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PLENARY LECTURES

Melatonin in Clinical Oncology

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Numerous studies clearly show that melatonin inhibits experimental tumor growth both under *in vivo* and under *in vitro* conditions if the pineal hormone is given at the correct circadian stage and if the respective malignant cells are susceptible. Animals have to be treated with melatonin towards the end of the light-phase to achieve tumor-inhibition and have to be kept preferably under short photoperiods (less than 12 hours of light per 24 hours). Administration of melatonin at other times can either lead to no effect or even to tumor-stimulation. Tumor cells that are

inhibited by melatonin were found to show the presence of either membrane-bound or nuclear melatonin receptors and/or a functional estrogen-response system. Recent studies indicate that the tumor-inhibitory action is also exerted via a modified metabolism of unsaturated fatty acids (i.e. linoleic acid). First clinical trials essentially performed by the group of Lissoni in Monza, Italy mainly treating patients with advanced and incurable malignancies showed that melatonin leads to an improvement of the quality of life by reducing pain and inducing sleep. In combination

with interleukin-2 melatonin improved the therapeutic efficacy of this type of therapy and reduced side-effects. The analysis of melatonin in cancer patients as well as in tumor-bearing animals revealed that the circulating levels of the hormone are reduced if large and poorly differentiated tumors without metastases are present. As opposed to that nocturnal melatonin appears to be elevated in case of small and/or highly differentiated tumors. The mechanisms involved in this phenomenon seem to include complex neuroimmunoendocrine interactions whereas the depression of melatonin may be due to a modified peripheral metabolism, perhaps

under the direct influence of the tumor. These metabolic changes leading to a degradation of the hormone may in part explain why large and undifferentiated tumors are not inhibited by the pineal hormone. Details of the underlying biochemical mechanisms require urgent clarification and could help to devise methods to overcome tumor-refractoriness to melatonin. Since melatonin acts best on early stages of tumor growth it would be highly desirable to extend further clinical trials to patients with freshly diagnosed tumors administering it in combination with established standard oncological therapies.

Melatonin and Alzheimer's Disease (AD)

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Sleep disturbances are common among persons with AD. Cross-sectional studies report that about 45% of patients have disruptions in their sleep. When sleep disturbances do occur, they constitute a significant physical and psychological stress for the caregiver and are related to patient institutionalization. A phenomenon related to the sleep disturbances in AD patients is "sundowning". Symptoms of sundowning agitation include a reduced ability to maintain attention to external stimuli, disorganized thinking and speech, a variety of motor disturbances including agitation, wandering and repetitious physical behaviors, and perceptual and emotional disturbances. The delirium-like symptoms associated with sundowning are most prevalent in the late afternoon to early evening. Management of sundowning is difficult because most drugs have undesirable side effects in AD. Shifts in the basic circadian sleep-wake rhythm of AD patients can be severe, and in extreme cases may lead to complete day/night sleep pattern reversals. In the later stages of disease, AD patients spend approximately 40% of their time in bed awake, and a significant proportion of their daytime hours asleep. This increased daytime sleep consists almost exclusively of stage 1 and 2 sleep, and poorly compensates for night-time losses of sleep, particularly of non REM sleep. Since we previously reported in older insomniacs that melatonin augmented non-REM sleep, we have used melatonin to treat sleep disorder and sundowning in AD patients since 1995. In our first study [Fainstein et al., *Current Ther Res* 1997] we reported that 7 out of 10 dementia patients having sleep disorders and treated with melatonin (3 mg p.o. at bed time) showed a decreased sundowning. In a retrospective study of 14 AD patients receiving 9 mg melatonin daily for 22 to 35 months we observed a significant improvement of sleep quality in all cases, sundowning being not longer detectable in 12 of them [Brusco et

al., *Neuroendocrinol Lett* 1998). Interestingly, in this latter study, clinical, neuropsychologic and neuropsychiatric evaluation indicated stabilization of behavioral and cognitive parameters. We also studied two monozygotic twins with AD and a similar cognitive and neuroimaging alteration [Brusco et al., *J Pineal Res* 1998]. Patients were treated with vitamin E and one of them received melatonin (6 mg orally) at bedtime daily for 57 months. At the time of publication (36 months of treatment), differences in functional stage of AD between twins were reported with improvement of sleep quality and abolition of sundowning after melatonin treatment. Melatonin is known to interfere *in vitro* with β -amyloid-related processes. In addition, melatonin treatment, which promotes non-REM sleep, can be beneficial by augmenting the restorative phases of sleep, including the augmented secretion of GH. Measurement of endogenous melatonin levels suggested that sleep and behavior disorders in the dementia group can be related to a decrease in the amplitude of the melatonin secretion rhythm. Melatonin treatment may constitute a selection therapy to ameliorate sundowning. In addition, melatonin treatment at a relatively moderate pharmacological dose appears to slow evolution of cognitive impairment in AD patients. To what extent this is due to an effect of melatonin on β -amyloid-related processes or is the consequence of an improvement in sleep quality and reduction of sundowning agitation awaits further investigation.

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Melatonin in Sleep Disorders and Jet-Lag

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Melatonin is synthesized and secreted during the dark period of the light/dark cycle. The rhythmic nocturnal melatonin secretion is directly generated by the circadian clock located in the suprachiasmatic nuclei (SCN), and is entrained to a 24-hour period by the light-dark cycle. The periodic secretion of melatonin can be used as a circadian mediator to any system than can "read" the message. In addition, direct effects of the hormone on the SCN could explain some of melatonin effects on the circadian system. Melatonin administration synchronizes the sleep-wake cycle in blind people and in individuals suffering from jet lag or delayed sleep phase syndrome. In elderly insomniacs, melatonin administration decreased sleep latency and increased sleep efficiency. The effect of melatonin on sleep is probably the consequence of increasing sleep propensity (by inducing a fall in body temperature) and of a synchronizing effect on the circadian clock (chronobiotic effect). Melatonin secretion correlates with sleepiness in sighted and blind people. Urinary levels of 6-sulphatoxymelatonin decrease with age and in chronic diseases like coronary heart disease. Data will be shown indicating that patients with coronary disease had a low melatonin production rate, with higher decreases in those with higher risk of cardiac infarction. Administration of melatonin (3 mg p.o.) for up to 6 months did not affect circulating prolactin, FSH, TSH or estradiol in elderly insomniac females. In this group of patients melatonin augmented sleep quality and duration, and decreased sleep latency and the number of awakening episodes, reducing significantly benzodiazepine use. Estimates of next-day function also improved significantly. Since

rapid transmeridian translocation through multiple time zones has a negative impact on athletic performance, we recently tested the timely use of three factors (melatonin treatment, exposure to light, physical exercise) to hasten the resynchronization of a group of elite sports competitors to a transmeridian flight comprising 12 time zones. Twenty-two male subjects were included in the study. They were professional soccer players and their coaches who traveled to Tokyo to play the final game of the Intercontinental Cup. Participants were asked to complete sleep log diaries from day -1 (pre-flight) to the day before returning to Buenos Aires (day 7). All subjects received 3 mg of melatonin p.o. daily at expected bedtime at Tokyo immediately after leaving Buenos Aires. Upon arrival at Tokyo the subjects performed a daily physical exercise routine outdoors at two restricted times of the day (from 0830 h to 1130 h in the morning and from 1500 h to 1800 h in the afternoon). Exposure to sunlight or physical exercise at other times of the day was avoided. Generally, there was an absence of significant changes in subjective sleep parameters as compared to pre-flight assessment. Sleep quality and morning alertness at Tokyo correlated significantly with pre-flight 6-sulphatoxymelatonin excretion. Mean resynchronization rate of sleep-wake cycle to the 12 h-time shift was 2.13 (0.88 days). The results indicate that the combination of melatonin treatment, an appropriate environmental light schedule and timely applied physical exercise are useful to help elite athletes to overcome the consequences of jet-lag.

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Regulation of IL-2 Production by Melatonin in Human Lymphocytes: A Possible Autocrine Mechanism

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Evidence has been accumulated demonstrating that melatonin is able to regulate the human immune system. Both *in vitro* and *in vivo* studies show that melatonin binds to immune cells by interacting with receptors located on the membrane surface (mt₁ and mt₂ receptors) and in the nucleus (RZR/ROR α nuclear receptor family). As a consequence of this interaction, melatonin increases IL-2 and IFN γ production by CD4 cells and IL-6 production by monocytes (CD14 cells). Under physiological conditions the origin of melatonin

interacting with these cells remains unknown. In this abstract we show that CD4 cells isolated and cultured in RPMI medium are able to bind melatonin, to express mRNA for the mt1 and mt2 membrane receptors, to express mRNA for the ROR (α 1, α 2, and α 3) and RZR α nuclear receptors, and synthesize IL-2 depending on the dose of melatonin added to the culture medium. Moreover, the same cells express the mRNA for hydroxyindole-O-methyltransferase (HIOMT), the last enzyme involved in the metabolic pathway syn-

thesizing melatonin. Finally, the cells release large amounts of melatonin to the medium, reaching melatonin concentrations higher than 100 nM in the culture

medium. In conclusion, analyzing all these data as a whole is possible to consider that melatonin behaves as an autocrine molecule in the immune system.

Melatonin and Magnetic Fields

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Extremely low frequency electric (ELF-EF) and magnetic fields (ELF-MF), e.g. generated by high-voltage transmission lines and household appliances, are present worldwide and receive increasing attention because of their potential consequences for human health. Moreover, magnetic fields are used in human physiotherapy because of some beneficial effects. There is substantial evidence that exposure to magnetic fields may alter melatonin secretion in animals. This has been shown for example in rats [Kato et al., *Neurosci Lett* 1994;**166**:59–62; Huuskonen et al., *Reprod Toxicol* 2001;**15**:49–59], hamsters [Yellon et al., *J Pineal Res* 1994;**16**:136–144], and fish [Lerchl et al., *Neurosci Lett* 1998;**256**:171–173]. However, large variations exist when studies are repeated [Loscher et al., *Radiat Res* 1998;**150**:557–567; Reiter et al., *Bioelectromagnetics* 1998;**19**:318–329]. Among other explanations [Anderson et al., *Environ Health Perspect* 2001;**108**:797–802], one reason may be the age of the animals since young rats seem to be more sensitive against magnetic fields than old ones [Selmaoui and Touitou, *Life Sci* 1999;**64**:2291–2297]. Recently, an investigation on isolated hamster pineal organs has shown variable results when the experiments were repeated under identical conditions at 16 2/3 and 50 Hz. However, exposure was found to suppress melatonin synthesis highly significantly when the results were pooled [Brendel et al., *J Pineal Res* 2000;**29**:228–233].

In contrast to animal studies, the data on the influence of magnetic fields on human melatonin concentrations are scarce and contradictory. Moreover, magnetic fields of various parameters have been employed in the experiments. Exposure to magnetic fields resulted in both decreases and increases in melatonin concentrations. Moreover, in several studies no effect of magnetic fields on melatonin levels was observed. These discrepancies may depend on different experimental paradigms, including differences in certain characteristics of the applied magnetic fields, such as field intensity, frequency, duration of exposure, timing of exposure, applied vector, etc. The results of our studies seem to support the hypothesis that the response of the human pineal to magnetic fields may depend on the field parameters because chronic exposure to 2.9 mT, 40 Hz magnetic field caused a significant decrease in nocturnal melatonin concentrations [Karasek et al., *J Pineal Res* 1998; **25**:240–244], whereas chronic exposure to 25–80 μ T, 200 Hz magnetic field did not influence melatonin levels [Karasek et al., *J Pineal Res* 2000; **29**:81–85]. Summarizing these data it seems that presently there are no convincing data showing a distinct effect of magnetic fields on melatonin secretion in humans, and more studies are needed to identify the specific circumstances under which such effects may occur.

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Melatonin and Aging

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Although many theories relating the pineal secretory product melatonin to aging have been put forward, the role of this agent in the aging process is not clear. There are several reasons for postulated potential importance of melatonin in this process. *First*, melatonin participates in many vital life processes, and its

secretion falls gradually over the life-span. Melatonin concentrations exhibit clear circadian rhythm with low values during the daytime, and 5–6 fold increase at night. This rhythm develops around 6th month of life and reaches the highest levels between 4th and 7th year of age. Around maturation there may be a drop in mela-

tonin concentrations, and thereafter its levels diminish gradually. In many individuals beyond 65 years of age, a day-night rhythm is almost absent. The amplitude of nocturnal melatonin secretion is believed to be genetically determined, and shows great differences among individuals. Thus, some individuals produce significantly less melatonin during their lifetime than others, which may have significance in terms of aging. *Second*, the diminished melatonin secretion in advanced age may be related to deterioration of many circadian rhythms, as a consequence of reduced function of suprachiasmatic nuclei. *Third*, the recently discovered role of melatonin in scavenging of free radicals, and the proposed link between oxidative stress and aging itself as well as age-related diseases (such as neoplastic disease, Alzheimer and Parkinson diseases) suggest a role for melatonin in these processes. Melatonin is a very effective antioxidant. It scavenges both hydroxyl radicals and peroxy radicals, although it is a more effi-

cient direct scavenger of highly toxic hydroxyl radicals. Additionally, melatonin stimulates a number of antioxidative enzymes, e.g. glutathione peroxidase and glutathione reductase. *Forth*, melatonin acts as endogenous sleep-inducing agent, and its reduced concentrations may result in reduced sleep efficacy very often associated with advancing age.

Finally, melatonin exhibits immunoenhancing properties, and suppressed immunocompetence has been implicated in the acceleration of aging processes. The aging process is multifactorial, and no single element seems to be of basic importance. It seems, however, that although melatonin cannot be univocally recognized as a substance delaying aging, some of its actions may be beneficial for the process of aging. However, the precise role of melatonin in the aging process remains to be determined.

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Potential Anticarcinogenic Action of Melatonin and Other Antioxidants Mediated by Antioxidative Mechanisms

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The complex process of carcinogenesis is, to a large extent, due to oxidative stress. Numerous indicators of oxidative damage are enhanced as the result of carcinogen action. Several antioxidants, with different efficacy, protect against oxidative abuse caused by carcinogenic agents. Recently, melatonin (N-acetyl-5-methoxytryptamine) and some other indoleamines, have gained particular meaning in the defense against oxidative stress and, consequently, carcinogenesis. It is noteworthy that certain antioxidants, like ascorbic acid, play a bivalent role in antioxidative defense, revealing, under specific conditions, prooxidative effects. Among known antioxidants, melatonin is particularly frequently applied

in experimental models on anticarcinogenic action. Having examined several parameters of oxidative damage in *in vitro* and *in vivo* models, numerous authors have concluded that this indoleamine is able to protect DNA and cellular membranes from the carcinogen-induced oxidative abuse. When preventing or decreasing oxidative damage to macromolecules, melatonin – at the same time – protects against cancer initiation. The protection caused by melatonin and some other antioxidants against cellular damage, following carcinogen action, make them potential therapeutic supplements in conditions of higher risk of cancer.

Extrapineal Melatonin in Pathology: New Perspectives for Diagnosis, Prognosis and Treatment of Illness

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During the last decade, attention has centered on melatonin – one of the hormones of the diffuse neuroendocrine system which for many years was considered only as a hormone of the pineal gland. Currently, melatonin has been identified not only in the pineal gland, but also in extrapineal tissues – retina, Harderian gland, gut mucosa, cerebellum, airway epithe-

lium, liver, kidney, adrenals, thymus, thyroid, pancreas, ovary, carotid body, placenta and endometrium as well as in non-neuroendocrine cells like mast cells, natural killer cells, eosinophilic leukocytes, platelets and endothelial cells. The above list of the cells storing melatonin indicates that melatonin has a unique position among the hormones of the diffuse neuroendocrine

system, being found in practically all organ systems. Functionally, melatonin-producing cells are certain to be part and parcel of the diffuse neuroendocrine system as a universal system of response, control and organism protection. Taking into account the large number of melatonin-producing cells in many organs, the wide spectrum of biological activities of melatonin and especially its main property as a universal regulator of biological rhythms, it should be possible to consider extrapineal melatonin as a key paracrine signal molecule for the local coordination of intercellular relation-

ships. Analysis of our long-term clinical investigations shows the direct participation and active role of extrapineal melatonin in the pathogenesis of tumor growth and many other non-tumor pathologies such as gastric ulcer, immune diseases, neurodegenerative processes, radiation disorders, etc. The modification of antitumor and other specific therapy by the activation or inhibition of extrapineal melatonin activity could be useful for the improvement of the treatment of illness.

Melatonin and Human Reproduction

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Melatonin is critically involved in reproductive processes of many animal species, especially in those living in polar and moderate habitats. The correct adaptation of reproduction to the appropriate time of the year is essential for surviving of the offspring. Also humans, although generally considered non-seasonal, show distinct seasonal variations of reproduction with amplitudes as high as $\pm 15\%$ from the annual mean [Roeneberg and Aschoff, *J Biol Rhythms* 1990;**5**:217–239]. These variations are likewise dependent on the latitude with lowest amplitudes being observed in equatorial regions. Since seasonal melatonin fluctuations are also observed in humans [Vondrasova et al., *Brain Res* 1997;**759**:166–170], the question arises whether melatonin has any effect on human reproduction. The pars tuberalis of the adenohypophysis plays the crucial role in transducing the melatonin signal into changes of pituitary hormones. However, due to technical reasons, so far no functional role of the human pars tuberalis has been identified, especially with respect to its possible modulation by melatonin. Nevertheless, there are a number of findings in men indicating that high levels of melatonin are in fact associated with low

levels of pituitary hormones [Lerchl et al., *J Pineal Res* 1995;**18**:41–48; Luboshitzky et al., *J Molec Neurosci* 1996;**7**:91–98; Ozata et al., *J Clin Endocrinol Metab* 1996;**81**:1877–1881). These findings are, however, no proof for any causal relationship [Luboshitzky and Lavie, *J Pediatr Endocrinol Metab* 1999;**3**:355–362]. In fact it has been shown that testosterone may suppress melatonin in male patients with GnRH deficiency [Luboshitzky et al., *Clin Endocrinol [Oxf.]* 1997;**47**:463–469]. On the other hand, exogenously administered melatonin suppresses LH and testosterone in men while comparatively high doses of the pineal hormone have been shown to suppress LH levels and ovulation in women [Voordouw et al., *J Clin Endocrinol Metab* 1992;**74**:108–117]. These data indicate that pathologically high or low levels of melatonin are associated with altered functions of the hypothalamus – pituitary – gonadal axis. Likewise, pharmacological doses of melatonin may influence this system. From that point of view, there is not enough information available to clearly exclude possible long-term effects of melatonin on the reproductive functions in humans.

Regulation of Melatonin Secretion in the Mammalian Pineal Gland – Phenomenon of Species Heterogeneity

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Melatonin (MEL) exhibits a prominent diurnal rhythm of its synthesis and secretion in the pineal gland of most investigated mammalian species. The rhythm is driven by the suprachiasmatic nucleus, which activity is modulated by the environmental light acting via the retina and neural pathways connecting the retina

with the hypothalamus. The output from the suprachiasmatic nucleus to the pineal gland is formed by a multisynaptic pathway, which reaches this gland as the sympathetic nerve fibers. Norepinephrine, released from these fibers, is the main neurotransmitter regulating the synthesis and secretion of MEL in mammals.

The majority of studies related to the mechanism of adrenergic control of MEL synthesis in the mammalian pinealocytes has been performed using the rat pineal gland. Their results enabled preparing a model of regulation of MEL synthesis and secretion, which seems to be rather coherent. However, an increasing number of evidence shows that this model is not universal and cannot be transposed to other mammalian species. In the rat pinealocytes MEL synthesis is regulated via the synergistic dual receptor mechanism involving β_1 and α_1 adrenoceptors. Stimulation of β_1 -adrenergic receptors leads to the increase in cAMP production. Activation of α_1 -adrenoceptor, which by itself does not alter the activity of adenyl cyclase, potentiates drastically β -adrenergic stimulation of cAMP formation. The elevated level of cAMP activates transcription of arylalkylamine N-acetyltransferase (NAT) – the enzyme limiting MEL synthesis and this process results in the stepwise increase in MEL secretion in the rat pineal. The dual (β and α) receptors model of adrenergic stimulation of MEL

secretion is not valid in the pineal gland of sheep, cattle and pigs, where MEL secretion is regulated by β -adrenoceptors and α_1 -adrenoceptors did not potentiate this process. In the sheep the level of MEL secretion is regulated mainly at a posttranscriptional level, which may explain the rapid increase in MEL secretion in this species after adrenergic stimulation or onset of night. Moreover, in the domestic pig the increase in MEL secretion *in vitro* following an adrenergic stimulation (which like in the sheep is very fast) is independent of the processes of transcription and translation of new proteins.

Summing up, the mechanisms of regulation of MEL synthesis in the mammalian pinealocytes differ significantly between species. These differences are probably responsible for the existence of three types (A, B and C) of diurnal patterns of MEL secretion. They may also explain other interspecies differences in MEL secretion as well as the variability in the ultrastructure of the mammalian pineal gland.

Melatonin and the Thyroid Gland

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This review briefly summarizes the published data on the influence of melatonin (Mel) – the main pineal hormone – on the thyroid function and growth processes. The prevailing part of studies was performed in our laboratory, however, some data result from a cooperation with other authors. The involvement of Mel in the control of proliferation of thyroid follicular cells has been – for a long time – the subject of intensive research.

We have shown that: **1.** Melatonin, when administered to mice in the late-afternoon (16.00–18.00) s.c. injections for 10 days, inhibited the basal and TSH-stimulated mitotic activity of the thyroid follicular cells; we succeeded to demonstrate a similar inhibitory effect of Mel on the rat thyroid follicular cells *in vitro* [Lewinski and Sewerynek, *J Pineal Res*, 1986;**3**:291–299]. **2.** Light restriction, which is known to increase the activity of the pineal gland, inhibited the thyroid growth in male mice [Lewinski et al., *Experientia* 1984;**40**:1284–1285]. **3.** Melatonin suppressed the pinealectomy-induced increase of mitotic incidence in the rat thyroid gland [Wajs et al., *Med Sci Res* 1989;**17**:61–62]. **4.** Short-term s.c. administration of Mel (5 days) resulted in a dose-dependent effect on ^3H -thymidine incorporation into DNA of rat thyroid lobes, transferred – after collecting – into incubation *in vitro*; Mel, at dose of 25 $\mu\text{g}/\text{daily}$, was effective in reducing ^3H -thymidine incorporation, Mel – at dose of 50 $\mu\text{g}/\text{daily}$, produced no effect, while the high-

est dose of Mel (100 $\mu\text{g}/\text{daily}$) brought about an increase of ^3H -thymidine uptake [Wajs et al., *Neuroendocrinol Lett* 1992;**14**:75–81]. **5.** The inhibitory action of Mel *in vitro* is not so pronounced as the *in vivo* effects, however, in the concentration of 10^{-9}M the hormone in question reduced ^3H -thymidine incorporation into DNA of rat thyroid lobes [Wajs and Lewinski, *Biochem Biophys Res Commun* 1991;**181**:1187–1191]. **6.** Melatonin pellets, after s.c. implantation to rats (under the skin of back), prevented the inhibitory effect of late-afternoon Mel injections on the thyroid growth processes [Wajs and Lewinski, *J Pineal Res* 1992;**13**:158–166; Lewinski et al., In: *Melatonin and the pineal gland – from basic science to clinical application*, *Excerpta Medica*, Amsterdam, 1993;265–268]. These results suggest a counterantithyroid action of Mel pellets on the growth-inhibiting response of the gland, following Mel injections. **7.** Both Mel and 5-methoxytryptamine decreased the mean nuclear volume of the thyroid follicular cells in Syrian hamsters; Mel was more efficient than 5-methoxytryptamine [Lewinski et al., *Neuroendocrinol Lett* 1986;**8**:63–68]. **8.** An administration of Mel and of its precursor – N-acetylserotonin (NAS) – decreased the mitotic activity of the thyroid gland male rats [Sewerynek et al., *Endokrynol Pol* 1988;**39**:269–275].

The inhibitory effect of short photoperiod on the thyroid growth processes in mice, as shown by us [Lewinski

et al., *Experientia* 1984;**40**:1284–1285], was confirmed by Haldar et al. [*J Neural Transm* 1992;**90**:45–52] in an experiment performed on Indian palm squirrels (*Funambulus pennanti*). It is to be stressed that the involvement of the pineal gland in the photoperiod response of the thyroid cannot be excluded, since the changes of Mel concentrations, following a short photoperiod exposure, were simultaneously observed [Haldar et al., *J Neural Transm* 1992;**90**:45–52]. Moreover, several lines of evidence speak in favour of a direct influence of Mel on thyroid follicular cells. The direct effect of the pineal gland on the thyroid can also be concluded from the observation that the pituitary is not required to demonstrate the increase in thyroid weight after pinealectomy in mice [Houssay et al., *J Invest Derm* 1966;**47**:230–234,] and rats [Houssay and Pazo, *Experientia* 1968;**24**:813–814]. The pineal gland exerts also antithyroid effects in rodents as regards hormone secretion and these effects are attributed to Mel action, as well. Late afternoon s.c. injections of Mel decreased circulating thyroid hormone concentrations in adult Syrian hamsters of both sexes (Mel – 25 µg/daily) [Vaughan et al., *Neuroendocrinology* 1984;**39**:361–366] and in male Wistar rats (Mel – 50 µg/daily) [Krotewicz et al., *Neuroendocrinol Lett* 1992;**14**:405–411].

We also investigated the influence of pinealectomy and mel treatment of pinealectomized male Wistar rats on thyroid hormone secretion. Serum L-thyroxine (T_4) concentrations were increased in 10 weeks after pinealectomy. The administration of Mel to rats subjected to pinealectomy prevented the elevation of T_4 level; the concentration of triiodothyronine (T_3) remained unchanged in the pinealectomized rats [Krotewicz and Lewinski, *Biomed Lett* 1994;**50**:101–107]. In male Wistar rats, we found that a chronic administration of Mel – released from s.c. implanted pellets – increased both T_3 and T_4 levels after 10 days and also, however to a lesser degree, after 10 weeks; this effect may be called the “prothyroid” action of Mel [Krotewicz et al., *Neuroendocrinol Lett* 1992;**14**:405–411]. On the other hand, the joint effect of late-afternoon Mel injections and Mel implants caused no changes in thyroid hormone concentrations [Krotewicz et al., *Neuroendocrinol Lett* 1992;**14**:405–411]. Not only chronic Mel availability, but also a short-term treatment with the hormone, may, under certain conditions, result in a “prothyroid” action. Unexpectedly, when Mel is injected at a dose of 25 µg/daily to rats for 5 consecutive days in the late light phase, it increases serum T_3 concentration and reveals a slight tendency towards serum T_4 rise [Krotewicz and Lewinski, *Neuroendocrinol Lett* 1994;**16**:263–268]. In the same study, also a 5-day treatment with NAS resulted in the “prothyroid” effect, concerning thyroid secretory processes.

All the above mentioned results, while proving the inhibition of thyroid growth and/or thyroid function by

the pineal hormone, as well as other reports – on the stimulation of the pineal gland activity and growth processes by the thyroid hormones (not discussed in this presentation), have allowed us to formulate a hypothesis on the existence of a reciprocal relationship between the thyroid and the pineal [Lewinski et al., *Med Hypothesis* 1984;**14**:141–160; Lewinski, *Adv Pineal Res* 1990;**4**:175–188]. In agreement with this hypothesis, Mel could act directly on thyroid follicular cells, inhibiting their proliferation. Accordingly, it is possible that the serum concentration of thyroid hormones is a direct modulator of the pineal function and growth. The clinical data on the pineal-thyroid relationship are scarce. No changes were observed in Mel levels in both hypothyroidism and hyperthyroidism in human subjects [Soszynski et al., *Acta Endocrinol (Copenh.)* 1988;**119**:240–244]. A decrease in nocturnal Mel concentration was observed in patients subjected to surgical treatment because of recurrent non-toxic nodular goitre when compared to healthy controls [Kuzdak, *Endokrynol Pol – Polish J Endocrinol* 1995;**46** (suppl. 2 to no. 2):59–68]. It is known that surgical removal of a very large goitre may traumatize adjacent anatomical structures. Assuming the above, i.e., taking into account that the manipulations involving superior cervical ganglia may alter Mel secretion, Karasek et al. [*Neuroendocrinol Lett* 2000;**21**:437–439] decided to study diurnal serum Mel profiles in patients with a very large goitre before and after the surgery (subtotal thyroidectomy). The authors recorded that nocturnal serum Mel concentrations (at 24:00, 02:00 and 04:00 hours) were significantly higher after the surgical treatment than before intervention. They have drawn a conclusion that the goitre of a very large size can – possibly – compress the superior cervical ganglia, and – in consequence – alter indirectly the Mel synthesis.

Summing up, much evidence has been accumulated, indicating – in experimental conditions – the mutual relationship between the pineal gland and the thyroid. Confirmation of these relations in clinical studies in humans meets many difficulties, resulting – among others – from the fact that – nowadays – human beings, as well as certain animal species used in experimental studies, left far away their natural habitat. It makes almost impossible to compare the results obtained in particular studies, performed in different species, on the pineal-thyroid interrelationship.

The Immunotherapeutic Potential of Melatonin

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The interaction between the brain and the immune system is essential for the adaptive response of the organism against environmental challenges. In this context, the pineal neurohormone melatonin (MEL) plays an important role. T-helper cells express G-protein coupled MEL cell membrane receptors and, perhaps, MEL nuclear receptors. Activation of MEL receptors enhances the release of T-helper cell type 1 (Th1) cytokines, such as gamma-interferon (γ -IFN) and interleukin-2 (IL-2), as well as of novel opioid cytokines. MEL has been reported also to enhance the production of interleukin-1 (IL-1), interleukin-6 (IL-6) and interleukin-12 (IL-12) in human monocytes. These mediators may counteract stress-induced immunodepression and other secondary immunodeficiencies and protect mice against lethal viral encephalitis, bacterial diseases and septic shock. Therefore, MEL has an interesting immunotherapeutic potential in both viral and bacterial infections. MEL may also influence haemopoiesis either by stimulating haemopoietic cytokines, including opioids, or by directly affecting specific progenitor cells such as pre-B cells, monocytes and NK cells. MEL may thus be used to stimulate the immune response during viral and bacterial infections as well as to strengthen the immune reactivity as a prophylactic procedure. In both mice and cancer patients, the haemopoietic

effect of MEL may diminish the toxicity associated with common chemotherapeutic protocols. Through its pro-inflammatory action, MEL may play an adverse effect in autoimmune diseases. Rheumatoid arthritis patients have increased nocturnal plasma levels of MEL and their synovial macrophages respond to MEL with an increased production of IL-12 and NO. In these patients, inhibition of MEL synthesis or MEL antagonists might have a therapeutic effect. In other diseases such as multiple sclerosis the role of MEL is controversial. However, the correct therapeutic use of MEL or MEL antagonists should be based on a complete understanding of their mechanism of action. It is not yet clear whether MEL acts only on T-helper type 1 cells or also on T-helper type 2 cells. This is an important point as the Th1/Th2 balance is of crucial importance in the immune system homeostasis. Furthermore, being MEL the endocrine messenger of darkness, its endogenous synthesis depends on the photoperiod and shows seasonal variations. Similarly, also the pharmacological effects of MEL might be season-dependent. No information is available concerning this point. Studies are, therefore, needed to investigate whether the immunotherapeutic effect of MEL changes with the alternating seasons.

Oncostatic Action of Melatonin: Facts and Question Marks

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Since the discovery of the pineal hormone, melatonin, numerous experiments investigating its effect on various animal tumors were performed. The majority of experimental tumors responded to the melatonin treatment with growth inhibition. However, some negative or opposite results (i.e. stimulation of tumor instead of inhibition) were also reported. Some of the negative results can be attributed to the improper timing of melatonin administration. Melatonin was also shown to inhibit the growth of several animal and human tumor cell lines in vitro. On the basis of these experiments, a hypothesis of the oncostatic action of melatonin was put forward. The mechanism of postulated oncostatic action of melatonin is complex and probably includes: (1) the modulation of the endocrine system, (2) the modulation

of the immune system, (3) the direct action of melatonin on tumor cells. The latter includes the recently recognized antioxidative action of melatonin which probably plays a role in the countering the DNA damage during the radiation challenge or the exposure to chemical carcinogens. It also includes the anti-proliferative and pro-apoptotic effects of melatonin exerted via the melatonin receptors. The involvement of mainly membrane melatonin receptors is assumed. However, the recent studies from our laboratories, performed on the murine colonic cancer, suggest rather the involvement of the nuclear signalling (via RZR/ROR receptors) in both melatonin-induced proliferation inhibition and apoptosis.

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Melatonin in Psychiatric Disorders

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Over the last years there has been a rapid development in studies about melatonin in psychiatric disorders. The main role of melatonin is the rhythm regulation which is disturbed in some psychiatric disorders. The second reason of the interest was the connection of different neurotransmitters involved in pathogenesis of some disorders, with melatonin synthesis. Pineal function was studied especially in major depression, bipolar affective disorder, eating disorders, panic disorder, obsessive compulsive disorder and schizophrenia. Although reduction in nocturnal melatonin has been reported in the majority of studies of major depression,

no change has been found in some studies, whereas in others elevated melatonin concentrations were observed. In bipolar disorder serum melatonin concentrations were lower in the depressive phase and lower or elevated in mania state. In bulimia and anorexia elevated mean plasma melatonin concentrations has been described, as in the majority of studies concerning panic disorder. A reduction in nocturnal melatonin was reported in chronic schizophrenic patients.

Further investigations are necessary to clarify melatonin secretion patterns in different psychiatric disorders.

Melatonin and Glucoregulation in Animals: Possible Applications in Mammals and Man

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The role of pineal and its hormone, melatonin, in glucoregulation and carbohydrate metabolism has been evaluated in various vertebrates. The animals used were: the frog, *Rana tigrina*, the lizard, *Hemidactylus flaviviridis*, the pigeon, *Columba livia* and the rat, *Rattus rattus norvegicus* (Charles Foster strain). Pinealectomy and melatonin administration have been employed as the experimental paradigms. Glucoregulatory role has been assessed by measurement of blood glucose, tissue glycogen content, *in vitro* uptake or release of glucose and differential staining of pancreatic islets and assay of insulin and glucagon. Islet cell function was assessed by glucose tolerance, insulin, glucagon, and adrenalin response tests. The results suggest that pineal and its hormone, melatonin has a definite glucoregulatory role and influence on carbohydrate metabolism across the vertebrate span. These influences are season specific and probably also related to reproductive activity, but with differential effects in

amphibians, reptiles and birds. This may depend on the metabolic adaptations of the vertebrate class concerned. Even in a non-seasonal mammalian breeder like the rat, melatonin has influence on carbohydrate homeostasis. Though the season specific effect of melatonin on carbohydrate metabolism cannot be accredited a pan vertebrate role, it nevertheless has a pan vertebrate hypoglycemic effect and a role in potentiating insulin sensitivity. Overall, the observations and results suggest a subtle but definite modulation of glucose homeostasis by melatonin. The observed effects suggest that melatonin has multiple effects on different levels, including pancreas and peripheral tissues. Effects on secretion of pancreatic hormones and on the sensitivity of the tissues to these hormones can be inferred. It is also likely that melatonin may exert an effect on the neuroendocrine axis regulating glucose homeostasis as well, a topic which is under investigation in our laboratory.

Melatonin: Its Role in Reducing Pathophysiology Induced by Free Radicals

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Melatonin is now known to be a widely acting free radical scavenger and broad spectrum antioxidant as documented in pure chemical systems, in *in vitro*

models containing tissue homogenates and under *in vivo* conditions. Melatonin, as a direct free radical scavenger, detoxifies the hydroxyl radical ($\bullet\text{OH}$) and nitric

oxide (NO•). The products generated from melatonin's interaction with the •OH and NO• have been identified; they are cyclic 3-hydroxymelatonin (3-OHM) and N-nitrosomelatonin, respectively. 3-OHM is excreted in the urine of animals (including humans) and the quantity excreted correlates with the degree of oxidative stress to which the animals are exposed. Melatonin also directly neutralizes other toxic reactive species including hypochlorous acid (HOCl), the peroxyxynitrite anion (ONOO⁻), peroxyxynitrous acid (ONOOH), singlet oxygen (¹O₂) and hydrogen peroxide (H₂O₂). The scavenging of H₂O₂ generates the product N¹-acetyl-N²-formyl-5-methoxykynuramine (AMFK) which itself is an efficient free radical scavenger. The scavenging of free radicals by melatonin as well as by the products generated greatly increases the efficacy of melatonin as a protector against oxidative damage; this repetitive process is referred to the antioxidant cascade. In addition

to its direct free radical scavenging actions, melatonin also protects against oxidative mutilation of macromolecules by stimulating a variety of antioxidative enzymes including superoxide dismutase, glutathione peroxidase and glutathione reductase. Each of these enzymes are important to the total antioxidant capacity of the cell. Melatonin also stimulates the rate-limiting enzyme in glutathione (GSH) synthesis, namely, γ-glutamylcysteine synthase. GSH is an important intracellular and extracellular antioxidant. Finally, melatonin increases the efficiency of the electron transport chain in the inner mitochondrial membrane thereby reducing electron leakage and free radical generation. This combination of actions makes melatonin an efficient protector of macromolecules from free radical damage. The implications of these findings are broad since free radical damage is associated with a large number of diseases.

Melatonin and Cardiovascular System

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Melatonin concentrations in serum and urinary levels of its main metabolite, 6-sulphatoxymelatonin, decrease with age. In the course of aging, the frequency of heart diseases, both acute and chronic, systematically increases. The evidence from the last 10 years suggests that melatonin influences the cardiovascular system. The presence of vascular melatonergic receptors/binding sites have been demonstrated; these receptors are functionally linked with vasoconstrictor or vasodilatory effects of melatonin. Melatonin can contribute in cardio-protection of the rat heart, following myocardial ischemia. It has been shown that patients with coronary heart disease have a low melatonin production rate, especially those with higher risk of cardiac infarction and/or sudden death. There are clinical data reporting some alterations of melatonin in human stroke and coronary heart disease. The suprachiasmatic nucleus and, possibly, the melatonergic system may also modulate cardiovascular rhythmicity. The other problems, related to age, are hypercholesterolemia and hypertension. People with high levels of LDL-

cholesterol have low levels of melatonin. It has been shown that melatonin suppresses the formation of cholesterol by 38% and reduces LDL accumulation by 42%. Cohen (1995) observed a 10–20% reduction of cholesterol in women using the B-oval pills containing melatonin. It is a very important fact, because, e.g., Angier (1995) suggested that even 10–15% depletion in blood cholesterol results in a 20 to 30% reduction in the risk of coronary heart disease. People with hypertension have lower melatonin levels than those with normal blood pressure. The administration of the hormone in question declines blood pressure to normal range. Arangino et al. (1999) observed that melatonin, even in a dose 1 mg, reduced blood pressure and decreased catecholamine level after 90 min in human subjects. Melatonin may reduce blood pressure via the following mechanisms: 1) by a direct effect on the hypothalamus; 2) as an antioxidant which lowers blood pressure; 3) by decreasing the level of catecholamines, or 4) by relaxing the smooth muscle lining the aorta.

Diagnostic Problems in Insomnia

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The International Classification of Sleep Disorders defines insomnia as "difficulty in initiating and/or maintaining sleep". Practically many variations on this

definition have been proposed, and a consensus definition of insomnia has not been reached. Generally, it is believed that insomnia is rather a symptom than

final diagnosis. Except for primary insomnia and sleep misperception, this sleep symptoms will always be found secondary to a medical, psychiatric, behavioral, environmental or circadian disorder. The table below

shows the major differential diagnostic categories of insomnia, according to the three classifications of sleep disorders:

ICSD [1]	ICD-10 [2]	DSM-IV [3]
Intrinsic Sleep Disorders	Nonorganic insomnia	Primary insomnia
Psychophysiological Insomnia		
Sleep State Misperception		Insomnia related to another mental disorders
Idiopathic Insomnia	Organic insomnia	
Extrinsic Sleep Disorders		Insomnia due to medical disorders
Inadequate Sleep Hygiene		
Environmental Sleep Disorder		Substance-induced insomnia
Altitude Insomnia		
Adjustment Sleep Disorder		
Insufficient Sleep Syndrome		
Limit-Setting Sleep Disorder		
Sleep-Onset Association Disorder		
Food Allergy Insomnia		
Stimulant-Dependent Sleep Disorder		
Alcohol-Dependent Sleep Disorder		
Toxin-Induced Sleep Disorder		
Sleep Disorders Associated with Medical/Psychiatric Disorders		

[1] American Sleep Disorders Association: ICSD – International Classification of Sleep Disorders, revised. Diagnostic and coding manual. American Sleep Disorders Association, Rochester 1997. [2] ICD-10, 1992, Polish version 1994 and 1997 [3] American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders. American Psychiatric Association, Washington 1994.

Melatonin in Immunity: A Comparative Aspect

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Photoperiodic information encoded in the circadian pattern of melatonin synthesis and release by the pineal gland is subsequently perceived as a regulatory factor involved in the control of several physiological processes exhibiting diurnal and seasonal rhythmicity. Among them there are immune parameters, and the photoperiod appears to play an important role in the seasonal changes in the immune status of several vertebrate species. Immunoregulatory activity of melatonin has been demonstrated in several experimental approaches and there is a good agreement that in mammalian species the immune function is often enhanced by melatonin treatment. In particular, in laboratory rodents melatonin seems to counteract the immunosuppressive effect of stress or corticosteroid treatment. However, similar effect has not been, demonstrated in avian species examined to date, but the antiinflammatory melatonin activity was recently described in young chickens. On the other hand, the effect of pinealectomy and melatonin administration on the circadian rhythm of some immune parameters was found in young, sexually immature chickens. The mechanisms involved in

the immunoregulatory function of melatonin seem to be numerous, including its direct influence on immune cell activity, via membrane-bound or/and nuclear receptors and using different intracellular second messengers, and the indirect action through the level of other hormones (e.g. corticosteroids, prolactin, sex steroids). Moreover, an association between the duration of environmental lighting conditions and immune parameters has been demonstrated, especially in seasonally breeding wide rodent species. Generally, it seems that in short days the immune function is enhanced because of increased melatonin synthesis, but it was postulated that this kind of melatonin effect is seen only in laboratory conditions. Field studies in mammals and birds have demonstrated a compromised immune function in winter period indicating that these stressful conditions might counteract the stimulatory effect of endogenously produced melatonin. Therefore, involvement of melatonin in the integrative system coordinating physiological processes responsible for survival and maintaining of internal homeostasis is postulated.

Melatonin Receptors and Signal Transduction

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Melatonin, the hormone secreted at night by the pineal gland, elicits potent circadian, reproductive and hypnotic effects in mammals. A family of high affinity

melatonin receptors has been cloned, all of which belong to the G-protein coupled receptor superfamily. Despite the potent physiological effects of the hormone

and its therapeutic potential, relatively little is known about the signal transduction pathways activated by the melatonin receptor family. Studies of the endogenous receptor in vertebrates indicate high affinity melatonin receptors negatively coupled to adenylyl cyclase by a pertussis toxin sensitive G protein. However, in cells expressing melatonin receptors as well as in cells stably transfected with melatonin receptor subtypes, additional pathways activated by melatonin have been found including facilitation of cGMP formation and activation of protein kinase C.

Of major scientific and clinical interest is the apparent interaction between melatonin and the sex steroid system. In the rat brain the density of a low and high affinity classes of melatonin receptors is modulated by circulating sex steroids in both males and females. Accumulating evidence indicate that melatonin may act as an androgen and estrogen protagonist and *inter alia* regulate androgen-dependent prostate epithelial cell and estrogen dependent breast cancer growth. Melatonin appears to suppress estrogen receptor DNA binding and efficacy in breast cancer MCF-7 cells and primes these cells to retinoic acid induced apoptosis.

We have recently found that human benign and cancer prostate epithelial cells express functional melatonin receptors, which effect suppression of growth and viability. Melatonin signal transduction in benign and cancer prostate cells included modulation of cGMP and

cAMP levels as well as activation of protein kinase C. These effects apparently differ in cells that express than in those that do not express the androgen receptor (AR). Because androgens, via the AR promote growth and functionality of androgen sensitive benign and cancer tissues, the interference of melatonin in AR activity was explored. Treatment of the human prostate cancer LNCaP cells that endogenously express a mutant form of the AR and of human prostate carcinoma PC3 cells stably transfected with the wild type AR with melatonin (1–100 nM) increased immunoreactive AR in the cells. DNA and androgen binding capacity of the AR were not suppressed by melatonin. However, the androgen-induced suppression of AR-mRNA and transactivation of an androgen-responsive reporter gene were attenuated by the melatonin treatment. Immunocytochemical analysis revealed that melatonin rapidly caused a persistent nuclear exclusion of AR in the cells. The nuclear localization of the AR is a hallmark of its biological activity. To our knowledge, this is the first demonstration of a melatonin-induced mislocalization of the AR and may explain the attenuation of androgen activity. The signal transduction pathways involved in this response are currently being explored. Together, these findings may set up a basis for the development of novel approaches to regulate AR localization and androgen efficacy in prostate cancer.

SHORT COMMUNICATIONS

The Effects of Zinc and Melatonin Deficiency on Thyroid Hormones in Rats

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AIM AND STUDY DESIGN: Zinc and melatonin were proposed to be interacting with each other in some metabolic and physiological processes. The study was done to evaluate the effects of melatonin and/or zinc deficiency on thyroid hormones in rats. Forty male, adult Sprague Dawley rats were divided into 4 groups. Group 1 consisted of zinc deficient rats (n=10). Group 2 consisted of melatonin deficient rats (n=10). Group 3 consisted of both melatonin and zinc deficient rats (n=10). Group 4 consisted of controls (n=10). Melatonin deficiency was held by pinealectomy and zinc deficiency was held by zinc deficient diet and by distilled water. At the end of 4 weeks blood samples were obtained and plasma melatonin, zinc, TSH, free and total T₃ and T₄ levels were measured. The study was done in Selcuk University Experimental Medicine Research Center. **RESULTS:** Plasma zinc levels of Group 1 and 3 were significantly lower than those of Group 2 (P<0.01). Plasma melatonin levels of Group 2 and 3 were signif-

icantly lower than those of Group 1 (P<0.01). Free and total T₃ levels were significantly lower in all three deficient groups than those in controls (P<0.01). Free T₄ levels were significantly lower in all three deficient groups than those in controls (P<0.01). Free T₄ was also significantly different among three deficient groups as Group 2>Group 3>Group 1 (P<0.01 for all). TSH levels were significantly lower in all three deficient groups than those in controls (P<0.01). **CONCLUSION:** Zinc deficiency may cause an inhibition on release of thyroid hormones and melatonin deficiency may reduce that inhibition. Melatonin and zinc may have opposite effects on thyroid hormones.

The Effects of Zinc and Melatonin Supplementation on Thyroid Hormones in Rats

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AIM AND STUDY DESIGN: The study was aimed to evaluate the effects of melatonin and/or zinc supplementation on thyroid hormones in rats. Forty male, adult Sprague Dawley rats were divided into 4 groups. Group 1 consisted of zinc-supplemented rats (n=10). Group 2 consisted of melatonin-supplemented rats (n=10). Group 3 consisted of both melatonin and zinc supplemented rats (n=10). Group 4 consisted of controls (n=10). Melatonin supplementation was done at a daily dose of 3mg/kg s.c. and zinc supplementation was done at a daily dose of 3 mg/kg intraperitoneally. At the end of four weeks blood samples were obtained and plasma melatonin, zinc, TSH, free and total T₃ and T₄ levels were measured. The study was done in Selcuk University Experimental Medicine Research Center. **RESULTS:** Free T₃ levels of zinc-supplemented group (Group 1) were significantly higher than those of Group 2 and 3 (P<0.01). Free T₃ levels of controls (Group 4) were also significantly higher than Group 2 (P<0.01).

Free T₄ levels of the control and zinc supplemented group were significantly higher than those of Group 2 and 3 (P<0.01 for all). Free T₄ levels of zinc+melatonin-supplemented group (Group 3) were significantly higher than those of Group 2 (P<0.01). Total T₃ levels of Group 1 and 4 were higher than those of melatonin-supplemented group (Group 2) (P<0.05). Total T₄ levels in Group 1 and 4 were higher than those of Group 2 and 3 (P<0.01). TSH levels were not significantly different among all three groups. Plasma zinc levels of Group 4 were significantly lower than those of other three groups (P<0.01). Plasma melatonin levels of Group 4 were significantly lower than those of Group 1, 2 and 3 (P<0.01). **CONCLUSION:** The results of this study support that melatonin supplementation may have an inhibitory effect on thyroid hormones and zinc supplementation may reduce such inhibition. Melatonin and zinc may have opposite effects on thyroid hormones.

Melatonin and Zinc Deficiency Increase Nitric Oxide Production in *Toxoplasma Gondii* Infected Rats

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AIM AND STUDY DESIGN: The study was done to investigate the effects of melatonin and/or zinc deficiency on plasma nitric oxide (NO) levels in *Toxoplasma gondii* infected rats. Five groups of rats were held for the study. Group 1 consisted of zinc deficient-infected rats (n=10). Group 2 consisted of melatonin deficient-infected rats (pinealectomized) (n=10). Group 3 consisted of both melatonin and zinc deficient-infected rats (n=10). Group 4 consisted of just infected rats as infected controls (n=10). Group 5 consisted of non-infected rats as non-infected controls (n=10). The study was done in Selcuk University Experimental Medicine

Research Center. Four weeks later blood samples were taken and plasma melatonin (RIA), zinc (AAS) and NO levels (Spectrofotometric Greiss reaction) were measured. **RESULTS:** Nitric oxide levels of all infected groups (Groups 1,2,3, and 4) were significantly higher than those of non-infected controls (Group 5) (P<0.01). Comparing the infected groups, NO levels were higher in Group 2 and 3 than Group 4 and 1 (P<0.01 for all). **CONCLUSION:** The results of the study supports that NO levels increase in *Toxoplasma gondii* infected rats. Melatonin deficiency may exaggerate such effect.

The Excretion of Sulfatoxymelatonin in Healthy Young Men Exposed to Electromagnetic Fields Emitted by Cellular Phone: An Experimental Study

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It is quite likely that non-visible electromagnetic fields (EMF) may affect melatonin production. Some

studies confirmed this hypothesis and showed that extremely low EMF altered pineal function in animals

and humans. Thus, it is reasonable to suppose that EMF emitted by cellular phones may also influence secretion of melatonin. The present study sought to evaluate possible effect of the exposure to EMF emitted by cellular phone on 6-hydroxymelatonin sulfate (6-OHMS) excretion, which reflects melatonin levels in blood. The examined group consisted of 9 healthy males aged 19–29 years. The experiment was performed under controlled conditions (the light intensity – 50 lx till midnight and 0 lx during night). Each person was examined twice: on a day without exposure (control day - Cday) and on a day with continuous exposure (60 min. exposure from cellular phone, frequency 900 MHz, output power 5 mW/cm³ - Eday). From 7 p.m. to 8 p.m. they used a cellular phone. The subjects did not know which day was Eday, and which was Cday. From 8 p.m. till midnight the subjects listened to music and then they slept till 7 a.m. next day. Urine samples were collected at 7 p.m., at midnight and at 7 a.m. in the same way in Cday as in Eday. Samples were frozen for later ELISA analysis of 6-OHMS. The 6-OHMS ELISA kit from Immuno-Biological Laboratories (Hamburg) was used for measurement of 6-OHMS. The data were analysed using Wilcoxon matched-pairs signed-ranks test for each subject and for the whole group. We com-

pared 6-OHMS level on the Eday and on the Cday separately for 3 time-points – 7 p.m., midnight, 7 a.m. Mean 6-OHMS level in both experiments did not differ significantly for any of the respective time points. However, we noted that 6-OHMS level at 7 a.m. was lower on the exposed than on the non-exposed day ($p=0.08$). The 6-OHMS concentrations during the E- and Cdays varied between the individuals. The greatest differences were recorded at midnight on the Eday. Circadian variations of 6-OHMS level were detected in all subjects, however, a significant flattening of the 6-OHMS concentration curve was recorded in two subjects on the Eday. Besides, on the Eday at midnight – 4 subjects, and at 7 a.m. – 5 subjects showed lower 6-OHMS concentration than on the Cday. When analysing the increase of 6-OHMS concentration between midnight and 7 a.m. in the individual subjects we found that in 7 subjects it was lower on E – than Cday. The results of our investigation so far has demonstrated that EMF emitted by cellular phones has an influence on the melatonin level. A tendency to lower melatonin excretion during the night hours following exposure to EMF emitted by cellular phone could be observed. Further research is required to confirm our observations.

Functional Melatonin Receptor in the Brain at Different Periods of Hibernation Of Female Frogs (*Rana Temporaria* L.)

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The occurrence of spontaneous activation of gonadal, thyroid, and adrenal neuroendocrine regulatory axes observed in hibernating frogs (*Rana temporaria* L.; 0–4°C, darkness) may be associated with the modulatory action of melatonin. The aim of the presented study was to analyze the presence functional melatonin receptor (MR) in the brain of female frogs at the early (November 20; $n=10$), middle (January 21; $n=9$), and late (March 22; $n=10$) period of hibernation. All animals were kept in dark containers filled with air saturated water. Tissue samples were collected either at darkness (less than 15 luxes) or after 7 hours of light exposition (250 luxes). The cytoplasmatic membrane fractions were prepared by homogenization in the buffer (50 mM Tris-HCl, 1 mM EDTA, pH 7.4), two centrifugations at 50 000 x g, and suspension of the resulting pellets in the buffer with an addition of 4 mM CaCl₂. The measurement of the MR was performed using radioligand binding assay and the obtained data were calculated according to Scatchard. The results were expressed in relation to fresh tissue weight, and to

the amount of membrane proteins determined according to Lowry. It was found that the exposition of hibernating animals to light had no effect on the MR parameters. Therefore, the results were expressed as average values corresponding to the respective period of hibernation. The calculated content of MR in the whole brain at the early, middle and late period was 4.2 ± 0.6 fmol, 6.8 ± 0.3 fmol and 3.9 ± 0.2 fmol, respectively. The corresponding concentration values were 55.3 ± 5.0 , 105.7 ± 7.4 , and 52.6 ± 3.0 fmol/g of tissue or 1.86 ± 0.30 , 4.60 ± 0.52 , and 1.61 ± 0.06 fmol/mg of membrane protein, respectively. The association constants differed with the time of hibernation and was 12.5 ± 2.9 , 24.4 ± 3.2 , and $7.9 \pm 1.7 \times 10^9$ l/mol, respectively. The analysis of different brain regions showed that the highest amounts of MR are localized in the mesencephalon and diencephalon, lower in the rhombencephalon, and the lowest in the telencephalon. The elevation of MR observed in the middle period of hibernation was the most pronounced in the part of mesencephalon

The data obtain showed that functional MR is widely

distributed within the brain of hibernating frog and its binding capacity as well as affinity is significantly increased at the middle period of hibernation – the time corresponding to spontaneous activation of neuroendocrine regulatory axes (week 14). It is suggested that

melatonin take part in those regulatory changes and might have significant influence on the processes leading to full reproductive activity in the period following hibernation.

Nocturnal Changes in Pineal Melatonin Synthesis in Girls

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Many studies have examined the role of MLT in the regulation of hypothalamic-pituitary-gonadal axis in animals. In humans it is unclear whether regulatory links exist between the hypothalamic-pituitary-gonadal axis and the pineal gland. Investigators working with several endocrine disorders, hypothalamic amenorrhea, idiopathic hypogonadotropic hypogonadism and delayed puberty have noted an association between hypogonadism and elevated MLT levels. Conversely investigators have reported an association between central precocious puberty and low MLT levels. However, the consistency of such findings remains unclear because they have yielded mixed results. The aim of the present study is to investigate the involvement of melatonin in the sexual maturation in children (girls), before puberty and at menarche by evaluating the excretion of 6-sulfatoxymelatonin, the main metabolite of melatonin. Our results show that in studied groups the excretion of 6-sulfatoxymelatonin exhibits a circadian pattern synchronised with the day-night cycle, with high levels during the

night and low levels during the day but rarely undetectable in the afternoon and early evening. The results suggested the optimal conditions for the measurement of 6-sulfatoxymelatonin in urine pools as follows: between 2 p.m. – 8 p.m. and 10 p.m. – 8 a.m. The 24, 48 and 72 h profiles of 6-sulfatoxymelatonin also show reproducibility from day to day in the same subject but a very large variability in amplitude of the rhythm between girls. Comparison of results between groups of girls revealed a significant decrease in nocturnal 6-sulfatoxymelatonin levels in menarcheal age compared with prepubertal age ($p=0.0004$). One possible explanation for this decrease of melatonin in the sexual maturation is that melatonin by its secretion regulates the oscillation and temporal organisation of maturity of hypothalamic-pituitary-gonadal axis. In conclusion, the present data sustain the role of melatonin in the onset of puberty in humans as in all other species and support the idea that 6-sulfatoxymelatonin may be a sensitive predictor in sexual maturation in humans.

New Evidence for Immunomodulatory Properties of Melatonin

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Clinical and experimental studies have provided evidence of immunomodulatory activity by the pineal neurohormone, melatonin. However, these studies have yielded contradictory results. The aim of this study was to evaluate the involvement of melatonin in cell-mediated immunity by both proliferation and cytotoxicity assays, following the effects of melatonin on responses of peripheral blood lymphocytes which were obtained from healthy subjects, patients with gastric cancer and immunosuppressed patients with kidney transplants. Lymphocytes from either healthy donors or patients with gastric cancer were incubated with the different doses of melatonin (5–25 μg MLT/ml). The dose of 5 μg /ml MLT enhanced to the maximum cell proliferation (as detected by ^3H thymidine uptake) by approximately 51% in healthy group, while in cancer group the maximal stimulation was about 10.12% at the dose of

10 μg /ml MLT. A significant decrease is noticeable in the proliferative response to PHA or MLT in lymphocytes of patients with gastric cancer (maximal stimulation: ~20% PHA and ~10% MLT). Interestingly, MLT did not alter PHA-induced lymphocytes proliferation when lymphocytes from either healthy or cancer donors were incubated with PHA and MLT. Also, MLT enhanced cell proliferation of lymphocytes from immune suppressed patients with kidney transplants and it counteracted the proliferative effect of lymphocytes from healthy subjects with hypersensitivity, in a dose dependent manner. Co-culture of the lymphocytes from gastric cancer donors with ^{51}Cr -labelled K562 tumor cells in the presence of the different doses of MLT resulted in a significant enhancement of NK cell cytotoxicity (60–76% at the ratio of 100:1 effector:target cells) while in the healthy group the release of ^{51}Cr was insignifi-

cant (8–19%). Taken together, these results support the modulatory action of MLT on immune responses and

further studies are needed to clarify the mechanisms of MLT in cell-mediated immunity.

The Effect of Neurokinin A on the Vasopressin and Oxytocin Response to Melatonin: *In Vitro* Studies

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Tachykinins have been demonstrated to influence the secretory activity of hypothalamic neurones; their role as regulators of the posterior pituitary endocrine function is also postulated. The aim of the present investigations was to study the role of neurokinin A (NKA), a member of a family of tachykinins, in the regulation of basal and K^+ -evoked vasopressin (AVP) and oxytocin (OT) secretion as a response to melatonin (MLT). Male Wistar rats served as donors of the hypothalamo-neurohypophysial (HN) explants which were incubated *in vitro* in 1 ml of Krebs-Ringer fluid (KRF) which, in the first experimental series, was enriched with NKA at the concentrations of 10^{-7} , 10^{-8} , 10^{-9} , 10^{-10} or 10^{-11} M/L. The HN explants were incubated successively in: 1 – normal KRF (B1); 2 – modified KRF containing the excess (56 mM) of K^+ (S1); 3 – the incubation fluid as B1 alone or with NKA in the respective concentration (B2); 4 – the KRF as S1 alone or with NKA in the same concentrations (S2). For the second experimental series, only two (10^{-9} and 10^{-7} M/L) concentrations of NKA have been chosen to be added to

B2 and S2 fluids which additionally contained MLT at a concentration of 10^{-9} M/L. After 20 minutes of incubation in each medium (i.e., 1, 2, 3 and 4, respectively), samples were collected and frozen before estimation of AVP and OT by the radioimmunoassay.

In agreement with previous *in vitro* studies high K^+ concentration stimulated both AVP and OT secretion from the isolated HN explants. Under basal conditions, the OT release was increased by NKA at a concentration of 10^{-9} and 10^{-7} M/L while the release of AVP was stimulated by NKA only at a concentration of 10^{-7} M/L. Melatonin significantly inhibited the effect of NKA on the *in vitro* AVP and OT secretion. K^+ -evoked release of AVP or OT was not further modified by either NKA or MLT. The present results show that neurokinin A may be involved in the pineal-neurohypophysial interactions. As yet, however, the way of NKA action on the response in question requires further studies.

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Melatonin in Human Cancer Patients: New Promising Marker for Tumor Diagnosis and Prognosis

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The radioimmunoassay of the main peripheral melatonin metabolite – 6-sulfatoxymelatonin ($aMT6_s$) was carried out from the frozen urine aliquots of the female and male patients which were suffering from cancer of the thyroid, larynx, lung, stomach, colon, and rectum, as well as of the urinary bladder. At the same time the expression of melatonin and PCNA (proliferative cell nuclear antigen) in most tumor specimens from these patients also has been studied. A very low production of melatonin (as estimated by the nocturnal urinary excretion of $aMT6_s$) was found in male patients with lung or stomach cancer compared to aged-matched controls as well as in female patients with thyroid cancer. The levels of $aMT6_s$ in these women, however, did not differ from female patients with benign thyroid diseases indicat-

ing a general suppressive effect of thyroid disease on the pineal gland. A similar but opposite phenomenon was observed in male patients with primary unoperated colorectal cancer who showed an elevated production of melatonin as estimated by urinary $aMT6_s$ when compared to healthy men but not when compared to patients with colitis ulcerosa. The mechanisms involved in these phenomena are poorly understood and seem to include central as well as peripheral components. This view is supported by the finding that in spite of varying urinary $aMT6_s$ excretion measured in patients with different types of tumors, $aMT6_s$ shows comparable positive correlations with the degree of tumor cell proliferation (as estimated by the number of PCNA-immunopositive cells). This means that the amount of $aMT6_s$

excretion (as well as the corresponding concentration of circulating melatonin) can be regarded as the net result of a number of different effects exerted by the tumor on the system, e.g., the secretion of pineal melatonin, its peripheral metabolism as well as the production and release by tumor cells. It is felt that a deeper understand-

ing of these processes will help to better understand the complex systemic effects of different types and stages of tumor growth upon the neuroimmunoendocrine network, and might in future even be applied for both diagnostic as well as prognostic purposes.

Effect of Melatonin on Experimental Peritonitis in Young Chickens

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To examine the effect of melatonin (Mel) on avian inflammatory reaction, experimental peritonitis was induced by a single intraperitoneal injection of thioglycollate (TG) into sexually immature chickens of both sexes, pre-treated (30 min.) with melatonin (MelTG) or injected with TG supplemented with the same Mel dose (TGMel). Control birds received Mel alone, PBS or were intact. Chickens were sacrificed at specific postinjection intervals, and total WBC number and the percentage of blood lymphocytes and granulocytes counted. Peritoneal leukocytes (PTLs) were obtained by flushing the peritoneal cavity, counted and used for an *in vitro* assay of activity (respiratory burst). Both, the inflammatory reaction and Mel influence were gender- and treatment-dependent. In males, the inflammatory reaction developed quickly, reached a maximum at 6h and gradually decreased for the following 24h. Pre-treatment with Mel diminished the intensity of reaction and delayed the maximal point until 24h after the injection. When Mel was injected simultaneously with TG, the reaction became bi-phasic with a second peak observed at 24h. In females, both the inflammatory reaction and Mel influence were similar but less expressed. TG-induced peritonitis was accompanied by non-signif-

icant changes in the total WBC number in males and a decrease at the beginning of peritonitis (3h) in females. Mel supplementation, regardless of time of hormone injection, generally increased the WBC number in males, and in females had no effect or significantly decreased this number at 3h of peritonitis in group TGMel. Mel increased the percentage of lymphocytes at the beginning of inflammation in males whereas later the percentage of granulocytes was higher. In females the proportion between lymphocytes and granulocytes caused by Mel addition, was inversed. Mel alone antagonized the effect of sham PBS injection on the PTL number and respiratory burst.

Mel effect on inflammatory reaction in young chickens appeared to be exerted in two complementary phases: firstly, when the anti-oxidant properties of Mel block the inflammation, and secondly, a pro-inflammatory effect, most probably mediated *via* some compound(s) (cytokines, endogenous opioids) synthesized and secreted by immune cells under Mel influence.

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Relationship Between cAMP Formation and Melatonin-Induced Inhibition of Proliferation in Chicken Lymphoid Cells

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The immunoregulatory function of melatonin (Mel) is a well known phenomenon. Studies performed to date on mammals and birds demonstrated the anti-proliferative effect of Mel on lymphocytes stimulated with mitogen *in vitro*, but the mechanism of this process still remains unclear. On the other hand there are evidences that the proliferative response of mammalian lymphoid cells *in vitro* is attenuated by the elevation of intracellular cAMP level. The aim of this study was to examine the correlation between cAMP content and prolifera-

tion of melatonin-treated chicken lymphoid cells. The experiments were performed on male chickens (2-4-week-old) kept from hatch in controlled light (L:D = 12:12) condition. Splenocytes and peripheral blood mononuclear cells (PBMC) were used in this present study. Proliferation of lymphocytes cultured for 24 or 72 h in the presence of phytohemagglutinin (PHA, 16 mg/ml and 31.25 mg/ml), dbcAMP (10^{-4} M), forskolin (10^{-4} M), and/or melatonin (10^{-9} M and 10^{-7} M) was estimated by measurement of [3 H]-thymidine incorpo-

ration. Furthermore, in splenocytes cultured for 24 h with various concentrations of Mel and/or PHA cyclic AMP concentration was examined (RIA assay). It was shown that in chicken splenocytes and PBMCs the cAMP increase (addition of dbcAMP or forskolin) caused the inhibition of cell proliferation. On the other hand, splenocyte proliferation stimulated by PHA was correlated with a decrease in cAMP formation. Melatonin exerted the inhibitory effect on proliferation of splenocytes cultured with higher PHA concen-

tration, however it increased the proliferative response in cells cultured in the presence of lower PHA dose. These effects were correlated with cAMP changes – an increase and a decrease, respectively. In conclusion, the results presented herein indicate the involvement of intracellular cAMP in the proliferative response of chicken splenocytes and suggest that the antiproliferative Mel effect is mediated, at least partially, by intracellular cAMP increase.

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Two Serotonin N-Acetyltransferase Transcripts are Present in Oocytes and Early Embryos of Japanese Quail (*Coturnix Coturnix Japonica*)

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Serotonin N-acetyltransferase [arylalkylamine N-acetyltransferase (AA-NAT; EC 2.3.1.87)] is a key enzyme controlling rhythmic synthesis of melatonin (MEL), high at night and low in the daytime. In vertebrates, MEL is produced mainly in the pineal gland

and in retina, but low levels of AA-NAT mRNA were also found in the testis and brain of some mammals and in the testis of quail (by the RNase protection assay). Recently, by Reverse Transcription-Polymerase Chain Reaction (RT-PCR), we have found the presence of 3

AA-NAT cDNA	ccgctgcacctggatgagat ccggcacttctctgacgctgtgcccggagctctctctcggctgggttcgaggaaggt	75	
DNA-blood	CCGCTGCACCTGGATGAGAT CCGGCACTTCTGACGCTGTGCCCGGAGCTCTCTCTCGGCTGGTTCGAGGAAGGT	75	
cDNA-germ	CCGCTGCACCTGGATGAGAT CCGGCACTTCTGACGCTGTGCCCGGAGCTCTCTCTCGGCTGGTTCGAGGAAGGT	75	
cDNA-pineal	CCGCTGCACCTGGATGAGAT CCGGCACTTCTGACGCTGTGCCCGGAGCTCTCTCTCGGCTGGTTCGAGGAAGGT	75	
AA-NAT cDNA	cgcttggtggcttttatcatcggctccctatgggaccaggacaggctcagccagg-----	130	
DNA-blood	CGCTGGTGGCTTTTATCATCGGCTCCCTATGGGACCAGGACAGGCTCAGCCAGGTAGGAGCCGGGCACAGGGTA	150	
cDNA-germ	CGCTGGTGGCTTTTATCATCGGCTCCCTATGGGACCAGGACAGGCTCAGCCAGGTAGGAGCCGGGCACAGGGTA	150	
cDNA-pineal	CGCTGGTGGCTTTTATCATCGGCTCCCTATGGGACCAGGACAGGCTCAGCCAGG-----	130	
AA-NAT cDNA	-----cagcgctgac	140	
DNA-blood	AGTGGAGCGGAGACTCGTGCTGCCCTGCACGGCCCCACTAATGGCCCCGTTCCCTCCCTTACAGGCAGCGCTGAC	225	
cDNA-germ	AGTGGAGYGGAGACTCGTGCTGCCCTGCACGGCCCCACTAATGGCCCCGTTCCCTCCCTTACAGGCAGCGCTGAC	225	
cDNA-pineal	-----CAGCGCTGAC	140	
AA-NAT cDNA	cctgcacaagcctcggggcacggcagtgacatccatgtgctggtgctgacaccgcaccttccgacagcagggcaa	215	
DNA-blood	CCTGCACAAGCCTCGGGGCACAGCAGTGACATCCATGTGCTGGCTGTGCATCGCACCTTCCGACAGCAGGGCAA	300	
cDNA-germ	CCTGCACAAGCCTCGGGGCACRGCAGTGACATCCATGTGCTGGCYGTGCATCGCACCTTCCGACAGCAGGGCAA	300	
cDNA-pineal	CCTGCACAAGCCTCGGGGCACRGCAGTGACATCCATGTGCTGGCYGTGCATCGCACCTTCCGACAGCAGGGCAA	215	
AA-NAT cDNA	ggg ctccatcctgatgtggcgg t 238	R=A/G; Y=T/C;	----- intron location,
DNA-blood	GGG CTCCATCCTGATGTGGCGGT 323		bold letters - primer location,
cDNA-germ	GGG CTCCATCCTGATGTGGCGGT 323		lower case: Gene Bank sequence._
cDNA-pineal	GGG CTCCATCCTGATGTGGCGGT 238		

MEL receptor transcripts (mel-1a,b,c) in quail oocytes and early embryos. The present work also reports the presence of AA-NAT mRNA in oocytes (germinal discs) and blastoderms (~40 000-cell embryo) from freshly laid, unincubated eggs. RNA was isolated from the biological material in daylight and RT performed with the RNA of a single germinal disc or blastoderm and from the pineal gland (~1µg of RNA). The primers were chosen on the basis of quail AA-NAT sequence (acc. nr. AF 007068). With the same pair of primers we detected one cDNA band, corresponding to the theoretical length (238 bp) for the RNA from the pineal gland, and 2 bands (238 and 323 bp) for the RNA from germinal discs and blastoderms. The single PCR product obtained for genomic DNA (from blood) was also 323 bp, suggesting that the longer, 323-bp product revealed

in the germinal disc and blastoderm comprised an intron of 85 bp and probably originated from an unprocessed mRNA present in the sample. To confirm or reject this hypothesis, the PCR product from genomic DNA, and the RT-PCR products from the pineal gland (238 bp) and germinal disc (323 bp) were sequenced and compared to the AA-NAT sequence from the Gene Bank. The results are shown below and confirm that both AA-NAT sequences found in the germinal discs and blastoderms of quail differ by an 85 bp intron present in the longer RT-PCR product and are homologous to the published AA-NAT cDNA sequence (except for the intron). Alternative splicing of the nascent AA-NAT transcript is possibly included in a mechanism regulating expression of the enzyme and thus, melatonin synthesis in birds.

Detection of Melatonin Receptor mRNAs in Avian Oocytes and Fertilized Eggs

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Melatonin (MEL) is a multifunctional hormone regulating most circadian and seasonal processes in animals, including reproduction. It is produced mainly in the pineal gland and its synthesis and secretion is the highest at night and lowest at the daytime. Its physiological effects on the action of various hormones involved in reproduction occur through the membrane receptors coupled to G-proteins. Recently, two MEL receptors for mammals (mt₁, MT₂) and three for birds (mel-1a, mel-1b and mel-1c) have been identified and sequenced. This has made it possible to discriminate between expression of 2 or 3 different types of MEL receptors. The present work reports, for the first time, the presence of MEL receptor transcripts in the RNA from oocytes and fertilised freshly laid eggs of the Japanese quail (*Coturnix coturnix japonica*) determined by RT-PCR method. Total RNA was isolated using InViSorb RNA II kit (InViTek GmbH, Berlin) from germinal discs of the *in vitro* ovulated quail oocytes, from blastoderms (~40 000-cell embryos) of freshly laid fertilised eggs and from the cytoplasmic layer localised around the yolk under the vitelline membrane. A special procedure for collecting the biological material excluded the possibility of contamination with cells of somatic origin (blood or granulosa cells). The chick DNA sequences were used to design the primers for the MEL receptors: mel-1a (acc. no. U31 820), mel-1b (acc. no. U30 609) and mel-1c (acc. no. U31 821) since the quail DNA sequences have not been determined. The primers are located within a putative 2nd exon and they contain no intron. The products of the gene sequences for quail clusterin and chick β-actin (both containing the introns) were used in the control reactions to esti-

mate DNA contamination and integrity of the isolated RNA. The identity of the RT-PCR products for the MEL receptor transcripts was verified by the presence of restriction sites for Hinf I and Tru9 I and the size of the restriction fragments. All three MEL receptor transcripts were detected in the RNA from quail oocytes and blastoderms. The amount of these transcripts was approximately ~2¹⁵ times lower as compared with the amount of β-actin transcript. The main mel-1c transcript was always present in germinal discs and blastoderms, the mel-1a and mel-1b products were always detected in blastoderms and sometimes, but not always, in the maternal RNA from germinal discs, i.e. their transcription should occur *de novo* after fertilisation. The results varied from sample to sample for the RNA extracted from the cytoplasmic layer around the yolk. The presence of MEL receptor transcripts in the oocyte and uterine embryo RNA seems unusual but their presence does not necessarily imply the existence of a functional receptor protein. An avian oocyte till ovulation is closely connected with its follicle and may undergo the influence of MEL circulating in the female organism, but an avian egg is a separate unit not undergoing any melatonin action (assuming that there is no MEL accumulated in the yolk or albumen). Thus the questions arise about the role and function of MEL receptor transcripts in early development. The avian embryo seems to be well suited for these kinds of studies as it forms a separate unit, isolated from maternal influence, and can be easily manipulated under defined experimental conditions.

Suppression of Nighttime Melatonin Concentrations in Patients with Hypertension

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BACKGROUND: Few reports, especially experimental ones suggest, that melatonin (Mel) can influence blood pressure. **OBJECTIVE:** The connection between a daily profile of Mel concentration and the 24-hour cycle of blood pressure in 27 men with hypertension was assessed. **METHODS:** Studies were performed in 8 patients with mild hypertension, 10 men with moderate hypertension, 9 patients with severe hypertension and 12 men with normal blood pressure. The age of studied patients oscillated between 34 and 49 (mean age 42 ± 1.4 years). Mel concentrations were assessed in saliva collected during the day with intervals of 3 hours with the use of the RIA method. Blood pressure assessment was done at the same time. **RESULTS:** A significant

decrease of mesor's value and amplitude of Mel rhythm without essential changes in acrophase's values were observed in patients with moderate and severe hypertension. Mean concentrations of Mel in saliva were significantly decreased from 11 pm to 5 am. Lower values of nighttime Mel concentrations in saliva were shown in patients with severe hypertension. Significant, negative correlation between nighttime Mel concentrations in saliva and diastolic and systolic blood pressure was shown. Correlation coefficients values were in general higher in patients with severe hypertension. **CONCLUSION:** Results of our studies seem to confirm the concept that decreased Mel secretion can be one of the causes of hypertension.

Influence of Pinealectomy and Melatonin Administration on the Dynamic Pattern of Biochemical Markers of Bone Metabolism in a Rat Model of Postmenopausal Osteoporosis

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BACKGROUND: There have been suggestions in the literature that characteristic changes of bone mass in osteoporosis may be related to the melatonin (Mel) level. **OBJECTIVE:** The purpose of this study was to demonstrate whether pinealectomy (Px) and Mel administration can affect postmenopausal osteoporosis processes induced in female rats by way of ovariectomy (Ovx). **METHODS:** The study included 150 animals; 6 remained unaffected (C), 72 were ovariectomized and the remaining ones underwent sham operation. Following surgery, rats were divided into 6 groups: 1 - Ovx, 2 - Ovx + Px, 3 - Ovx + Px + Mel, 4 - C, 5 - C + Px, 6 - C + Px + Mel. Animals from the 2nd, 3rd, 5th and the 6th group were pinealectomized while the remaining ones underwent sham operation. After 2 weeks following surgery animals in the 3rd and 6th group were administered Mel ($50\mu\text{g}/100\text{g}$ of bw) while the remaining animals were administered solvent only (daily between 5 and 6 pm during a 4-week period). At the appropriate time, i.e.

prior to surgery and after 6, 12, 18 and 24 weeks from operation the animals were placed in metabolic cages (from 6.30 until 9.30 am) in order to collect urine samples for HYP and Ca levels determinations. Within the next 24 hours the rats were decapitated (at 8 am) and their blood was collected and centrifuged down. Mel and remaining markers of bone turnover (ALP, PICP, ICTP) were then determined in serum. **RESULTS:** The study has shown that pinealectomy had an inducing, while exogenous Mel a suppressing effect upon the level of investigated markers. Administration of Mel only partially leveled changes of bone metabolism caused by Ovx. In rats with preserved pineal gland the effect of Mel upon bone metabolism was more pronounced.

CONCLUSION: Melatonin is an important modulator of postmenopausal osteoporosis processes induced in female rats by way of ovariectomy.

Effects of Six Months Melatonin Treatment on Sleep Quality and Serum Concentrations of Estradiol, Cortisol, DHEAS and Somatomedin-C in Aged Women

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INTRODUCTION: Nocturnal melatonin secretion is known to be depressed in aged subjects. It is suggested that melatonin deficiency is involved in the age-related sleep disturbances. Moreover, the role of melatonin in aging processes, in general, is also discussed. Therefore, the aim of the present study was to evaluate the effect of melatonin treatment on sleep and certain hormonal parameters in aged women. **SUBJECTS AND METHODS:** The study was performed on 15 women (volunteers) aged 71.4 ± 4.6 years (mean \pm SEM). Melatonin (2 mg daily) was administered orally at 19:00 h during 6 months. All but one subjects finished the study. One volunteer disrupted the melatonin treatment because of abdominal pain. No subject received hormonal replacement therapy. Before and after 6 months of melatonin therapy the subjects responded to a questionnaire concerning sleep parameters. Serum concentrations of the following hormones were determined before, after 3 months and 6 months of the melatonin treatment: 17-beta-estradiol, dehydroepiandrosterone sulfate (DHEAS), cortisol, and somatomedin C (IGF-I). Additionally, before and after melatonin treatment peripheral venous blood samples were taken in the morning (approx. at 08.00 h) after the overnight fast. The blood morphology and the serum concentrations of

glucose, total, LDL- and HDL-cholesterol and triglycerides were estimated. The study was approved by a Local Ethical Committee and a written consent was obtained from each subject. **RESULTS:** Nine out of 14 subjects reported sleep disturbances before the therapy. In 7 out of those 9 subjects marked improvement of sleep quality was noted after the therapy completion. Significant decrease in estradiol concentration was observed both after 3 and 6 month in comparison with initial levels (64.7 ± 24.6 and 88.8 ± 19.3 vs 175.6 ± 36.1 pmol/l; respectively). An increase in somatomedin-C concentration was observed following 6 months of treatment in comparison to initial levels (264.7 ± 100.8 vs 232.3 ± 99.9 ng/ml). A tendency towards higher, although not statistically significant, DHEAS/cortisol ratio was found after 6 months of treatment (11.1 ± 12.1 vs 7.2 ± 7.4). The melatonin treatment did not influence significantly either the parameters of blood morphology or glucose and serum lipids levels. **CONCLUSIONS:** Six months of treatment with melatonin improved sleep quality in the majority of studied aged women, lowered serum estradiol concentration, increased serum somatomedin-C level, and increased DHEAS/cortisol ratio. Further studies on melatonin treatment effects in aged subjects are needed.

Effect of Melatonin and Pineal Tetrapeptide Epitalon on Chemically Induced Colon Carcinogenesis in Rats

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Earlier it was shown that melatonin (MLT) significantly inhibited intestinal carcinogenesis induced by 1,2-dimethylhydrazine (DMH) in rats. Recently, the effect of the synthetic pineal tetrapeptide Epitalon (was evaluated in CBA mice. It was found that the preparation reduced the incidence of spontaneous tumors. The goal of our study was to compare the effect of MLT and epitalon on colon carcinogenesis induced by DMH in rats. In experiments with MLT (experiment 1, groups 1 and 2), and in experiments with epitalon (experiment 2, groups 1-4) two-months-old outbred LIO rats were exposed to five s.c. injections of DMH at a single dose of

21 mg/kg of body weight. From the day of the first injection of the carcinogen, rats from group 2 (experiment 1) were given melatonin 5 days a week during the nighttime with drinking water (20 mg/l). In experiment 2, the rats from groups 2, 3 and 4 received epitalon s.c. at a dose of $1 \mu\text{g}/\text{rat}$ 5 days a week. Rats from group 2 were exposed to epitalon during the whole experiment, animals from group 3 - after termination of DMH injections, and rats from group 4 - during the period of DMH exposure. All experiments were finalized 6 months after the first injection of the carcinogen. In experiment 1, MLT decreased the total incidence of colon tumours

(from 100% to 60%), as well as the tumours multiplicity (from 3,8 to 1,5 tumours per rat, $p < 0.05$). The incidence and the multiplicity of neoplasms in the ascending and descending colon were significantly reduced as well. In experiment 2, epitalon failed to influence the total incidence of colon tumours. However, in rats from group 2 in comparison with group 1, the incidence of tumours in ascending colon was decreased (from 85% to 60%), as well as the incidence of carcinomas in descending colon. The size of all colon tumours, carcinomas in ascending and descending parts of the colon in animals from group 2 was also decreased. The slight inhibitory effect of

epitalon was observed in groups 3 and 4. The multiplicity of neoplasms in the descending colon as well as the incidence of neoplasms in jejunum and ileum were decreased in the group 3. In conclusion, our experiments firstly demonstrated the inhibitory effect of epitalon on chemically induced intestinal carcinogenesis in rats. As suggested, the inhibitory effect of epitalon on the colon carcinogenesis is mediated by its potent antioxidative properties and depends on the stage of carcinogenesis. The inhibitory potential of epitalon was comparable with the effect of MLT.

Effects of Melatonin Administration in Patients with Sleep Disorders

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The influence of melatonin on the sleep was observed in 45 patients aged 25 to 87 (mean age 52 years), 22 females and 23 males, with symptoms of chronic insomnia. Diagnosis in the studied (acc. DSM-IV) group was as follows: 32 patients - chronic insomnia (including: 4 subjects with post traumatic stress disorder, 3 subjects dependent on benzodiazepines, 14 patients with dystymia, 7 patients with depression, 3 subject with insomnia related to medical disorders, 1 person with idiopathic insomnia); 10 patients - circadian rhythm sleep disorders (including: 2 patients with delayed sleep phase syndrome and 8 patients with irregular sleep-wake pattern); 2

patients - primary diagnosis of depression, 1 patient - schizophrenia. The majority of patients came to the outpatient clinic after beginning of melatonin administration by themselves. The period of observation was from 1 month to 6 months. In all patients melatonin was an additional drug to basic treatment. In none of the patients but one adverse events were observed (in one patient the insomnia was exaggerated). In 17 patients' considerable improvement was observed (including all 10 patients suffering from sleep-wake rhythm disorder). In 13 patients no effect, and in 15 patients only mild effect of melatonin was observed.

Assessment of Connection between Neopterin and Melatonin Concentrations and Risk Factors in Patients with Atherosclerosis

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BACKGROUND: Few reports concern connection between neopterin and melatonin concentrations and progression of atherosclerotic changes. It is known, that neopterin is one of many substances produced and secreted to circulatory system by stimulated blood cells in the inflammatory process taking place in arteries. Melatonin seems to have protective action against atherosclerosis. There are some suggestions that this hormone influences stimulatingly neopterin production. **OBJECTIVE:** Assessment of the connection between neopterin and melatonin concentrations and risk factors in men with atherosclerosis. **METHODS:** 30 patients with

atherosclerosis treated at Silesian Center of Cardiac Diseases were studied. The control group consisted of 30 healthy men. Age of studied patients oscillated between 45 and 63 (mean 47 ± 8.5 years). Concentrations of melatonin (at 9 am and 2 pm), and neopterin and chosen parameters of lipids' concentration balance that is: total cholesterol - TCh and its content in HDL and LDL fractions and triglycerides -TG (at 9 am) were assessed in serum in all studied patients. **RESULTS:** In patients with clinically recognized atherosclerosis significant decrease of mean HDL fraction of cholesterol concentration and increase of LDL cholesterol was shown. Moreover, sig-

nificant increase of mean neopterin concentration and decrease of difference between day/night values of melatonin were observed. It was shown that changes in neopterin and melatonin concentrations observed in patients with atherosclerosis correlated only in small degree with changes in concentrations of chosen parameters of lipids' balance. **CONCLUSION:** A significant

increase of neopterin concentration and suppression of melatonin secretion were shown in patients with clinically recognized atherosclerosis; these changes correlated, however, only in small degree with parameters describing disturbances of lipoprotein's balance.

The Proapoptotic Effect of Melatonin on Murine Transplantable Colon 38 Cancer: Possible Involvement of Nuclear RZR/ROR Receptor

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OBJECTIVES: In the earlier study from our laboratory it was shown that melatonin (MLT) exerts its oncostatic effect not only via inhibition of tumor cell proliferation but also via induction of apoptosis. Recently, the experimental evidence indicates that MLT may act directly through membrane and nuclear receptor as well. Because MLT is suggested to be endogenous natural ligand of nuclear RZR/ROR receptor, the nuclear signaling may be involved in the oncostatic action of this hormone. To verify this hypothesis, in the present study we examined the effects MLT and two specific receptor antagonists: UCM 386 (mt1 and MT2 membrane receptors antagonist) and CGP 55644 (nuclear RZR/ROR receptors antagonist) on murine transplantable Colon 38 cancer. We assessed the cell proliferation, apoptosis and proliferation/apoptosis ratio. **MATERIAL AND METHODS:** The experiment was performed on adult male B6D2F1 mice strain. The animals were implanted subcutaneously with suspension of Colon 38 cells. Ten days after induction of tumors, the examined substances were given once daily at 18:00 h during ten days. The animals were treated with MLT, UCM 386 and CGP

55644 alone and MLT together with UCM 386 or CGP 55644 at the same doses of 25 µg/animal per day. The cell proliferation was assessed by incorporation of bromodeoxyuridine into tumor cell nuclei (labeling index-LI). The labeling of apoptotic cells according to the Tunel method was considered as an index of apoptosis (AI). **RESULTS:** It was found that MLT and UCM 386 given either alone or together inhibited cell proliferation and strongly decreased P/A ratio as compared control group. MLT given alone or together with UCM 386 significantly increased the AI, but UCM 386 given separately did not change significantly the number of apoptotic cells. On the other hand, the nuclear receptor antagonist CGP 55644 did not significantly change LI and P/A but decreased apoptosis vs control. **CONCLUSIONS:** This finding confirm our earlier observation that MLT exerts anti-proliferative and also proapoptotic effects on murine colonic cancer cells. Moreover, the presented data may suggest that the proapoptotic action of MLT is mediated probably by nuclear RZR/ROR receptors.

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