Influence of pinealectomy and long-term melatonin administration on GH-IGF-I axis function in male rats

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Abstract

BACKGROUND: A relationship between circadian GH-IGF-I axis activity and pineal gland function in rats is not sufficiently elucidated, particularly in the aspect of melatonin (MEL) participation with relation to a possible mechanism of these dependencies. **OBJECTIVE**: Influence of pinealectomy and long-term MEL administration on circadian GH-IGF-I axis function in male rats was evaluated. An attempt was also made to determine whether the effect of exogenous MEL is dependent on the pineal gland presence. **METHODS**: Studies were performed in 192 sexually mature male Wistar rats, which were subjected to pinealectomy or sham operation. In half of the animals from each group MEL (Sigma, USA) in dose of 50 μg/100g b.m. was administrated intraperitoneally (daily between 5 and 6 pm during a 4-week period). Blood for RIA assays of MEL, GH and IGF-I concentrations was collected every 3 hours during a day-beginning at 8 am (rats killed by decapitation). **RESULTS**: Significant influence of pinealectomy and exogenous MEL on a daily profile of endogenous MEL in rats was confirmed. Distinct, dependent on the time of the day anomalies in circadian oscillations of GH and IGF-I showing positive correlation with changes in endogenous MEL concentrations were also shown. GH rhythm was suppressed in a group of rats with removed pineal gland; after pinealectomy distinct decrease and after MEL use - distinct increase of GH and IGF-I concentrations during the day were observed. It had influenced mean daily concentrations and values of amplitude of circadian GH and IGF-I oscillations in all studied groups of animals. In rats with preserved pineal gland the effect of exogenous MEL action was more intensified. **CONCLUSIONS**: Pineal gland can influence circadian function of GH-IGF-I axis, and in mechanism of this dependence, changes in endogenous MEL concentrations seem to play an important role. Administration of MEL in rats after pinealectomy only partly prevents changes of GH-IGF-I axis function caused by gland removal, which can indicate participation of other pineal gland substances in generating disturbances. Influence of exogenous MEL on GH-IGF-I axis function during the day is dependent on the presence of pineal gland, which can be connected with indirect and/or direct influence of administrated hormone on this gland.

ORIGINAL ARTICLE

ABBREVIATIONS

A   amplitude
ACTH   adrenocorticotropin hormone
ALP   alkaline phosphatase
Ca   total calcium
EGF   epidermal growth factor
FSH   folitropin
f (T)   average hormone concentration at the given time point
GH   growth hormone
5HIAA   5-hydroxyindolo-acetic acid
HIOMT   hydroxyindole-O-methyltransferase
HYP   hydroxyproline
ICTP   cross-linked carboxyterminal propeptide of type I collagen
IGF-I   insulin-like growth factor
LD   light:dark cycle
LH   lutropin
M   mesor
MEL   melatonin
NAT   N-acetylotransferase
NE   norepinephrine
p   probability
PDGF   platelet-like growth factor
PICP   carboxyterminal propeptide of type I procollagen
PTH   parathyroid hormone
PRL   prolactin
Px   pinealectomized rats
RIA   radioimmunological methods
SE   standard error
SPx   sham operated rats
TGF   transforming growth factor
TNF   tumor necrosis factor
TSH   thyrotropin
φ   acrophase
ω   angular frequency

Introduction

Many studies indicate connection between pineal gland function and GH-IGF-I axis in mammals, however their results are not always synonymous [1, 2]. Some investigators have shown that pinealectomy (Px) abolishes a decrease of GH concentration in rats' pineal gland, as well as in plasma caused by constant darkness [3]. Others obtained the decrease of GH secretion in rats after Px during the day and a lack of the effect at night hours [4]. Others indicate intensification of circadian fluctuations of GH after Px in rats with small increase of daily secretion [5].

Griffits's et al. [6] and Smythe's and Lazarus's [7] studies revealed that liberation of GH from hypophysis in rats is inhibited by MEL and stimulated by serotonin. Griffits et al. [6] also confirmed that MEL inhibited basic and TRH stimulated secretion of this hormone *in vitro*. During prolonged (24 h) incubation a weak inhibitory effect of MEL on GH secretion by pituitary cells of neonatal but not adults rats was observed [8]. Injection of MEL to rats in perinatal period did not influence the daily rhythm of GH in mature animals [9]. Significant disturbances in the profile of daily GH secretion after MEL administration in grown-up individuals have also not been shown [5].

Results concerning assessment of pineal gland and MEL influence on synthesis and liberation of GH in other animals are also not synonymous. In lemmings (*Dicrostonyx groehlandicus*) kept in short day conditions GH concentrations during the day were increased. After exposure to long light cycle, daily profile of GH similar to that in control animals was observed [10]. In *in vitro* studies MEL did not influence GH liberation from mink's pineal gland during a short and a long day [11]. Subcutaneous implantation of MEL granules to pigeons (2mg for a period of 12 months) caused significant increase of GH concentrations [12].

It was shown in clinical studies that stimulation of GH secretion after postinsulin hypoglycaemia, arginin infusions, clonidin or GHRH administration is accompanied by decrease of MEL concentrations [13–16], which could indicate existence of inversely proportional dependence between MEL and GH. However, after MEL administration, changes have not been observed [17], but only the decrease [13] or stimulation of GH secretion [18].

Smythe et al. [19] observed the stimulating influence of serotonin and inhibiting of MEL on insulin-like growth factors also called somatomedins, through which GH influences tissues and target organs. Vriend et al. [20] showed the essential increase of GH and IGF-I concentrations in male Syrian hamsters after MEL administration at evening hours for a 10 week period. On the other hand in female Syrian hamsters a short day induced the decrease of IGF-I concentration and Px partly abolished this effect [21]. MEL administration in dose of $25\mu g$ caused suppression of IGF-I concentrations in these animals [21, 22].

The aim of the performed studies was to show: 1) if and in what degree Px in rats and long-term MEL administration influence daily profile of GH and IGF-I concentrations, 2) if effect of MEL administration is dependent on pineal gland presence.

Material and methods

Studies were performed in 192 male Wistar rats with the initial body mass of circa 150 g. Before the beginning of the studies animals stayed for 2 weeks in a place with a temperature of 20–22°C, air humidity of 80–85% and regulated light cycle: 12 hours of light: 12 hours of dark (LD 12:12, light from 7 am to 7 pm) with the aim of adaptation. Rats were fed every day at the beginning of dark phase with chow produced by Altromin firm 1324 (Standard-Diäten, Austria) and drank filtered water *"ad libitium"*.

In a period of adaptation in half of the animals (96 rats) pinealectomy according to Kuszak and Rodin's method [23] in intraperitoneal narcosis with the use of 2% chloral hydrate and in the rest of them – sham

operation were performed. Two weeks after operation the rats were divided into four equal groups with regard to quantity (48 animals in each): 1) SPx - control group after sham operation, which received intraperitonealy 5% solution of ethyl alcohol in physiological saline; 2) SPx + MEL – after sham operation – which received intraperitonealy MEL (Sigma, USA) in amount of 50 μg/100g b.m. in solvent mentioned above; 3) Px – after pinealectomy – which received only solvent; 4) Px + MEL – after pinealectomy – which received 50μg/100g b.m. MEL in solvent mentioned above. Rats were administered the MEL solution or the solvent daily between 5 and 6 pm during a 4-week period. During experiment as well as during adaptation period rats stayed in the same zoohigienical, alimentary and illumination conditions.

After the end of the experiment animals were killed by decapitation and blood was collected (to tubes with granulated mass produced by Sarstedt firm) for MEL, GH and IGF-I concentrations assays. Blood was collected every 3 hours during the day, from 8 am, in 6 rats in each of time points. Collected blood was immediately centrifuged and obtained serum kept in temperature of –75 °C till assays.

All actions during the dark period were done in red light obtained with the use of photographic lamps with dark-room filter F-05.

The study was conducted with the permission of the Ethics Committee at the Silesian Academy of Medicine in Katowice.

Hormones concentrations were assessed in doublets with the use of commercial RIA kits: MEL–DRG (USA), rGH–IMMUNOTECH (France) and rIGF-I–DSL (USA).

Sensitivity of the method, within-assay and between-assay error were for MEL of – 1 pg/for sample, 11 and 12%, for GH of – 1 ng/ml, 6.2 and 8.1% and for IGF-I of - 2.73 nmol/l, 3.7 and 6.1% .

The statistical significance of the results was assessed on the base of variance analysis for parametric tests when distribution of the variable was normal. When the variable distribution was not normal variance analysis was conducted according to Kruskal-Wallis method for nonparametric tests. The dependency of MEL levels of GH and IGF-I was assessed with the use of the Pearson correlation test (when variable distribution was normal) or Spearman correlation test (when variable distribution was not normal).

The statistical analysis of circadian rhythms of MEL, GH and IGF-I concentrations was carried out with the use of cosinor method according to Halberg et al. [23]. Cosinor analysis was carried out for a fixed average time group value by fitting the main cosinor function f (T) = M + A cos (tω + φ), where f (T) is the average hormone concentration at the given time point; M is the mesor, arithmetic average of actual values describing oscillations within the cycle; A is the amplitude, the difference between maximum (or minimum) value of a given hormone concentration within 24 hours; ω is angular frequency. The appearance of a rhythm was deducted following the rejection of zero amplitude hypothesis.

Results

Significant daily rhythm of MEL with acrophase at 3.25 am, mesor value of 25.19 pg/ml and amplitude of 25.19 pg/ml (Tables 1 and 2, Figure 1) was shown in rats with preserved pineal gland (SPx - control group). Administration of MEL to rats from control group (SPx + MEL) caused suppression of endogenic MEL rhythm with increase of mean daily concentration. Increase of MEL concentrations in relation to control group was observed from 8 am till 11 pm. Distinct decrease of MEL concentrations from 8 pm till 11am was shown in rats after pinealectomy (Px). MEL secretion at these time points differed statistically from that observed in control group, which caused suppression of the rhythm and decrease of mean daily concentration. Administration of MEL to rats after pinealectomy (Px+MEL) caused increase of mean daily endogenic MEL concentration, increase of amplitude value and displacement of the peak of circadian oscillations for 3.20 pm; rhythm was still suppressed. The increase of endogenic MEL concentrations in comparison to Px group was observed during the whole day.

In rats after sham operation, GH secretion was subjected to significant circadian fluctuations with acrophase at 6.20 am, mesor value of 23.44 ng/ml and amplitude of 7.91 ng/ml (Tables 1 and 2, Figure 1). MEL administration caused stimulation of GH secretion from 11 am till 5 am and suppression at 8 am in rats with preserved pineal gland. GH concentrations were statistically increased from 5 pm till 2 am and at 11 am. The increase of mesor and decrease of amplitude values with a preserved daily rhythm of GH was observed. The suppression of GH secretion rhythm with distinct decrease of concentrations from 2 am till 8 pm and increase at 11 pm was shown in rats after Px. Differences were significant only from 2 am till 2 pm and at 11 pm. Administration of MEL to rats after Px caused the increase of amplitude of circadian GH oscillations, the increase of mean daily concentration and the intensification of circadian fluctuations. At all periods of the day except 11 pm distinct increase of GH concentrations in relation to group after Px was noted. GH secretion was statistically increased from 2 am till 8 am and at 2 pm.

IGF-I concentrations in the serum of rats after sham operation were subjected to not significant fluctuations during the day with maximum at 3.19 pm.

Table 1. Mean serum concentrations (mean ± SE) of melatonin (MEL), growth hormone (GH) and insulin-like growth factor-I (IGF-I) in sham operated (SPx) and pinealectomized (Px) rats after exogenous melatonin (MEL) administration

| Variables | Hours | Groups (LD 12:12) | | | |
		SPx	SPx + MEL	Px	Px + MEL
MEL (pg/ml)	2.00	70.73 ± 4.11	74.28 ± 3.77	8.99 ± 0.36***	37.87 ± 0.74***.
	5.00	49.54 ± 5.58	57.59 ± 1.80	9.00 ± 0.47***	44.49 ± 0.92***
	8.00	20.83 ± 1.56	60.07 ± 4.41***	8.31 ± 0.12***	39.00 ± 6.14***
	11.00	16.18 ± 1.99	63.99 ± 3.81***	8.00 ± 0.16**	52.93 ± 2.71***
	14.00	8.72 ± 1.11	42.15 ± 3.43***	9.02 ± 0.10	71.51 ± 1.52***
	17.00	7.73 ± 1.22	53.14 ± 4.79***	8.89 ± 0.25	59.33 ± 1.80***
	20.00	12.30 ± 1.00	49.26 ± 5.33***	9.12 ± 0.17*	58.37 ± 6.03***
	23.00	20.47 ± 1.84	51.09 ± 2.27***	8.73 ± 0.22***	37.92 ± 1.88***
GH (ng/ml)	2.00	29.64 ± 2.09	36.23 ± 2.21*	18.60 ± 1.76***	22.00 ± 1.65
	5.00	32.76 ± 1.03	34.86 ± 3.06	18.00 ± 1.99***	22.40 ± 1.03*
	8.00	30.30 ± 1.88	26.98 ± 4.56	22.00 ± 2.00**	27.80 ± 1.05**
	11.00	21.46 ± 1.54	26.33 ± 2.21**	15.51 ± 1.67**	16.02 ± 3.44
	14.00	22.46 ± 2.00	24.11 ± 3.79	14.68 ± 1.54**	17.90 ± 0.41*
	17.00	19.20 ± 1.98	24.20 ± 1.60*	15.64 ± 3.68	17.00 ± 2.89
	20.00	14.31 ± 1.11	19.60 ± 1.97*	12.26 ± 3.55	11.66 ± 1.99
	23.00	17.46 ± 1.32	27.21 ± 1.48***	20.56 ± 0.49**	17.99 ± 4.06
IGF-I (nmol/l)	2.00	113.39 ± 0.47	160.32 ± 5.10***	77.08 ± 3.59***	95.21 ± 3.44**
	5.00	71.63 ± 7.30	97.96 ± 4.42*	61.32 ± 7.73	57.63 ± 4.80
	8.00	89.15 ± 10.20	136.44 ± 13.56*	102.06 ± 4.87	93.35 ± 5.87
	11.00	149.82 ± 4.20	181.13 ± 15.37	92.94 ± 4.11***	112.33 ± 7.53*
	14.00	164.02 ± 3.67	173.93 ± 13.94	89.87 ± 8.65***	110.24 ± 8.65*
	17.00	144.25 ± 6.88	137.12 ± 7.35	105.82 ± 9.39**	135.64 ± 6.50**
	20.00	107.52 ± 7.05	138.84 ± 9.90*	76.49 ± 3.47**	78.36 ± 3.81
	23.00	112.02 ± 10.86	117.44 ± 7.88	70.34 ± 5.43**	104.83 ± 7.22**

* p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001   vs control group

Table 2. Chronobiologic parameters (M - mesor, A - aplitude and φ - acrophase) of the mean rhythms of melatonin (MEL), growth hormone (GH) and insulin-like growth factor-I (IGF-I) in sham operated (SPx) and pinealectomized (Px) rats after exogenous melatonin (MEL) administration.

| Variables | Chronobiologic parameters | Groups (LD 12:12) | | | |
		SPx	SPx+MEL	Px	Px+MEL
MEL	M (pg/ml)	25.83	↑ 57.32	↓ 8.80	↑ 51.51
	A (pg/ml)	25.19	↓ 8.69	↓ 0.36	↑ 14.44
	φ (h.min)	3.25	4.49	→ 21.30	15.20
	p	0.043*	0.233	0.258	0.059
GH	M (ng/ml)	23.44	↑ 27.44	↓ 17.16	↑ 19.03
	A (ng/ml)	7.91	↓ 6.20	↓ 3.01	↑ 5.25
	φ (h.min)	6.15	4.21	4.42	6.09
	p	0.018*	0.045*	0.179	0.049*
IGF - I	M (nmol/l)	119.00	↑ 143.17	↓ 85.11	↑ 99.68
	A (nmol/l)	33.98	↓ 20.99	↓ 14.99	↑ 20.85
	φ (h.min)	5.19	13.35	13.28	15.26
	p	0.066	0.340	0.139	0.231

↑ - significantly higher values versus control group
↓ ⁻ significantly lower values versus control group
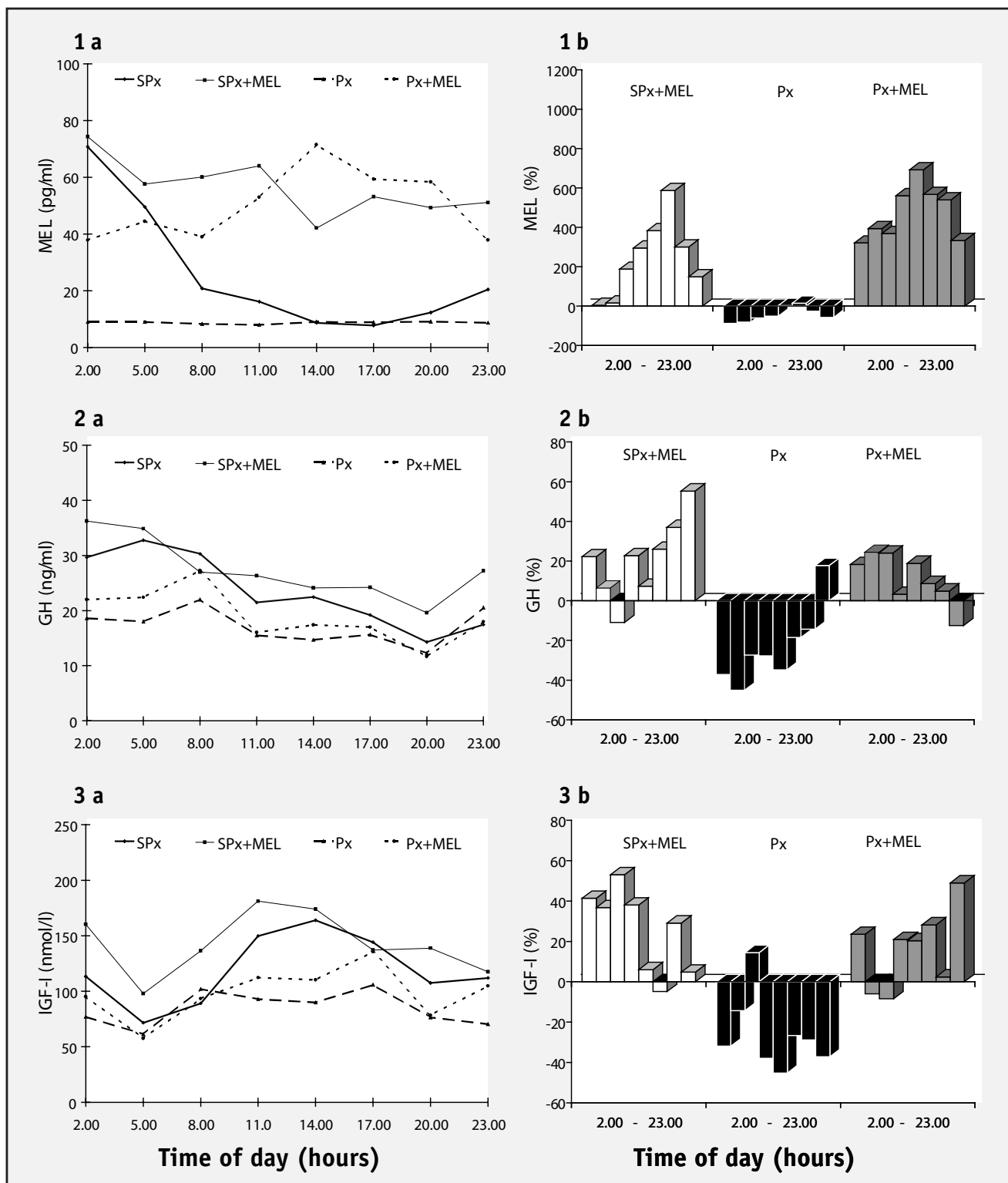← - acrophase shift to earlier hours
→ - acrophase shift to later hours
p - probability (anything less than 0.05 indicates a statistically rhythm detection)

Table 3. Correlation coefficients (r) between circadian variations of serum melatonin (MEL), growth hormone (GH) and insulin-like growth factor-I (IGF-I) concentrations during the day in sham operated (SPx) and pinealectomized (Px) rats after exogenous melatonin (MEL) administration

| Independent variable | Dependent variables | Groups (LD 12:12) | | | |
		SPx	SPx + MEL	Px	Px + MEL
MEL (pg/ml)	GH (ng/ml)	0.461**	0.317*	0.308*	0.250
	IGF-I (nmol/l)	0.516**	0.287*	0.456**	0.352**
IGF-I (nmol/l)	GH (ng/ml)	0.755***	0.426**	0.297*	0.205

* p ≤ 0.05;   ** p ≤ 0.01;   *** p ≤ 0.001

Fig. 1. Mean serum concentrations (mean ± SE) of: 1 - melatonin (MEL); 2 - growth hormone (GH); 3 - insulin-like growth factor-I (IGF-I) in sham operated (SPx) and pinealectomized (Px) rats after exogenous melatonin (MEL) administration expressed in: a – pg, ng/ml and nmol/l, respectively; b – as percent of the control values

Mean daily concentration was of 119.00 nmol/l and amplitude value of 33.98 nmol/l (Tables 1 and 2, Figure 1). Administration of MEL to rats with preserved pineal gland caused the increase of mean daily concentration and decrease of amplitude of circadian oscillations of IGF-I. Increase of IGF-I concentrations in relation to control group was observed at all time points except 5 pm. Increase of IGF-I values was sig-

nificant from 2 am till 8 am and at 8 pm. Decrease of mean daily concentration and amplitude of circadian fluctuations of IGF-I as a result of decrease of concentrations of this protein at most time points except 8 am was shown after Px. Significant decrease of IGF-I values was observed at 2 am and from 11 am till 11 pm. Administration of MEL to rats after Px stimulated IGF-I synthesis except 5 am and 8 am, which is tes-

tified by changes of IGF-I concentrations in relation to group after Px presented in figure 1. IGF-I values were significantly increased from 11 pm till 2 am and from 11 am till 5 pm. Described anomalies caused the increase of mean daily IGF-I concentration and the increase of values of circadian oscillations amplitude.

Changes of endogenous MEL concentrations induced by long-term exogenous MEL administration correlated positively, statistically significant with changes of GH concentrations (SPx, SPx + MEL and Px + MEL groups – r = 0.308–0.461), as well as of IGF-I (all groups: r = 0.287–0.516) (Table 3). Significant dependence between IGF-I and IGF-I concentrations was also shown (SPx, SPx + MEL and Px groups – r = 0.297–0.755).

Discussion

Not many experimental works have been devoted to the problem of assessment of influence of Px and MEL administration on GH-IGF-I axis function. They mainly regarded GH secretion, more rarely IGF-I in rodents at morning and evening hours [1–4, 6–8]. Influence of Px was not observed in these studies, but stimulating or suppressive effect of this operation on GH or/and IGF-I concentrations, dependent on the time of the day was shown [3–5, 21]. Results concerning the effects of exogenous MEL action were also not always synonymous, which may be a result of performing studies on animals of different species, sex, age, in different lighting conditions with the use of different doses and times of MEL administration [5–12, 19–20, 22].

Similar to other authors' reports [25–28] in own studies the significant influence of Px and MEL administration on daily profile of endogenous MEL in rats was shown. Distinct, dependent on the time of the day anomalies in circadian oscillations of GH and IGF-I indicating directly proportional dependence with endogenous MEL concentrations were also shown. GH rhythm was suppressed only in the group of rats with removed pineal gland; after Px significant decrease and after MEL administration – distinct increase of GH and IGF-I concentrations during the day were observed. It had influenced mean daily concentrations and values of amplitude of circadian GH and IGF-I oscillations in all groups of animals. Obtained results of own studies indicate that the pineal gland can modify the function of GH-IGF-I axis during the day, and in mechanism of this dependence changes in endogenous MEL concentrations seem to play an important role. However, MEL administration in rats after Px only partly prevented changes in GH-IGF-I axis function caused by gland removal, which most probably indicates participation also of other pineal gland substances in generating these disturbances.

Vriend et al. [20] have also shown significant increase of GH and IGF-I concentrations performing studies on Syrian male hamsters after MEL administration at evening hours for the period of 10 weeks. In the authors' opinion the increase of IGF-I concentrations induced by exogenous MEL administration is probably secondary to GH secretion. GH is a basic regulator of IGF-I concentration in plasma; stimulates IGF-I synthesis at endo-, para- and autocrinic level; IGF-I is, however, a main mediator of GH action [29, 30]. It was shown that increase of GH and IGF-I concentrations was accompanied by diminished norepinephrine (NE) turnover in hypothalamus [20]. However, it is not in the authors' opinion a cause of GH and IGF-I concentrations increase after MEL. It is confirmed by the fact that after blocking NE synthesis (by alpho-methyl-p-tyrosine) it comes to reduction of the GH concentration in serum. The significant increase of 5-hydroxy-indolo-acetic acid (5HIAA) and of index 5HIAA/serotonin in hypothalamus and brain stem extracts after MEL administration suggests, rather that rather serotoninergic component is responsible for increase of IGF-I concentrations induced by increased GH secretion [20]. Obtained in own studies in male rats, positive correlation between changes in MEL and GH and IGF-I, and GH and IGF-I concentrations in all studied groups of animals seems to confirm Vriend's et al. [20] conception, that MEL indirectly by inducing changes in GH concentrations, can influence IGF-I production.

On the other hand, by performing studies on female Syrian hamsters, Vriend et al. [22] have shown that administration of 25μg of MEL causes reduction of IGF-I concentrations. In authors' opinion induced by exogenous MEL changes in secretion and liberation of thyroid, gonadal, hypothalamic hormones, generally more intensified than in case of male rats, can secondarily contribute to decrease of GH secretion and subsequent decrease of IGF-I production in these animals; hence the different effects dependent on sex.

Data from literature and results of own studies allow to consider, that MEL probably manifests multidirectional action on the synthesis and secretion of IGF-I; MEL can directly and/or indirectly influence, by inducing changes in other hormones and growth factors concentrations. It is known that MEL is an important modulator of many endocrine glands action [1, 2, 5, 12, 31–36] and produced in them and liberated to blood hormones can on the other hand influence IGF-I secretion [29, 30]. Gonadotropins, TSH, PTH, estrogens, androgens, cortisol, insulin [29, 30, 37] influence, except GH, IGF-I secretion and their secretion during the day, as mentioned above, is subjected to regulating influence of MEL. It is not excluded that MEL is also one of the factors regulating production of other, except IGF-I, polypeptide growth factors, although there are not many studies in this field. It

was proved that some of the polypeptide growth factors can take part in regulating the GH secretion, so indirectly also IGF-I. They are: epidermal growth factor (EGF), platelet-like growth factor (PDGF), transforming growth factor α (TGF α) – influencing stimulatorily IGF-I secretion and TGF β-acting inhibitorily. Interferone and tumour necrosis factor (TNF) can also inhibit proliferative action of IGF-I [38].

The presented results of own studies also indicate that effect of exogenous MEL action on circadian GH-IGF-I axis function depends on pineal gland.

Some studies indicate that the pineal gland itself, being the target organ for MEL, could modulate effects of its own action by places specifically binding this hormone [36, 39–43]. In fact, the presence of receptors specifically binding MEL was shown in the pineal gland, which seems to confirm the conception mentioned above [42]. 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 [39–41].

Other studies suggest the possibility of indirect influence of administered MEL on the pineal gland by inducing changes in hormones concentrations [1, 2, 5, 12, 31–36], which could influence secondarily on pineal gland [44–47]. Results concerning influence of these hormones on pineal gland function are, however, not synonymous. In rats after hypophysectomy weakening of nocturnal increase of N-acetylotransferase (NAT) as well as MEL was shown, which could indicate that tropic hormones of hypophysis influence this hormone biosynthesis in the pineal gland [48]. However, PRL and GH administration (separately or altogether) to rats after hypophysectomy did not restore high NAT and MEL values during the night [48]. However, in rats with hyperprolactinemia significant changes in daily/nocturnal NAT activity and MEL content in pineal gland were shown [49]. Other studies [50] performed *in vitro* have shown that ACTH does not influence, FSH inhibits and LH, TSH and GH stimulate synthesis and liberation of pineal gland MEL, when they are administered in a dose of 100ng/ml. On the other hand PRL administrated in a dose of 1ng/ml stimulates and in a dose of 100ng/ml inhibits MEL production in pineal gland. Bauer's et al. studies [51] have not shown significant influence of hypophyseal-adrenal and -thyroid axis hormones on synthesis and liberation of MEL. Other studies [47] indicate, that glycocorticosteroids influence *in vitro* on MEL liberation from pineal gland and the effect of their action is dependent on the dose. It was also shown that glycocorticosteroids inhibit stimulated by β-agonists administration increase of NAT activity in pineal gland however, they do not have significant influence on hydroxyindolo-O-metylotransferase (HIOMT) activity [52]. Some authors' studies

[44, 46] indicate that MEL synthesis in the pineal gland and its secretion, particularly during the night, are dependent on thyroid hormones. It was shown that in *in vitro* conditions triiodothyronine stimulates synthesis and secretion of MEL during the day and inhibits during a period of darkness [46]. In rats with hypothyreosis increase and after thyroxine administration – decrease of nocturnal peak of MEL secretion was observed [1, 2]. Rom-Bugoslavskaia et al. [53] have shown that thyroxine stimulates and calcitonin inhibits o-methylation of serotonin in pineal gland.

It seems that the effect of exogenous MEL action on circadian GH-IGF-I axis function is dependent on pineal gland presence, which can be connected with indirect – by other hormones – and/or direct influence of administrated hormones on this gland.

Conclusions

1. Pineal gland can influence GH-IGF-I axis function during the day and in the mechanism of this dependence the changes in endogenous melatonin concentrations seem to play an important role.
2. Melatonin administration in rats after pinealectomy only partly prevents changes of GH-IGF-I axis function caused by gland removal, which can indicate participation also of other pineal substances in generating disturbances.
3. Effect of exogenous melatonin action on circadian GH-IGF-I axis function is dependent on the pineal gland presence, which can be connected with direct and/or indirect influence of the administrated hormone on this gland.

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