0.001$ (Student t-test).
[a] The difference from the age of 7 months in the same group is significant, $P < 0.05$ (Student t-test).
[b] The difference from the parameter at phase estrus of the same group and age, $P < 0.05$ (Student t-test).

fatal tumor-bearing mice, and (3) for fatal tumor-free mice. We composed the groups of animals without consideration of possible effects caused by dependence between these groups. The Gompertz model shows a slow down (by 18.4%) of the population aging rate (calculated as α in the Gompertz equation) and corresponding increase in MRDT under the influence of Deltaran. The mortality rate of fatal tumor-free mice treated with Deltaran was increased as compared with the controls Fig. 3.

4. Discussion

The results of our study show that long-term administration of Deltaran increases survival and maximum life span and decreases the rate of development of spontaneous tumors, mainly mammary adenocarcinomas and leukemias, in female SHR mice. The results concerning the effects of Deltaran on life span and carcinogenesis have never been published or even discussed before.

The important feature of the effect of Deltaran on survival is that it does not influence the food consumption during almost the entire life span. No reduction in food consumption was observed from the age of 3 to 16 months in the group treated with the peptide. Only at the age of 18 months the decrease in the food consumption was registered in Deltaran-treated group. The body weight was decreased by 7–14% in mice treated with Deltaran as compared with the control after the age of 7 months. A positive correlation between excessive body weight and tumor incidence is observed in rodents and human females (Weindruch and Walford, 1988; Dilman, 1994). Our data on the decrease of spontaneous tumor incidence in mice exposed to Deltaran are in agreement with these observations.

It is worth noting that decrease in the body weight observed in Deltaran-treated mice is not related to the decrease of food consumption: the latter was the same in both groups practically during the entire period of observation. The effect could be related to the influence of Deltaran on basal metabolism of animals. The decrease of mean body temperature in the Deltaran-treated mice at the age of 7 months suggests the inhibitory effect of Deltaran on the basal metabolism. However, there was no difference in the body temperature between both groups at the age of 12, 15 and 17 months, and the body temperature was even higher in Deltaran-treated mice at the age of 19 months (Table 2). Another explanation of the decrease in body weight in mice exposed to Deltaran could be the stimulatory effect of DSIP on locomotor activity (Graf et al., 1981). This question needs additional studies.

Another interesting new finding obtained in our study is the slowing down of the age-related disturbances in estrous function in mice treated with Deltaran (Table 3). The measurements of rectal temperature confirmed the cyclic pattern of estrous function in Deltaran-treated mice as compared with acyclic temperature pattern in the control. Our observations are in agreement with data on stimulatory effect of DSIP on hypothalamic neural structures responsible for stimulation of ovulatory luteinizing hormone releasing hormone and luteinizine hormone release (Iyer and McCann, 1987; Sahu and Kalra, 1987).

The aging process predisposes cells to accumulate mutations; some of them stimulate initiation of tumor growth in target tissues (Vijg, 2000; Bodyak et al., 2001). The incidence of chromosome aberrations increases with age in different strains of mice (Crowley and Curtis,

Table 5
Survival distribution of female SHR mice treated with saline or Deltaran

Group	No. of survivors at the age of												
	4 mo	5 mo	8 mo	10 mo	12 mo	14 mo	16 mo	18 mo	20 mo	22 mo	24 mo	26 mo	28 mo	30 mo	31 mo
Saline	50	47	36	36	34	33	28	20	16	10	2	0	0	0	0
Deltaran	50	50	40	40	39	37	29	13	10	8	5	4	4	1	0

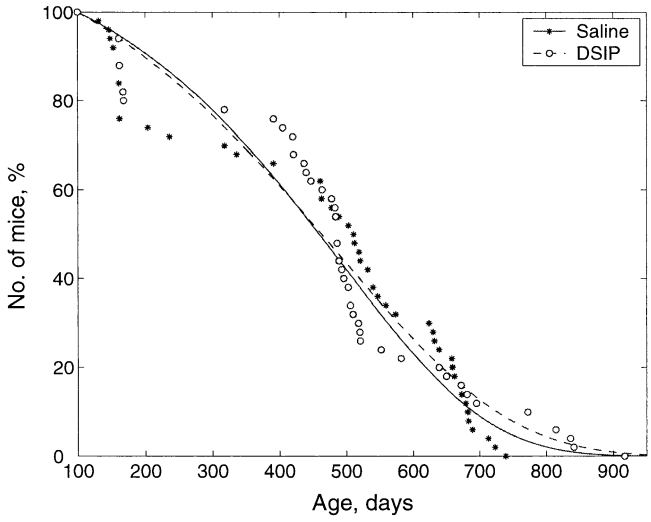

Fig. 1. Effect of DSIP on the survival of female SHR mice.

Table 6
Parameters of life span in female SHR mice treated and not treated with delta ran

Parameters	Saline	Deltaran
Number of mice	50	50
Mean life span, days (M ± S.E.)	456 ± 29	474 ± 29
Median	512	486
Mean life span of last 10% of survivors, days	709 ± 10.8	844 ± 19.8*
Maximum life span, days	739	917

* The differences from saline is significant, $P < 0.01$ (Student t-test).

1963; Sato et al., 1995). Earlier we found age-related increases in chromosome aberrations in hone marrow cells and in primary spermatocytes in male SHR mice (Rosenfeld et al., 2001). In this paper we observed a significant increase in the frequency of chromosome aberrations in the bone marrow cells in 12-month-old female SHR mice compared to 3-month-old ones. Treatment with Deltaran revealed a significant inhibition (by 22.6%) of the age-associated increase in chromosome aberrations in female SHR mice. It is worthy of note that the treatment with melatonin started at the age of 3 months had no effect on the chromosome aberrations in 12-month-old SHR mice (Rosenfeld et al., 2002).

The long-term administration of Deltaran was followed by significant decrease in spontaneous tumorigenesis and by the increase of longevity in female SHR mice (Tables 5–8). This effect was manifested mainly in relation to mammary carcinomas and leukemias. This is in agreement with the data on inhibitory effect of Deltaran on frequency of chromosome aberrations in the bone marrow cell. Deltaran significantly inhibited metastasizing of mammary carcinomas into lungs (Table 7). It was shown earlier that DSIP significantly de-

Table 7
Incidence, localization and type of tumors in female SHR mice treated and not treated with Deltaran

Parameters	Saline	Deltaran
Number of mice	50	50
Number of tumor-bearing trice	18 (36%)	7 (14%)*
Number of malignant tumor-hearing mice	15 (30%)	5 (10%)*
Total number of tumors	25	8
Total number of malignant tumors	18	5
Number of tumors per tumor-bearing mice	1.39	1.14
Mean life span of fatal-tumor-bearing mice, days	549±29	693±109
Mean life span of fatal tumor-free-animals, days	416±38	450±128
Localization and type of tumors		
Mammary gland		
Adenocarcinoma	12 (10)[a]	2**
Nos. of metastases	7	1*
Leukemia	6	0
Lung		
Adenoma	1	0
Adenocarcinoma	0	2
Utery		
Polyp	1	0
Adenocarcinoma	0	1
Ovary		
Cyst	5	2
Granuleso-cell tumor	0	1

The differences from saline is significant, $*P < 0.01$; $**P < 0.002$ (Fischer's exact test).
[a] Two mice had 2 mammary tumors each.

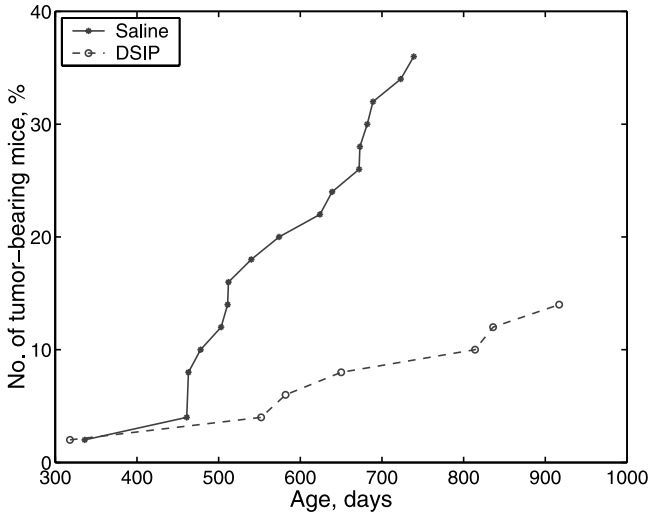

Fig. 2. Effect of DSIP on total tumor incidence in female SHR mice.

creased the number of lung metastases of Lewis lung carcinoma transplanted into C57BL, mice (Shmal'ko and Mikhaleva, 1988; Prudchenko et al., 1993). We suggest that the capacity of Deltaran to prevent the development of spontaneous malignant tumors in female SHR mice is related to DSIP antioxidative activity

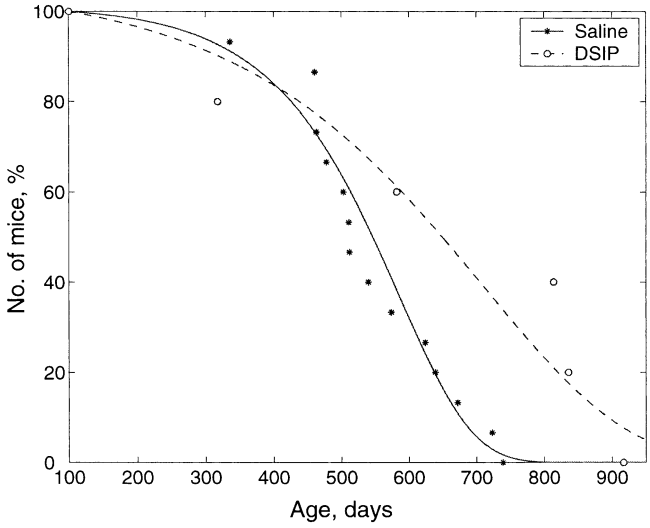

Fig. 3. Effect of DSIP on the survival of fatal tumor-hearing SHR mice.

(Rikhireva et al., 1993; Bondarenko et al., 1999; Lysenko et al., 1999; Shustanova et al., 2001). Some crude pineal peptide preparations and melatonin are also potent antioxidants, which inhibit spontaneous and chemically induced carcinogenesis and increase longevity in mice and rats (Anisimov et al., 1994, 1998, 2001a; Cos and Sanchez-Barcelo, 2000; Bartsch et al., 2001). It is worth noting that administration of DSIP was followed by stimulation of synthesis and secretion of melatonin in rat pineal gland (Oaknin et al., 1986; Ouichou et al., 1992). Therefore, certain effects of DSIP-containing preparation Deltaran (such as antioxidant, anticarcinogenic and geroprotector actions) can he mediated by synthesis of endogenous melatonin. There are no available data on the synthesis of melatonin in our outbred Swiss-derived SHR mice. However the observation of small but significant peak of melatonin in the middle of the dark phase in outbred OF1 Swiss mice (Vivien-Roels et al., 1998) is in accord with the above suggestion. Recently we demonstrated a clear geroprotective effect of melatonin administration (2 mg/l in night drinking water) in our Swiss mice (Anisimov et al., 2003b). Long-term administration of melatonin was also followed by the slow down of age-related switching-off of reproductive function in SHR and CBA mice and rats (Anisimov et al., 2001b, 2003b; Meredith et al., 1998). Thus, some effects of DSIP-containing preparation Deltaran (antioxidant, anticarcinogenic and geroprotector) as well as action of some other peptides regulating pineal function can be mediated by synthesis of endogenous melatonin. It should be noted that melatonin in pharmacological doses can reveal both anticarcinogenic activity and increase spontaneous tumor development (Anisimov, 2001; Anisimov et al., 2003a,b). Deltaran on the contrary showed in our experiment no adverse effect and in contrast to melato-

Table 8
Parameters of life span in female SHR mice treated and not treated with Deltaran

Group	Total no. of cases	Fatal tumor-bearing mice	Fatal tumor-tree mice
Number of mice			
Saline	50	15	35
Deltaran	50	5[a]	45
Mean life span (days)			
Saline	456±29	549±29	416±38
Deltaran	474±29	693±109	450±28
Mean life span of the last 10% of survivors (days)			
Saline	709±10	731±8	692±7
Deltaran	844±19*	917±0**	761±32*
Aging rate $\alpha \times 10^3$ ($days^{-1}$)			
Saline	4.55 (4.43; 4.86)	9.15 (9.02; 10.4)	2.80 (2.74; 3.20)
Deltaran	3.84 (3.83; 4.16)[#]	4.67 (3.66; 9.48)	4.48 (4.10; 5.04)[#]
MRDT (days)			
Saline	152.41	75.71	247.71
Deltaran	180.65[#]	148.57	154.82[#]

Note: Mean life spans are given as mean±standard error; 95% confidence limits are given in parentheses; MRDT, mortality rate doubling time. The difference with controls is significant: [a]$P < 0.05$ (Fischer's exact test); *$P < 0.05$; **$P < 0.01$ (Student t-test); [#]$P < 0.05$ (Cox's method).

nin it inhibited spontaneous tumor development. Some additional mechanisms not related to the synthesis of endogenous melatonin might be involved in Deltaran or DSIP positive effects on biomarkers of aging, used in this work, life span and spontaneous tumor incidence as well as in cases of stress-induced metabolic disturbances (Pollard and Pomfrett, 2001; Sudakov et al., 2001). Further research is required for better understanding molecular and physiological mechanisms of geroprotector and anticarcinogenic effects of Deltaran.

Acknowledgements

The authors are very thankful to James W. Vaupel for the opportunity to use the facilities of the Max Planck Institute for Demographic Research to complete this paper, and to I.I. Mikhailova and O.V. Novikova for excellent technical assistance.

References

Anisimov, V.N., 1987. Carcinogenesis and Aging, vol. 1 and 2. CRC Press, Boca Raton, FL.

Anisimov, V.N., 2001. Life span extension and cancer risk: myths and reality. Exp. Geront. 36, 1101–1136.

Anisimov, V.N., Khavinson, V.Kh., Morozov, V.G., 1994. Twenty years of study on effects of pineal peptide preparation: epithalamin in experimental gerontology and oncology. Ann. NY Acad. Sci. 719, 483–493.

Anisimov, V.N., Mylnikov, S.V., Khavinson, V.Kh., 1998. Pineal peptide preparation epithalamin increases the lifespan of fruit flies, mice and rats. Mech. Ageing Dev. 103, 123–132.

Anisimov, V.N., Arutjunyan, A.V., Khavinson, V.Kh., 2001a. Effect of pineal peptide preparation epithalamin on free radical processes in animals and humans. Neuroendocr. Lett. 22, 9–18.

Anisimov, V.N., Zavarzina, N.Y., Zabezhinski, M.A., Popovich, I.G., Zimina, O.A., Shtylik, A.V., Arutjunyan, A.V., Oparina, T.I., Prokopenko, V.M., Mikhalski, A.I., Yashin, A.I., 2001b. Melatonin increases both life span and tumor incidence in female CBA mice. J. Gerontol. Biol. Sci. 56A, B311–B323.

Anisimov, V.N., Alimova, I.N., Baturin, D.A., Popovich, I.G., Zabezhinski, M.A., Manton, K.G., Semenchenko, A.V., Yashin, A.I., 2003a. The effect of melatonin treatment regimen on mammary adenocarcinoma development in HER-2/neu transgenic mice. Int. J. Cancer 103, 300–305.

Anisimov, V.N., Alimova, I.N., Baturin, D.A., Popovich, I.G., Zabezhinski, M.A., Rosenfeld, S.V., Manton, K.G., Semenchenko, A.V., Yashin, A.I., 2003b. Dose-dependent effect of melatonin on life span and spontaneous tumor incidence in female SHR mice. Exp. Gerontol. 38, 449–461.

Arendt, J., 1995. Melatonin and the Mammalian Pineal Gland. Chapman and Hall, London.

Armstrong, S.M., Redman, J.R., 1991. Melatonin: a chronobiotic with anti-aging properties? Med. Hypotheses 34, 300–309.

Bartsch, H., Bartsch, C., Simon, W.E., Flehmig, B., Ebels, I., Lippert, T.H., 1992. Antitumor activity of the pineal gland: effect of unidentified substances versus the effect of melatonin. Oncology 49, 27–30.

Bartsch, C., Bartsch, H., Blask, D.E., Cardinali, D.P., Hrushesky, W.J.M., Meeke, D. (Eds.), The Pineal Gland and Cancer. Neuroimmunoendocrine Mechanisms in Malignancy. Springer, Berlin 2001.

Benson, B., 1977. Current status of pineal peptides. Neuroendocrinology 24, 241–248.

Bodyak, N.D., Nekhaeva, E., Wei, J.Y., Khrapko, K., 2001. Quantitation and sequencing of somatic deleted mtDNA in single cells: evidence for partially duplicated mtDNA in aged human tissues. Human Mol. Genetics 10, 17–24.

Bondarenko, T.I., Miliutina, N.P., Mikhaleva, I.I., Noskova, N.V., 1998. Meanbrane-stabilizing effect of delta sleep-inducing peptide during stress. Bull. Exp. Biol. Med. 126, 325–327.

Bondarenko, T.I., Miliutina, N.P., Shustanova, T.A., Mikhaleva, I.I., 1999. Regulatory effect of delta sleep-inducing peptide on the activity of antioxidant enzymes in erythrocytes and tissues of rats during cold stress. I.M. Sechenov' Russ. Physiol. J. 85, 671–679.

Butler, R.N., Fossel, M., Harman, S.M., Hewad, C.B., Olshansky, S.J., Perl, T.T., Rothman, D.J., Rothman, S.M., Warner, H.R., Wcst, M.D., Wright, W.E., 2002. Is there an antiaging medicine? J. Gerontol. Biol. Sci. 57A, B333–B338.

Chiodera, P., Volpi, R., Carpetti, L., Giacalone, G., Caffarri, G., Davoli, C., Nigro, E., Coiro, V., 1994. Different effects of delta-sleep-inducing peptide on argininc–vasopressin and ACTH secretion in normal men. Horm. Res. 42, 267–272.

Cos, S., Sanchez-Barcelo, E.J., 2000. Melatonin and mammary pathological growth. Front. Neuroendocrin. 17, I33–170.

Cox, D.R., Oakes, D., 1996. Analysis of Survival Data. Chapman and Hall, London.

Crowley, C., Curtis, H.J., 1963. The development of somatic mutations in mice with age. Proc. Natl. Acad. Sci. USA 49, 625–628.

De Grey, A.D.N.J., Baynes, J.W., Berd, D., Heard, C.H., Pawelec, G., Stock, G., 2002. Is human aging still mysterious enough to be left only to scientists? BioEssays 24, 667–676.

Dilman, V.M., 1994. Development, Aging and Disease. A New Rationale for and Intervention Strategy. Harwood, Chur, Switzerland.

Gart, J.J., Krewski, D., Lee, P.N., Tarone, S., Wahrendorf, J., 1986. Statistical Methods in Cancer Research. Vol. III—The Design and Analysis of Long-Penn Animal Experiments. IARC Scientific Publication 79. IARC, Lyon.

Gauss System and Graphic Manual, 1994. Aptech Systems, Inc., Maple Valley.

Goubler, E.V., 1978. Computing Methods of Pathology Analysis and Recognition. Meditsina, Leningrad.

Graf, M.V., Kastin, A.J., 1986. Delta-sleep-inducing peptide (DSIP): an update. Peptides 7, 1165–1187.

Graf, M., Christen, H., Tobler, H.J., Maier, P.F., Schoenenberger, G.A., 1981. Effects of repeat DSIP and DSIP-P administration on the circadian locomotor activity of rats. Pharmacol. Biochem. Behav. 15, 717–721.

Iyer, K.S., McCann, S.M., 1987. Delta sleep inducing peptide (DSIP) stimulates the release of LH but not FSH via a hypothalamic site of action in the rat. Brain Res. 19, 535–538.

Khvatova, E.M., Rubanova, N.A., Prudchenko, I.A., Mikhaleva, I.I., 1995. Effect of DSIP and some analogues on the activity of monoamine oxidase type A in rat brain under hypoxia stress. FEBS Lett. 368, 367–369.

Kruger, J.M., Karnovsy, M.L., 1995. Sleep as a neuroimmune phenomenon: a brief historical perspective. Adv. Neuroimmunol. 5, 5–12.

Lapin, V., Ebels, I., 1976. Effect of some low molecular weight sheep pineal fractions and melatonin on different tumours in rats and mice. Oncology 33, 11–13.

Lesnikov, V.A., Pierpaoli, W., 1994. Pineal cross-transplantation (old-to-young and vice versa) as evidence for an endogenous 'aging clock'. Ann. NY Acad. Sci. 719, 461–473.

Lysenko, A.V., Alperovich, D.V., Uskova, N.J., Mendzheritski, A.M., 1999. Metabolic features of the adaptive effect of delta-sleep inducing peptide and piracetam under hyperoxic conditions. Biochemistry (Moscow) 64, 652–657.

Malm, O.J., Skaug, O.E., Lingjaerde, P., 1959. The effect of pinealectomy on bodily growth. Acta Endocrinol. 30, 22–28.

Meredith, S., Jackson, K., Edwards, P., 1998. Long-term supplementation with melatonin delays reproductive senescence in female rats, without an effect on number of primordial follicles. Exp. Gerontol. 35, 343–352.

Moller, M., 1997. Petidergic cells in the mammalian pineal gland. Morphological implications for a paracrine regulation of the pinealocyte. Biol. Cell 89, 561–567.

Noteborn, H.P.J.M., Bartsch, L.L., Bartsch, C., Mans, D.R.A., Weusten, J.J.A.M., Flehmig, B., Ebels, I., Salemnik, C.A., 1988a. Partial purification of a low molecular weight ovine pineal compounds) with an inhibiting effect on the growth of human melanoma cells in vitro. J. Neural. Transm. 73, 135–155.

Noteborn, H.P., Graf, M.V, Ernst, A., Schoenenberger, G.A., Wensten, J.A., Ebels, I., Salemnik, C.A., 1988b. Purification and characterization of DSIP-like material from ovine pineal glands: possible peptide–protein interaction. J. Pineal Res. 5, 161–177.

Oaknin, S., Troiani, M.E., Webb, S.M., Reiter, R.J., 1986. Influence of delta-sleep inducing peptide on melatonin synthesis in the rat pineal gland. Neurosci. Lett. 70, 127–131.

Ouichou, A., Pevet, P., 1992. Implication of tryptophan in the stimulatory effect of delta sleep-inducing peptide (DSlP) on indoles secretion from perfused rat pineal glands. Biol. Signals 1, 78–87.

Ouichou, A., Zitouni, M., Raynaud, F., Simonneaux, V., Gharib, A., Pevet, P., 1992. Delta-slecp-inducing peptide stimulates melatonin, 5-methoxytryptophol and serotonin secretion from perfused rat pineal glands. Biol. Signals 1, 65–77.

Oxenkrug, G., Requintina, P., Bachurin, S., 2001. Antioxidant and antiaging activity of N-acetylserotonin and melatonin in the in vivo models. Ann. NY Acad. Sci. 939, 190–199.

Pevet, P., 1983. The different classes of proteic and peptidergic substances present in the pineal gland. In: Axelrod, J., Fraschini, F., Velo, G.P. (Eds.), The Pineal Gland and its Endocrine Role. Plenum press, New York–London, pp. 113–159.

Pierpaoli, W., 1998. Neuroimmunomodulation of aging. A program in the pineal gland. Ann. NY Acad. Sci. 840, 491–497.

Pierpaoli, W., Regelson, W., 1994. Pineal control of aging: effect of melatonin and pineal grafting on aging mice. Proc. Natl. Acad. Sci. USA 91, 787–791.

Pierpaoli, W., Dall'Ara, A., Pedrinis, E., Regelson, W., 1991. The pineal control of aging: the effects of melatonin and pineal grafting on survival of older mice. Ann. NY Acad. Sci. 621, 291–313.

Pollard, D.J., Pomfrett, C.J., 2001. Delta sleep-inducing peptide. Eur. J. Anaesthesiol. 18, 419–422.

Prudchenko, I.A., Stashevskaya, L.V., Shepel, E.N., Mikhaleva, I.I., Ivanov, V.T., Shmal'ko, Iu.P., Ckalyi, A.P., Umanskii, V.Iu., Grinzhevskaya, S.N., 1993. Synthesis and biological properties of delta-sleep peptide analogs. 2. Antimetastatic effect. Bioorg. Khim. 19, 1177–1190.

Reiter, R.J., 1995. The pineal gland and melatonin in relation to aging: a summary of the theories and of the data. Exp. Gerontol. 30, 199–212.

Reiter, R.J., Tan, D.X., Kim, S.J., Manchester, L.C., Qi, W., Garcia, J.J., Cabrera, J.C., El-Sokkary, G., Rouvier-Garay, V., 1999. Augmentation of indices of oxidative damage in life-long melatonin-deficient rats. Mech. Ageing Dev. 110, 157–173.

Reiter, R.J., Tan, D.X., Allegra, M., 2002. Melatonin: reducing molecular pathology and dysfunction due to free radicals and associated reactant. Neuroendocr. Lett. 23 (Suppl. 1), 3–8.

Reppert, S.M., Weaver, D.R., 1995. Melatonin madness. Cell 83, 1059–1062.

Rikhireva, G.T., Makletsova, M.G., Mendzheritskii, A.M., Vartanian, L.S., Gurevich, S.M., Lozovskaia, E.L., Kopylovskii, S.A., Rylova, A.V., Prudchenko, I.A., Mikhaleva, I.I., 1993. Changes in the intensity of free-radical reactions in the organs of rats under hypokinetic stress and with protection by the delta sleep-inducing peptide and its tyrosine-containing analog. Izv. Akad. Nauk Ser. Biol. 2, 243–256.

Rikhireva, G.T., Sokolova, I.S., Rylova, A.V., Kopylovslii, S.A., Mikhaleva, I.I., Prudchenko, I.A., 1995. Changes in the rate of protein biosynthesis in the organs of mice under the action of the delta sleep-inducing peptide and psychocmotional stress. Izv. Akad. Nauk SSSR, Ser. Biol. 2, 142–148.

Rosenfeld, S.V., Togo, E.F., Mikheev, V.S., Popovich, I.G., Zabezhinski, M.A., Anisimov, V.N., 2001. Dynamics of chromosomal aberrations in male mice of various strains during aging. Bull. Exp. Biol. Med. 131, 482–483.

Rosenfeld, S.V., Togo, E.F., Mikheev, V.S., Popovich, I.G., Khavinson, V.Kh., Anisimov, V.N., 2002. Effect of epitalon on the incidence of chromosomal aberrations in senescence-accelerated mice. Bull. Exp. Biol. Med. 133, 274–276.

Sahu, A., Kalra, S.P., 1987. Delta sleep-inducing peptide (DSIP) stimulates LH release in steroid-primed ovariectomized rats. Life Sci. 40, 1201–1206.

Sato, S., Taketomi, M., Nakajima, M., Kitazawa, M., Shimada, H., Itoh, S., Igarashi, M., Higashikuni, N., Sutou, S., Sisaki, Y.F., 1995. Effect of aging on spontaneous micronucleus frequencies in peripheral blood of nine mouse strains. Mutat. Res. 338, 5I–57.

Schoenenberger, G.A., Monnier, M., 1977. Characterization of a delta-electroencephalogram (-sleep)-inducing peptide. Proc. Natl. Acad. Sci. USA 74, 1282–1286.

Shmal'ko, Iu.P., Mikhaleva, I.I., 1988. Antimetastatic effect of the delta-sleep peptide during stress in mice with Lewis' lung carcinoma. Exp. Onkol. 10 (2), 57–60.

Shustanova, T.A., Bondarenko, T.I., Milyutina, N.P., Mikhaleva, I.I., 2001. Regulation of free radical processes by delta-sleep inducing peptide in rat tissue under cold stress. Biochemistry (Moscow) 66, 632–639.

Simonneaux, V., Ribeylaga, C., Miguez, J.M., 1997a. Peptidergic modulation of rat pineal synthetic and secretory activities: example of VIP and NPY. In: Webb, C., Puig, M., Moller, M., Pevet, P. (Eds.), Pineal Update: From Molecular Biology to Clinical Medicine. PJD Publication, Westbury, NY, pp. 149–160.

Simonneaux, V., Vuillez, P., Eder, U., Miguez, I.M., Pevet, P., Fischer-Colbrie, R., 1997b. Secretoneurin: a new neuropeptide in the rodent pineal gland. Cell Tissue Res. 288, 427–434.

Simonneaux, V., Rodeau, J.L., Calgari, C., Pevet, P., 1999. Neuropeptide Y increases intracellular calcium in rat pinealocytes. Eur. J. Neurosci. 11, 725–728.

Skagerberg, G., Bjartell, A., Vallet, P.G., Charnay, Y., 1991. Immunohistochemical demonstration of DSIP-like immunoreactivity in the hypothalamus of the rats. Peptides 12, 1155–1159.

Sudakov, K.V., 1991. The antistress effects of the delta sleep-inducing peptide. I.M. Sechenov Physiol. J. SSSR 77 (3), 1–13.

Sudakov, K.V., Umrykhin, P.E., Koplik, E.V., Anoknin, K.V., 2001. Expression of the e-fos gene during emotional stress in rats: the clocking effect of delta sleep-inducing peptide. Neurosci. Behav. Physiol. 31, 635–640.

Turusov, V.S., Mohr, U., (Eds.), (1994). Pathology of Tumours in Laboratory Animals, Tumours of the Mouse. Volume 1., second ed., IARC Sci. Pub. No. 111. IARC, Lyon.

Vijg, J., 2000. Somatic mutations and aging: a re-evaluation. Mutat. Res. 447, 117–135.

Vivien-Roels, B., Malan, A., Rettori, P., Delagrange, P., Jeanniot, J.P., Pevet, P., 1998. Daily variations in pineal melatonin concentrations in inbred and outbred mice. J. Bio. Rhythms 13, 403–409.

Weindruch, R., Walford, R.L., 1988. The Retardation of Aging and Disease by Dietary Restriction. C.C. Thomas, Springfield, IL.

Yehuda, S., Carasso, R.L., 1988. DSIP—a tool for investigating the sleep onset mechanisms: a review. Int. J. Neurosci. 38, 345–353.

Yeguda, S., Kastin, A.J., Coy, H., 1980. Thermoregulatory and locomotor effect of DSIP: paradoxal interaction with amthetamine. Pharmacol. Biochem. Behav. 13, 895–900.

Yeguda, S., Caspi, T., Carasso, R.L., 1988. The circadian cycle effects of DSIP on colonic temperature blood pressure and heat rate in control and area postrema lesioned rats. Int. J. Neurosci. 42, 259–265.

Yuwiler, A., Brammer, G.L., 1993. Neurotransmitters and peptides in the pineal gland. In: Wetterherg, L. (Ed.), Light and Biological Rhythms in Man. Pergamon Press Ltd, Oxford, pp. 133–144.