Neuroendocrinology Letters ISSN 0172–780X

ORIGINAL ARTICLE

The influence of pinealectomy and melatonin administration on the dynamic pattern of biochemical markers of bone metabolism in experimental osteoporosis in the rat

Zofia Ostrowska,[1] Beata Kos-Kudla,[2] Bogdan Marek,[2] Dariusz Kajdaniuk,[2] Pawel Staszewicz,[2] Bozena Szapska[1] & Janusz Strzelczyk[2]

1. Department of Clinical Biochemistry, Silesian Medical Academy, Zabrze, Poland.
2. Department of Pathophysiology and Endocrinology, Silesian Medical Academy, Zabrze, Poland.

Correspondence to: Zofia Ostrowska
Department of Clinical Biochemistry
Silesian Medical Academy,
Pl. Traugutta 2, 41-800 Zabrze, Poland.
TEL / FAX +48 32 2786126
E-MAIL ozdrasiek@poczta.onet.pl

Submitted: *November 12, 2001*
Accepted: *January 10, 2002*

Key words: **pinealectomy; melatonin; bone metabolism; experimental osteoporosis; female rats**

Neuroendocrinology Letters 2002; ***23****(suppl 1):104–109 pii:NEL230702A08 Copyright © Neuroendocrinology Letters 2002*

Abstract

BACKGROUND AND OBJECTIVES: There have been suggestions in literature that characteristic changes of bone mass in osteoporosis may be related to the melatonin (Mel): The aim of this study was to demonstrate whether pinealectomy and Mel administration can affect postmenopausal osteoporosis processes induced in female rats by way of ovariectomy.

METHODS: The study included 198 animals; 6 remained intact (0), 96 were ovariectomized (Ox), and the remaining ones underwent a sham operation (SOx). Two weeks after surgery, the rats were divided into eight groups: 1) SOx + SPx, 2) SOx + SPx + Mel, 3) Ox + SPx, 4) Ox + SPx + Mel, 5) SOx + Px, 6) SOx + Px + Mel, 7) Ox + Px, 8) Ox + Px + Mel. Animals from the 5th, 6th, 7th and 8th groups were pinealectomized (Px) while the remaining ones underwent a sham operation (SPx). Two weeks after surgery Mel (50μg/100g of bm) were administered intraperitoneally in rats in the 2nd, 4th, 6th and 8th groups while the remaining animals were administered with solvent only (5% solution of ethyl alcohol in physiological saline). Rats were administered the Mel solution or the solvent daily between 5 and 6 pm during a 4-week period. At the appropriate time, i.e. prior to surgery (group 0) and after 6, 12, 18 and 24 weeks from Px or SPx (time subgroups a, b, c and d) the animals were placed separately in metabolic cages (from 6.30 until 9.30 am) in order to collect urine aliquots for HYP and Ca determinations. The blood for the assay of ALP, PICP and ICTP was collected within the next 24 hours at 8 am (rats killed by decapitation).

RESULTS: The study has shown that pinealectomy had inducing, while exogenous Mel suppressing effect upon the level of investigated markers of bone metabolism; these changes were more pronounced in ovariectomized rats. Administration of Mel only partially levelled changes of bone metabolism caused by pinealectomy. In rats with preserved pineal gland effect of Mel on bone turnover markers was less pronounced. After discontinuing administration of Mel distinct tendency to increase studied biochemical markers of bone metabolism was shown.

CONCLUSION: Our findings suggest that Mel is an important modulator of experimental osteoporosis processes induced in female rats by way of ovariectomy.

Abbreviations

ALP	alkaline phosphatase
Ca	total calcium
HYP	hydroxyproline
5-HT	5-hydroxytryptamine
ICTP	cross-linked carboxyterminal telopeptide of type I collagen
Mel	melatonin
Ox	ovariectomized rats
PICP	carboxyterminal propeptide of type I procollagen
Px	pinealectomized rats
RIA	radioimmunological methods
SOx	sham ovariectomized rats
SPx	sham pinealectomized rats

Introduction

A few studies, particularly experimental, suggest that the main neurohormone of pineal gland – melatonin (Mel) can influence bone tissue metabolism. Our own studies performed previously on starved male rats have shown a significant suppressive effect of Mel on processes of bone formation and resorption [1]. Furthermore, it has been found [unpublished data] that illumination conditions, pinealectomy, and long-term Mel administration influence the circadian metabolism of bone tissue in male rats, and the changes in endogenous Mel concentrations seem to play an important role in the mechanism of this dependence. In an experimental model of postmenopausal osteoporosis, generated by ovariectomy in female rats, a successive lowering of nocturnal Mel concentration was observed [2]. These changes correlated, in an inversely proportional manner, with the values of biochemical markers of bone metabolism, particularly markers of bone resorption. As a consequence of these studies it seems that Mel deficiency, occurring probably as a result of switching off female sex hormone function, may be a cofactor in inducing bone mass changes in female rats with removed ovaries [2].

Clinical studies suggest that characteristic changes of bone mass in postmenopausal osteoporosis, resulting from predominance of resorptive processes over those leading to bone tissue formation, may be related to the Mel [3]. It has been suggested that pineal Mel is an anti-aging hormone [2-6] and that the menopause is associated with a substantial decline in Mel secretion and an increased rate of pineal calcification [8-10]. Studies by Sandyk et al. [3] indicate that the fall of Mel plasma levels during the early stage of menopause may be an important contributory factor in the development of postmenopasual osteoporosis. Consequently, plasma Mel levels in the early menopause could be used as a marker for susceptibility to postmenopausal osteoporosis [3]. In women who were taking M-Oval specimen elaborated by Cohen et al. [11] (containing 75 mg of Mel and a small amount of estrogens) for a period of three years, an increase of bone density was observed. It has been demonstrated that in premenopausal obese women there exists a significant correlation between Mel and type I collagen metabolism [12]. It has also been found in postmenopausal obese women with more than 20% overweight that increased Mel secretion has protective significance in loss of bone mass after menopause [13].

The aim of this study was to demonstrate whether pinealectomy and long-term Mel administration can affect experimental osteoporosis processes (assessed on the base of assays of biochemical markers of bone turnover), induced in female rats by way of ovariectomy.

Material and methods

198 sexually mature female Wistar rats weighing 145 ± 9g were included in the study. During the experiment the animals were housed under conditions of uniform temperature (20 to 22°C), air humidity (80-85%) and light (LD 12:12, light from 7 am to 7 pm). Rats were fed at the onset of the dark phase using a standard diet suitable for conducting bone metabolism research in experimental animals (Altromin Standard Diäten, Austria) and were given drinking water *ad libitum*.

After 2 weeks of adjustment vaginal swabs were taken daily between 8 and 8.30 am. After establishing the estrus cycle, 6 animals were left intact (group 0), 96 underwent ovariectomy in the estrus phase (Ox) and the remaining ones underwent a sham operation (SOx). Two weeks after surgery the rats were divided into eight equal groups with regard to quantity (24 animals in each):

1) SOx + SPx, 2) SOx + SPx + Mel, 3) Ox + SPx,
4) Ox + SPx + Mel, 5) SOx + Px, 6) SOx + Px + Mel,
7) Ox + Px, 8) Ox + Px + Mel.

Animals from the 5^{th}, 6^{th}, 7^{th} and 8^{th} groups were pinealectomized (Px) according to Kuszak and Rodins' method [14] while the remaining ones underwent a sham operation (SPx). Two weeks after surgery Mel (Sigma, USA; 50 μg/100g b.m.) was administered intraperitoneally in the animals of 2^{nd}, 4^{th}, 6^{th} and 8^{th} groups while the remaining animals were administered with solvent only (5% solution of ethyl alcohol in physiological saline). Rats received the Mel solution or the solvent daily between 5 and 6 pm during a 4-week period.

At the appropriate time (with regard to time from Px or SPx), i.e. prior to surgery (group 0) and after 6, 12, 18 and 24 weeks from operation (time subgroups a, b, c, and d) animals were marked and placed separately in metabolic cages for 3 hours (from 6.30 until 9.30 am) in order to collect urine aliquots for hydroxyproline (HYP) and total calcium (Ca) determinations. Within the next 24 hours rats were decapitated (at 8 am). The blood was collected into test tubes with granulated mass (Sarstedt) and centrifuged immediately. The obtained serum was stored frozen at –75°C until determination of alkaline phosphatase (ALP) activity, carboxyterminal propeptide of type I procollagen (PICP) and cross-linked carboxyterminal telopeptide of type I collagen (ICTP) concentrations.

PICP and ICTP concentrations were measured with commercially available RIA kits (Farmos, Finland). Serum ALP activity and urinary excretion of Ca were determined using ALPHA DIAGNOSTICS kits (Poland). Urinary excretion of HYP was determined according to Drózdz et al. [15]. The sensitivity of assays was as follows: PICP 1.2 μg/l, ICTP 0.34 μg/l, HYP 6.6 μmol/l. The linearity for ALP and Ca methods were: up to 1000 U and up to16 mg/dl, respectively. The respective intraassay and interassay coefficients of variations were: PICP 3.1 and 5.8 %, ICTP 4.5 and 6 %, HYP 5.5 and 7.2 %, ALP 7.8 and 8.5 %, Ca 4.7 and 6.8 %.

The results were analysed statistically using variance analysis for parametric tests. After rejecting the variance uniformity hypothesis, further analysis of statistical significance was performed using Student t-test.

Results

In a model of experimental osteoporosis induced by ovariectomy in female rats a distinct tendency to increase the studied biochemical indices of bone metabolism was shown; it was more pronounced in regard to resorption markers, especially ICTP and HYP (Tables 1 and 2). After ovariectomy (Ox + SPx group), the significant increase in ALP values was observed in the beginning of the 20th week (time subgroup c) while in the 8th week (time subgroup a) in regard to ICTP, HYP and Ca values. The ALP values remained at a similar level until the end of observation, whereas ICTP, HYP and Ca concentrations decreased gradually. There were no statistically significant differences between ovariectomized rats and the animals from the control group in regard to changes of PICP concentrations during the time of the study.

Pinealectomy stimulated studied indices of bone turnover (Tables 1 and 2). ALP and PICP values were significantly increased in the 18th week after pinealectomy, whereas ICTP and HYP concentrations - on the 6th and 12th week, respectively (SOx + Px group). Pinealectomy in these rats produced only a slight increase in calcium urine excretion. In rats with removed ovaries (Ox + Px group) the effect of pinealectomy on bone metabolism was more pronounced. ALP activity and PICP, ICTP, HYP as well as Ca concentrations were significantly increased on the 6th week after pinealectomy. Changes in the values of the studied indices of bone turnover (generated by pinealectomy in SOx and Ox female rats) remained at a similar level until the end of observation.

Long-term Mel administration suppressed studied markers of bone turnover, and these changes were more pronounced in rats with removed ovaries (Tables 1 and 2). After the 4th week of Mel administration (time subgroup a) a significant decrease in ALP activity and PICP, ICTP, HYP as well as Ca concentrations, versus appropriate control groups, was observed. Administration of Mel only partially levelled changes of bone metabolism caused by pinealectomy. In rats with preserved pineal gland effect of Mel action on bone metabolism was less pronounced, especially in SOx + SPx + Mel group.

After discontinuing Mel administration, a distinct tendency to increase the studied biochemical markers of bone metabolism was shown (time subgroups b - d). In the 6th week after discontinuing Mel administration (time subgroup b) an increase of ALP activity as well as an increase of PICP, ICTP, Ca and HYP concentrations were generally observed. These alterations were more pronounced within the next weeks of observation (time subgroups c and d). In the 24th week after discontinuing Mel administration (time subgroup d) there were no significant differences in regard to ALP activity as well as concentration of PICP, ICTP, HYP and Ca in comparison to appropriate control groups.

Discussion

In a model of experimental osteoporosis induced by ovariectomy in female rats a distinct tendency to increase the studied biochemical markers of bone metabolism was shown; it was more pronounced in regard to resorption markers, especially ICTP and HYP. These data indicate that ovariectomy and decreased estrogen levels result in increased bone turnover. Although there is evidence that estrogen deficiency is an important contributory factor, the pathogenesis of postmenopausal osteoporosis is multifactorial and presently poorly understood [2, 3, 16-18]. Our previous studies showed evidence that not only estrogen deficiency, but also Mel deficiency induced by ovariectomy in female rats can change in a substan-

Table 1. Dynamic patterns of serum alkaline phosphatase (ALP) activity and carboxyterminal propeptide of type I procollagen (PICP) concentrations in ovariectomized (Ox), pinealectomized (Px) and sham operated (SOx and/or SPx) rats after long-term melatonin (Mel) administration. Data are expressed as mean±SD

		Time from Px or SPx (weeks) [time subgroups]				
	Groups	0	6 [a]	12 [b]	18 [c]	24 [d]
ALP [U/l]						
1.	SOx + SPx		187.24 ± 21.13	178.96 ± 28.98	150.21 ± 17.95	150.99 ± 30.00
2.	SOx + SPx + Mel		149.73 ± 29.50*	144.96 ± 18.01*	125.77 ± 18.03	135.47 ± 19.43
3.	Ox + SPx		209.80 ± 29.87	197.20 ± 22.07	186.01 ± 10.06*	189.43 ± 39.23*
4.	Ox + SPx + Mel	210.01 ± 20.01	161.55 ± 20.11^	155.55 ± 18.36^	155.69 ± 19.07^	162.87 ± 20.33
5.	SOx + Px		200.51 ± 20.32	191.50 ± 20.31	190.37 ± 21.32*	192.06 ± 24.17*
6.	SOx + Px + Mel		158.40 ± 19.78#	153.20 ± 19.81#	157.40 ± 18.70#	168.69 ± 22.39
7.	Ox + Px		260.32 ± 30.21*	247.36 ± 20.32*	223.66 ± 16.89*	214.05 ± 26.37*
8.	Ox + Px + Mel		190.89 ± 22.46~	189.48 ± 20.52~	178.70 ± 28.46~	185.80 ± 25.18
PICP [µg/l]						
1.	SOx + SPx		9.60 ± 1.76	9.44 ± 0.47	9.99 ± 0.76	10.20 ± 0.54
2.	SOx + SPx + Mel		7.66 ± 0.65*	7.54 ± 0.66*	8.26 ± 0.59*	9.17 ± 0.98
3.	Ox + SPx		10.70 ± 1.96	10.30 ± 0.76	8.80 ± 1.19	9.40 ± 0.64
4.	Ox + SPx + Mel	9.72 ± 1.76	8.20 ± 0.81^	7.96 ± 0.55^	7.20 ± 0.88^	8.24 ± 1.02
5.	SOx + Px		10.57 ± 1.67	11.25 ± 2.00	13.03 ± 1.45*	13.21 ± 1.05*
6.	SOx + Px + Mel		8.31 ± 0.99#	8.98 ± 1.12#	10.90 ± 0.87#	11.73 ± 1.37
7.	Ox + Px		12.83 ± 0.98*	12.73 ± 1.74*	13.20 ± 1.60*	14.13 ± 2.07*

tial manner the bone tissue metabolism [2]. In an experimental model of experimental osteoporosis (generated by ovariectomy in female rats) we have shown a decrease of nocturnal concentrations of Mel appearing two weeks after the ovariectomy. However, from the 4th week after surgery the difference in Mel concentrations decreased, in comparison to the control group. The nocturnal changes in Mel concentrations observed in ovariectomized rats within 8 weeks of surgery correlated significantly and negatively with serum ICTP and both urinary HYP and Ca levels, and only slightly - with serum ALP activity and PICP concentrations. These results suggest that secondary changes in Mel concentration, due to the deficiency of sex hormones, co-participating in the development of bone mass changes are characteristic for postmenopausal osteoporosis [2].

Results presented in our recent study seem to support the above-mentioned hypothesis. It has been shown that pinealectomy stimulated serum ALP activity, PICP, and ICTP concentrations and urinary excretion of HYP and Ca; these changes were more pronounced in ovariectomized rats. It is most probably connected with the influence of secondary changes in endogenic factors concentrations including hormones (also Mel), growth, and immunological factors, induced by ovariectomy [1-3, 11-13, 16-24].

Intensification of suppression of studied bone turnover markers by long-term Mel administration observed in rats with removed ovaries (especially in Ox+Px+Mel group) is probably due to synergistic, independent Mel and sex hormones influence on bone metabolism as well as interactions between these hormones. Several studies have shown the effect of estrogen on Mel synthesis in female peripubertal and perimenopausal rats [25-27]. It has been shown that ovariectomy produced a large estradiol-reversible decrease in ^{125}I-Mel binding in the medulla-pons and hypothalamus. In contrast, ^{125}I-Mel binding sites in the other brain regions were generally unaffected by ovariectomy or estradiol [28]. Both 5-hydroxytryptamine (5-HT) and Mel concentrations were reduced during proestrus [29]. Ovarian hormones blocked the isoproterenol-induced elevation of pineal Mel production in the female rat and reduced 5-HT concentrations in ovariectomized rats [29-31]. Moreover, ovariectomy increased the density of β-adrenoreceptors, whereas estradiol replacement blocked these effects. These data suggest that estradiol directly modulates the responses to dopaminergic neurosecretory system in the hypothalamus to Mel [29-31]. On the other hand, it was observed that Mel decreased ovarian weight in rats and inhibited compensatory hypertrophy of the remaining ovary after unilateral ovariectomy [32]. Several investigations have also demonstrated an inhibitory effect of the pineal gland/Mel on the hypothalamo-pituitary-ovarian axis function [5, 6]. It is widely stated that antigonadotropic effect of Mel is achieved by the reduced hypothalamic GnRH release and/or synthesis [5].

Differences in Mel effects on bone tissue metabolism observed in female rats with preserved ovaries in comparison to those after ovariectomy can also be related

Table 2. Dynamic patterns of serum concentrations of cross-linked crocarboxyterminal telopeptide of type I collagen (ICTP) and urinary excretion of hydroxyproline (HYP) and total calcium (Ca) in ovariectomized (Ox), pinealectomized (Px) and sham operated (SOx and/or SPx) rats after long-term melatonin (Mel) administration. Data are expressed as mean±SD

		Time from Px or SPx (weeks) [time subgroups]				
	Groups	0	6 [a]	12 [b]	18 [c]	24 [d]
ICTP [µg/l]						
1.	SOx + SPx		16.44 ± 1.65	14.44 ± 1.11	14.99 ± 0.91	16.00 ± 2.00
2.	SOx + SPx + Mel		12.90 ± 1.43*	11.45 ± 1.73*	12.52 ± 1.23*	13.95 ± 1.07
3.	Ox + SPx		21.30 ± 1.15*	20.34 ± 0.99*	19.90 ± 0.87*	20.18 ± 1.43*
4.	Ox + SPx + Mel	14.72 ± 0.73	16.03 ± 1.87^	15.64 ± 1.26^	16.29 ± 1.79^	17.31 ± 1.58
5.	SOx + Px		18.67 ± 1.33*	17.37 ± 1.26*	17.90 ± 1.74*	20.60 ± 1.97*
6.	SOx + Px + Mel		14.47 ± 1.50#	14.20 ± 1.77#	14.80 ± 1.44#	17.51 ± 2.62
7.	Ox + Px		23.54 ± 1.92*	20.09 ± 1.84*	21.06 ± 2.53*	22.26 ± 0.96*
8.	Ox + Px + Mel		17.48 ± 2.02~	15.44 ± 1.34~	17.14 ± 1.42~	19.21 ± 2.99
HYP [µmol/l]						
1.	SOx + SPx		15.81 ± 1.43	14.72 ± 1.77	17.30 ± 1.34	17.40 ± 1.43
2.	SOx + SPx + Mel		12.92 ± 1.82*	12.66 ± 1.96	14.92 ± 1.79*	15.53 ± 1.77
3.	Ox + SPx		20.15 ± 1.58*	19.19 ± 1.67*	21.50 ± 1.50*	20.40 ± 1.71*
4.	Ox + SPx + Mel	16.05 ± 1.93	15.89 ± 1.78^	15.92 ± 1.54^	19.23 ± 1.99	18.13 ± 1.68
5.	SOx + Px		17.31 ± 2.31	17.50 ± 1.94*	21.48 ± 1.79*	20.93 ± 2.36*
6.	SOx + Px + Mel		13.97 ± 1.63#	14.22 ± 1.80#	17.97 ± 2.37#	18.67 ± 2.06
7.	Ox + Px		20.73 ± 2.76*	19.99 ± 1.85*	23.21 ± 2.71*	23.66 ± 1.99*
8.	Ox + Px + Mel		15.50 ± 1.92~	14.88 ± 2.00~	18.48 ± 1.89~	20.29 ± 2.93
Ca [mmol/l]						
1.	SOx + SPx		2.83 ± 0.28	3.36 ± 0.22	3.35 ± 0.48	2.94 ± 0.86
2.	SOx + SPx + Mel		2.41 ± 0.30*	2.89 ± 0.29*	3.00 ± 0.34	2.70 ± 0.47
3.	Ox + SPx		3.31 ± 0.24*	4.27 ± 0.23*	3.72 ± 0.46	3.30 ± 0.59
4.	Ox + SPx + Mel	2.69 ± 0.19	2.76 ± 0.31^	3.62 ± 0.49^	3.21 ± 0.45	2.94 ± 0.32
5.	SOx + Px		3.07 ± 0.41	3.61 ± 0.36	3.78 ± 0.40	3.27 ± 0.37
6.	SOx + Px + Mel		2.51 ± 0.38#	3.08 ± 0.43#	3.25 ± 0.48	2.96 ± 0.40
7.	Ox + Px		3.64 ± 0.42*	4.16 ± 0.45*	4.19 ± 0.38*	3.83 ± 0.41*
8.	Ox + Px + Mel		2.75 ± 0.40~	3.16 ± 0.43	3.35 ± 0.46	2.94 ± 0.89

* $p \leq 0.05$ vs control group (SOx + SPx); ^ $p \leq 0.05$ vs group 3 (Ox + SPx); # $p \leq 0.05$ vs group 5 (SOx + Px); ~ $p \leq 0.05$ vs group 7 (Ox + Px)

to the effect of secondary (induced by ovariectomy) changes in endogenic factors concentrations including hormones, growth and immunological factors [2, 3, 13, 16-23].

In female rats with preserved pineal gland effect of Mel action on bone metabolism was less pronounced which suggests that the pineal gland itself can modify the effects of its own action most probably by specific receptors for this hormone [5, 6, 33-37]. It was shown that the density of these receptors is inversely proportional to Mel concentration in blood, hence the strongest effect of this hormone action is in the end of light phase [33-35]. Other studies suggest the possibility of indirect influence of administrated Mel on the pineal gland by inducing changes in hormones concentrations [38-42], which could influence secondarily the pineal gland [43-46].

Exogenous Mel administration only partly levelled changes in concentrations of biochemical markers of bone metabolism caused by pinealectomy, especially ICTP and HYP. That indicates most probably participation of other pineal factors in this mechanism. In the pineal gland of mammalians, other except Mel indoles substances presence was revealed [5, 6, 47]. The presence of polypeptide substances including those synthesized in the pineal gland (potential pineal hormones) was discovered [5, 6, 48]. However, their chemical structure and potential participation in regulatory mechanisms underlying the control of the pineal gland are still unknown.

The effect of estrogen and Mel on bone tissue metabolism seems to be synergistic, although achieved through independent mechanisms. It is well known that osteoblasts and osteoclasts have receptors to estrogens, which suggests the possibility of its direct influence on bone tissue [16, 17, 49-51]. It is claimed that estrogens can also cause changes in concentration of systemic and local factors such as: calcitonin, interleukin-1 and 6, transforming growth factor-β (TGF-β), prostaglandin E2 or insulin-like growth factors (IGFs), especially IGF-I [51-56]. Estrogens, through the receptor stimulate osteoblasts to autocrine secretion of IGF-1, which stimulates osteoblasts maturation, growth and collagen and alkaline phosphatase secretion [16, 51, 57].

However, there is still much to be revealed about the effect of Mel on the bone tissue metabolism rate. There could be two possible explanations of the mechanism of bone metabolism regulation by Mel. It seems that Mel can influence bone tissue metabolism indirectly, by inducing changes in concentrations of endogenic factors, mainly hormonal. Our own studies [unpublished data] show that Mel may influence the circadian rhythm of bone formation and resorption processes indirectly, by inducing changes in concentrations of hormonal factors that significantly influence these processes such as parathormone, thyroid hormones, corticosterone and IGF-I. We cannot exclude direct influence of Mel on bone tissue cells. Latest studies indicate that in *in vitro* conditions this hormone may play an essential role in regulating bone formation. It was shown that it stimulates osteoblast differentiation and mineralization of matrix in culture [58]. Mel enhances also synthesis of collagenic and noncollagenic proteins of bone matrix [59]. No reports were found, however, concerning the presence of Mel receptors on either osteoblasts or osteoclasts. It is known that lack of membrane receptors does not necessarily reflect the lack of Mel influence upon a given type of cells [5, 6]. This hormone easily penetrates intercellular components and may probably act after adhering to intracellular binding sites for example via nuclear receptors RZR/ROR.

After discontinuing administration of Mel a distinct tendency to increase the studied biochemical markers of bone metabolism was shown, which seems to confirm a previously formulated conception that Mel is one of the contributory factors in the development of postmenopausal osteoporosis.

In conclusion, our findings indicate that melatonin is an important modulator of experimental osteoporosis induced in female rats by ovariectomy.

REFERENCES

1 Ostrowska Z, Swietochowska E, Buntner B, Marek B, Zwirska-Korczala K, Spyra Z, et al. Assessment of relationship between secretion of melatonin, activity of thyroid, adrenal cortex as well as testes and chosen markers of collagen metabolism in starved rats. Endocrine Regul 1996; **30**:83-92.

2 Ostrowska Z, Kos-Kudla B, Swietochowska E, Marek B, Kajdaniuk D, Gorski J. Assessment of the relationship between dynamic pattern of nighttime levels of melatonin and chosen biochemical markers of bone metabolism in a rat model of postmenopausal osteoporosis. Neuroendocrinol Let 2001; **22**:131-8.

3 Sandyk R, Anastasiadis PG, Annimos PA, Tsagas N. Is postmenopausal osteoporosis related to pineal gland functions? Int J Neurosc 1992; **62**:215-25.

4 Schmid HA. Depresed melatonin biosynthesis, calcium flux, pineal gland calcification and aging: a hypothetical fromework. Gerontology 1993; **39**:189-99.

5 Karasek M. Szyszynka i melatonina. [(The pineal gland and melatonin.) (In Polish)] Warszawa-Lódz; PWN: 1997.

6 Karasek M, Reiter RJ. Melatonin and aging. Neuroendocrinol Lett 2002; **23** (suppl 1): 14–16.

7 Csaba G, Baráth P. The effect of pinealectomy on the parafollicular cells of the rat thyroid gland. Acta Biol 1977; **28**:143-4.

8 Reiter RJ. The pineal gland. In: Becker KL, editors, Principles and practice of endocrinology and metabolism, Lippincott, Philadelphia; 1990. p. 104-9.

9 Reiter RJ. Pineal melatonin: cell biology of its synthesis and of its physiological interactions. Endocrine Rev 1991; **12**:151-80.

10 Schmid HA, Requintina PJ, Oxenkurg GF, Sturner W. Calcium, calcification, and melatonin biosynthesis in the human pineal gland: a postmortem study into age-related factors. J Pineal Res 1994; **16**:178-83.

11 Cohen M, van Heusden AM, Verdok HER, Wijnhamer P. Melatonin/noretisterone contraception. In: Touitou Y, Arendt J, Pévet P, editors. Melatonin and the pineal gland – from basic science to clinical application. Amsterdam: Elsevier; 1993. p. 339-45.

12 Ostrowska Z, Swietochowska E, Zwirska-Korczala K, Buntner B, Marek B, Pardela M, et al. Assessment of the relationship between collagen metabolism and selected hormonal

factors in extremely obese women before and after jejunoileostomy. Endocrine Regul 1997; **31**:55-61.
13 Ostrowska Z, Kos-Kudla B., Marek B., Swietochowska E., Gorski J. Assessment of the relationship between circadian variations of salivary melatonin levels and type I collagen metabolism in postmenopausal obese women. Neuroendocrinol Let 2001; **22**:255-62.
14 Kuszak J, Rodin M. A new technique of pinealectomy for adult rats. Experientia (Basel) 1977; **33**:283-4.
15 Drozdz M, Kucharz E, Szyja J. A colorimetric micromethod for determination of hydroxyproline in blood serum. Z Med Labortechnik 1976; **17**:163-9.
16 Badurski J, Sawicki A, Boczon S. Osteoporoza. [(Osteoporosis.) (In Polish)]. 2nd ed. Bialystok: Osteoprint; 1994.
17 Galus K: Choroby metaboliczne kosci. [(Bone metabolic disease.) (In Polish)]. Warszawa: Med. Tour Press International; 1995.
18 Lukaszewicz J, Kaczmarewicz E, Lorenc RS. Nowoczesne markery obrotu kostnego. [(Modern markers of bone turnover.) (In Polish)]. Warszawa: Osteoforum; 1998.
19 Dietrich JW, Canalis EM, Maina DM, Raisz LG. Hormonal control of bone synthesis *in vitro*: effects of parathyroid hormone and calcitonin. Endocrinology 1976; **98**:943-9.
20 Raisz LG, Kream BE. Regulation of bone formation. N Engl J Med 1983; **309**:83-9.
21 Fujita T. Calcium regulating hormones. Nippon Sekeigeka Gakkai Zassi 1988; **62**:267-77 (Abstract Medline).
22 Fujita T, Matusui T, Nakao Y, Shizawa S, Imai Y: Cytokines and osteoporosis. Ann NY Acad Sci 1990; **587**:371-5.
23 Yashikawa H, Takaoka K. Bone remodeling and its regulators. Nippon Rinsho 1990; **48**:2797-803.
24 Kawaguchi H, Pilbeam CC, Woodiel FN, Raisz LG. Comparison of the effect of 3,5.3'-triiodothyroacetic acid and triiodothyronine on bone resorption in cultured fetal rat long bones and neonatal mouse calvariae. J Bone Miner Res 1994; **9**:247-53.
25 Okatani Y, Hayashi k, Watanabe K, Morioka N, Sagara Y. Estrogen modulates the nocturnal synthesis of melatonin in peripubertal female rats. J Pineal Res 1998; **24**:224-9.
26 Hayashi K, Okatani Y. Mechanisms underlying the effects of estrogen on nocturnal melatonin synthesis in peripubertal female rats: relation to norepinephrine and adenylate cyclase. J Pineal Res 1999; **26**:178-83.
27 Okatani Y, Marioka N, Hayashi K. Changes in nocturnal pineal melatonin synthesis during the perimenopausal period: relation to estrogen levels in female rats. J Pineal Res 1999; **27**:65-72.
28 Laudon M, Zisapel N. Impact of circulating estradiol on melatonin binding sites in discrete areas of the female rat brain. Brain Res 1987; **402**:146-50.
29 Moujir F, Bordon R, Santana C, Abreu P, Hernandez G, Alonso R. Ovarian steroids block the isoproterenol-induced elevation of pineal melatonin production in the female rat. Neurosci Lett 1990; **119**:12-4.
30 Zisapel N, Shaharabani M, Laudon M. Regulation of melatonin's activity in the female rat by estradiol: effects on neurotransmitter release and on iodomelatonin binding sites. Neuroendocrinology 1987; **46**:207-16.
31 Yie SM, Brown GM. Effects of sex hormones on the pineal response to isoproterenol and on pineal beta-adrenergic receptors. Neuroendocrinology 1995; **62**:93-100.
32 Lewinski A, Szymczykiewicz P, Sewerynek E, Wajs E. Effects of pinealectomy and melatoninadministration on certain indices of ovarian hyperplasia and/or hypertrophy in rats with both ovaries intact or after unilateral ovariectomy. J Pineal Res 1993; **14**:117-27.
33 Gauer F, Masson-Pévet M, Skene DJ, Vivien-Roels B, Pévet P. Daily rhythms of melatonin binding sites in the rat pars tuberalis and suprachiasmatic nuclei; evidence for a regulation of melatonin receptors by melatonin itself. Neuroendocrinology 1993; **57**:120-6.
34 Gauer F, Masson-Pévet M, Pévet P. Differential regulation of melatonin receptors by short- versus long-term pinealectomy in the rat suprachiasmatic nuclei and pars tuberalis. J Pineal Res. 1994; **16**:73-6.
35 Gauer F, Masson-Pévet M, Stehle J, Pévet P. Daily variation in melatonin receptor density of rat tuberalis and suprachiasmatic nuclei are distinctly regulated. Brain Res 1994; **641**:92-8.
36 Morgan PJ, Barret P, Howell E, Helliwell R. Melatonin receptors: localization, molecular pharmacology and physiological significance. Neurochem Int 1994; **24**:101-46.
37 Miguez JM, Simonneaux V, Pévet P. Changes in pineal indoleamines in rats after melatonin injections: evidence for a diurnal sensitivity to melatonin. J Neuroendocrinol 1996; **8**:611-6.
38 Reiter R.J. The pineal and its hormones in the control of reproduction in mammals. Endocr Rev 1980; **1**:109-31.
39 Mess B. Interaction between pineal and nonreproductive endocrine gland. In: Axelrod J, Frashini F, Velo GP, editors, The pineal gland and its endocrine role, New York: Plenum Press; 1983. p. 477-508.
40 Armstrong SM, Cassone VM, Chesworth MJ, Redman JR, Short RV. Synchronization of mammalian circadian rhythms by melatonin. J Neural Transm 1986; **21** (suppl):375-94.
41 Reiter R.J. Mechanisms of control of reproductive physiology by pineal gland and its hormones. Adv Pineal Res 1987; **2**:109-25.
42 John TM, Viswanathan M, George JC, Scanes CG. Influence of chronic melatonin implantation on circulating levels of catecholamines, growth hormone, thyroid hormones, glucose, and free fatty acids in the pigeon. Gen Comp Endocrinol 1990; **79**:226-36.
43 Vacas MI, Keller-Sarmiento MI, Pereyra EN, Etchegoyen GS, Cardinali DP. *In vitro* effects of adenohypophysial hormones on rat pineal melatonin content and release. Mol Cell Endocrinol 1987; **50**:23-7.
44 Bauer MS, Poland RE, Whybow PC, Frazer A. Pituitary-adrenal and thyroid effects on melatonin content of the rat. Psychoneuroendocrinology 1989;**14**:165-75.
45 Yuwiler A. Effects of steroids on serotonin-N-acetyltransferase activity of pineals in organ culture. J Neurochem 1989; **52**:46-53.
46 Rom-Bugoslavoskaia ES, Bondarenko LA, Sil'chenko TN. Epifizarno-tireoidnye vazimootnoshenie: vliianie kal'tsitonina na metabilizm indolov v norme i na fone izbytka tireoidnykh gormonov. [(Epiphyseal-thyroid interrelations: effect of calcitonin on indole metabolism in health and in the presence of an excess of thyroid hormones.) (in Russian with English abstract.)] Probl. Endokrinol Mosk 1991; **37**:33-5.
47 Beck O, Borg S, Lundman A. Concentrations of 5-methoxyindoles in the human pineal gland. J Neural Transm 1982; **54**:111-6.
48 Pévet P. 5-Methoxyindoles, pineal peptides and reproduction. In: Brown GM, Wainwright SD, editors, The pineal gland. Endocrine aspects, Pergamon Press, Oxford; 1985. p. 81-101.
49 Komm BS, Terpening CM, Benz DJ, Graeme BW, Gallegos A, Korc M, et al. Estrogen binding receptor mRNA and biologic response in osteoblast-like osteosarcoma cells. Science 1988; **1241**:81-4.
50 Kaye AM, Weisman Y, Harell A, Sojmen D. Hormonal stimulation of bone cell proliferation. J Steroid Biochem Mol Biol 1990; **37**:431-5.
51 Kajdaniuk D, Marek B, Swietochowska E, Buntner B, Matuszewska G. Insulinopodobny czynnik wzrostowy-I: aspekty patofizjologiczne i kliniczne. [(Insulin-like growth factor-I: pathophysiological and clinical aspects.) (In Polish with English abstract.) Pol Merk Lek 1999; **41**:205-7.
52 Paciifici R, Fifas L, Teitelbaum S, Slatopolsky E, Mc Cracken R, Bergfeld M, et al. Spontaneous release of interleukin 1 from human blood monocytes reflects bone formation in idiopathic osteoporosis. Proc Nat Acad Sci 1987; **84**:4616-20.
53 Ernst M, Heath JK, Rodan GA. Estradiol effects on proliferation, messenger ribonucleic acid for collagen and insulin-like growth factor-I, and parathyroid hormone stimulated adenylate cyclase activity in osteoblastic cells from calvariae and long bones. Endocrinology 1989; **125**:825-33.
54 Gray TK, Mohan S, Linkhart TA, Baylink DJ. Estradiol stimulates in vitro the secretion of insulin-like growth factors by the clonal osteoblastic cell line, UMR 106. Biochem Biophys Res Commun 1989; **158**:407-12.
55 Stock JL, Coderre JA, Mc Donald B, Rosenwasser LJ. Effect of estrogen in vivo and in vitro on spontaneous interleukin-I release by monocytes from postmenopausal women. J Clin Endocrinol Metab 1989; **68**:346-68.
56 Ernst M, Rodan GA. Estradiol regulation of insulin-like growth factor-I expression in osteoblastic cells: evidence for transcriptional control. Mol Endocrinol 1991; **5**:1081-9.
57 Marek B, Buntner B, Borgiel-Marek H, Kos-Kudla B, Ostrowska Z, Swietochowska E. Insulinopodobne czynniki wzrostowe (IGFs) i ich bialka wiazace (IGFBPs) w stanach fizjologii i patologii. [(Insulin-like growth factors (IGFs) and their binding proteins (IGFBPs) in physiology and pathology.) (In Polish.)] Pol Arch Med Wewn 1996; **96**:290-7.
58 Roth JA, Kim BG, Lin WL, Cho MI. Melatonin promotes osteoblast differentiation and bone formation. J Biol Chem 1999; **274**:22041-7.
59 Nakade O, Koyama H, Ariji H, Yajima A, Kaku T. Melatonin stimulates proliferation and type I collagen synthesis in human bone cells *in vitro*. J Pineal Res 1999; **27**:106-10.