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Effects of melatonin, testosterone and the two hormones administered in parallel on ventral prostate of the rat treated with stilbestrol in the first day of life

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Summary. Effects of melatonin, testosterone and the two hormones administered in parallel on ventral prostate were examined in the rats treated with estrogens in the first day of life. Thirty-nine-day long hormonal stimulation with melatonin, testosterone or the two hormones in parallel was started in rats aged 20, 28, 35 or 45 days. A single dose of estrogens led to atrophy of ventral prostate when the animals reached maturity, which was associated with high LH levels and low testosterone levels in the serum. Melatonin accentuated estrogen-induced changes in prostate morphology of ventral prostate while initemporal administration of melatonin and testosterone led to a resultant effect of testosterone-induced stimulation and melatonin-induced inhibition.

Key words: Neonatal estrogenization, Ventral prostate, Melatonin, Testosterone, LH

Introduction

A single dose of estrogens, introduced to males in the first day of life leads to pronounced changes in structure and function of the reproductive system when the males reach mature age. The phenomenon results from estrogen-induced disarrangement in hypothalamic sexual centre function, responsible for synthesis and secretion of GnRH (Dalterio et al., 1985; Handa et al., 1985). The hour «0» or the period just after delivery represents the most estrogen-sensitive stage in the rats (Corbier, 1985). As demonstrated in studies of our own, in adult male rats treated with a single dose of stilbestrol on the first day of life, spermatogenesis reaches the level of primary spermatocytes while Leydig cells become dedifferentiated. In the animals, serum testosterone level has been markedly lowered (Limanowski, 1978; Limanowski et al., 1990, 1994a). In such conditions prostate morphology demonstrates marked hypertrophy of connective tissue stroma as well as atrophy of glandular epithelium, paralleled by disappearance of its secretory functions (Limanowski et al., 1994a,b).

Melatonin represents a hormone, the role of which in the organism remains to be clarified. For many years it has been thought to exert an anti-gonadotropic activity (Glass and Knotts, 1987; Masson-Pevet et al., 1986; Glass and Dolan, 1988). In certain experimental conditions, however, melatonin has been capable of exerting stimulatory effect on the hypothalamo-hypophyseal-gonadal axis (Reiter et al., 1978; Amador et al., 1986; Lukaszyk, 1990). Studies by Withyschumnarukul et al. (1986) have demonstrated presence of receptors for melatonin in the prostate, which suggests direct effects of melatonin on the organ.

Considering the above, we thought it purposeful to examine effects of melatonin, introduced at various times, on ventral prostate in the rat treated with a single dose of stilbestrol in the first day of life.

Materials and methods

The studies were conducted on male rats of Wistar strain, which were injected subcutaneously with a single dose of 1 mg Stilbestrol dipropionate (Polfa-Poland) in the first day of life. After 20, 28, 35 or 45 days, groups of the rats were treated for 39 days with daily s.c. injections at 50 µg melatonin (Sigma), 3 mg Testosterone propionate (Polfa-Poland) or both hormones at the same doses. The controls consisted of intact rats and of estrogen-treated rats in the first day of life. Rats of all the groups were given free access to chow and water and were housed in standard illumination controls (14L:10D). At the end of the experiment, each rat was weighed out and exsanguinated from the heart, under ether anaesthesia. In the serum, LH gonadotropin and testosterone levels were estimated by RIA techniques. The RIA tests were conducted in the Department of Endocrinology, K. Marcinkowski

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University of Medical Sciences in Poznan, using ^{125}I-labelled antigens and antibodies of their own make. For histological studies, ventral prostate was isolated, weighed out and fixed in Bouin solution. The sections were stained with H+E. The data pertaining to body weight, relative weight of ventral prostate and levels of studied hormones were subjected to statistical analysis using Ducan's test.

Results

Results pertaining to body weight and relative weight of ventral prostate are listed in Table 1. Except of rats treated with estrogens in the first day of life and examined after 59 and 67 days, body weights of experimental rate were always lower than in control rats, independently of the type of administered hormones or timing of their administration. Melatonin-injected animals exhibited low body weight independently of the time of starting melatonin administration.

In the rats given estrogens in the first day of life relative weight of the ventral prostate was always lower that in the corresponding control. Administration of melatonin to estrogenized rats further lowered relative weight of ventral prostate in animals of all examined groups. On the other hand, in estrogen treated rats, subsequently even testosterone at various times, relative weight of the ventral prostate resembled normal value when testosterone was given beginning from days 20 or 28 or was lower than in the control but still higher than in the rats given a single dose of estrogens only, when testosterone was started on days 35 or 45. Parallel administration of melatonin and of testosterone to rats given a single dose of estrogens in the first day of life increased relative weight of ventral prostate as compared to the norm, if the stimulation started at days 20 or 28. In the remaining age groups, the relative weight of the ventral prostate was markedly lower than in the controls. Nevertheless, it never reached such low values as those noted in rats treated with estrogens only at the corresponding age. The levels of studied hormones in the serum are listed in Table 2. Note the clearly augmented level of LH gonadotropin in all rats treated with estrogens in the first day of life, as compared to the control maintained also in rats treated subsequently with melatonin, testosterone or both hormones in parallel. In estrogen-treated rats, as well as in rats treated with estrogens and, subsequently, with melatonin, testosterone level was always lower than in the corresponding controls. In animals treated with testosterone or with melatonin plus testosterone, serum testosterone levels clearly exceeded control values.

Histological pattern of the prostate in estrogen-treated rats documented atrophy of glandular epithelium and hypertrophy of stromal connective tissue (Fig. 2). Administration of melatonin stimulated stromal

Table 1. Body weight and relative weight of ventral prostate in control rats (C), estrogen-treated on the 1st day of life (E), and injected at different periods of time with melatonin or testosterone following estrogen treatment (EM and ET) or else with testosterone after injection of the two compounds (ETM). The results are the mean±SD. n=6.

TE BEGINNING OF HORMONAL STIMULATION/ AUTOPSY (days of life)	EXPERIMENTAL GROUP	BODY WEIGHT (BW) (g)	RELATIVE WEIGHT OF VENTRAL PROSTATE (mg/100g BW)
20	C	121±3	164±8
(59)	E	141±6*	66±3
	EM	89±5*	27±3*
	ET	74±4*	152±6
	EMT	109±3*	205±13*
28	C	138±5	131±6
(67)	E	180±6*	32±6**
	EM	84±10*	9±1*
	ET	118±8	143±44*
	EMT	125±5*	153±3*
35	C	235±2	364±7
(74)	E	165±3*	28±2**
	EM	135±6*	10±1*
	ET	129±8*	174±11*
	EMT	119±6*	77±11*
45	C	239±2	364±7
(84)	E	164±3*	30±1**
	EM	97±10*	7±3
	ET	154±18*	136±23*
	EMT	128±3*	91±5*

Differs from the control: *, p<0.05; **, p<0.01

Table 2. Serum level of LH and testosterone in control rats (C), estrogen-treated on the 1st day of life (E), and injected at different periods of time with melatonin or testosterone following estrogen treatment (EM and ET) or else with testosterone after injection of the two compounds (ETM). The results are the mean±SD. n=6.

TE BEGINNING OF HORMONAL STIMULATION/ AUTOPSY (days of life)	EXPERIMENTAL GROUP	LH (ng/ml)	TESTOSTERONE (ng/ml)
20	C	30±1	1.14±0.17
(59)	E	96±6*	0.53±0.01*
	EM	80±10*	0.64±0.15*
	ET	79±28*	12.40±1.02*
	EMT	45±4*	12.30±1.71*
28	C	32±2	0.83±0.06
(67)	E	100±11*	0.22±0.03*
	EM	106±16*	0.37±0.03*
	ET	83±9*	12.75±1.38*
	EMT	55±10*	11.38±1.68*
35	C	51±1	1.04±0.07
(74)	E	83±6*	0.69±0.12*
	EM	86±12*	0.57±0.21*
	ET	76±5*	11.10±0.15*
	EMT	79±7*	14.25±1.18*
45	C	62±1	1.22±0.11
(84)	E	217±23*	0.58±0.77*
	EM	93±27*	0.77±0.27*
	ET	69±11*	1.05±0.22*
	EMT	57±9*	4.20±1.34*

Differs from the control: *, p<0.05; **, p<0.01

hypertrophy in such rats, particularly hypertrophy of smooth muscle cells (Fig. 3). Administration of testosterone to rats given estrogens in the first day of life restored normal pattern of the ventral prostate (Fig. 4). Stimulation with testosterone and melatonin in parallel led to more pronounced stromal hypertrophy than in the normal control, and numerous intraepithelial cysts appeared in the glandular epithelium (Fig. 5). For comparison, a normal pattern of the ventral prostate in the adult control rat is shown in Fig. 1.

Discussion

Most of the studied animals have demonstrated decreased body weight, independently of the type of hormones administered and timing of their application. The phenomenon may be explained on grounds of the experimental results of Jansson et al. (1985), who demonstrated sexual dimorphism of rat hypothalamus in GH-RH secretion. Indispensable in the first day of life of males, androgenic stimulation of hypothalamic nuclei establishes male type of GH-RH secretion. This results in greater body weight in males than in females. In our experiments a single injection of estrogens in the first day of life disturbed androgenic activity of Leydig cells, thus disturbing GH-RH secretion and causing lower body weight in estrogen-treated rats, as compared to the control. Administration of melatonin to rats treated with estrogens in the first day of life has accentuated the decreased body weight which may indicate effect of the hormone on hypothalamic nuclei responsible for synthesis and secretion of GH-RH.

The decreased body weight of the examined animals has been accompanied by markedly decreased weight of the ventral prostate in estrogen-treated rats (in 74- and 84-day-old rats the weight was 12-fold lower than in appropriate controls). Similar results have been obtained by Dalterio et al. (1985) in mice treated in the first day of life with estrogens. In previous studies of our own (Limanowski, 1978), Leydig cells of rats treated in the first day of life with estrogens have been shown to undergo dedifferentiation toward fibroblast-like cells, which has been associated with low serum testosterone levels. This resulted in a markedly decreased relative weight of ventral prostate. Receptors for androgens and estrogens in the prostate are stimulated by the hormones and may participate in normal development of the organ (Ricciardelli et al., 1989). In the estrogen-treated rats the prostate exhibits a significant degree of atrophy in glandular epithelium and hypertrophy of stromal connective tissue and muscular tissue. Bartsch et al.

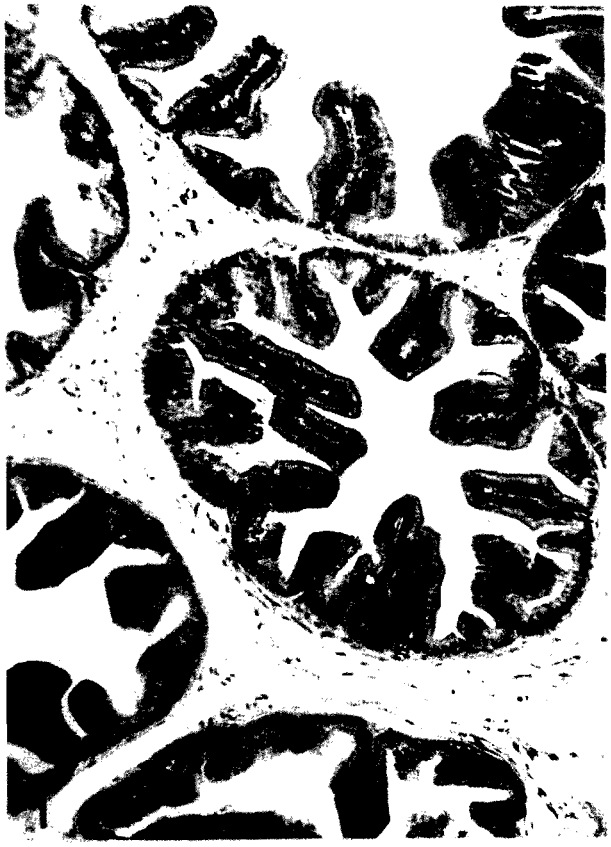

Fig. 1. Ventral prostate of an adult control rat. H+E, x 200

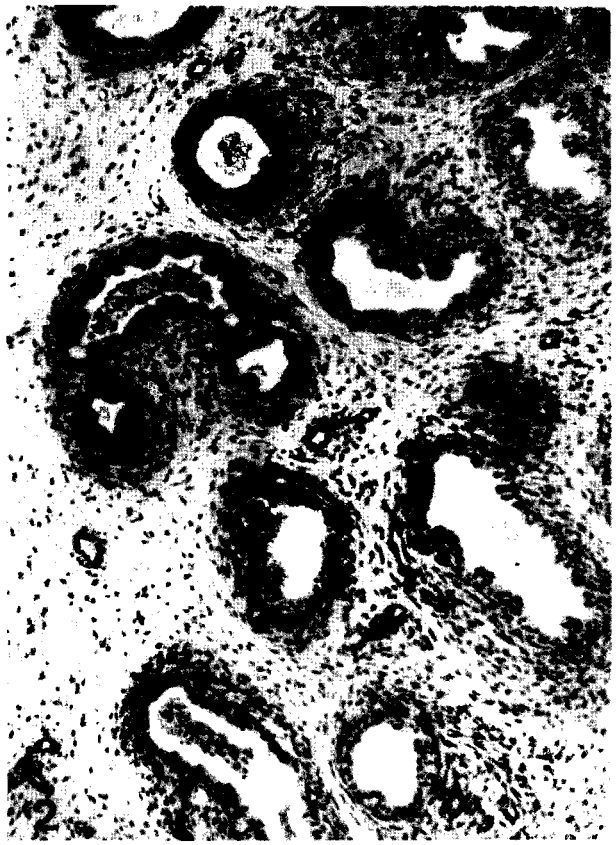

Fig. 2. Ventral prostate of 59-day old rat injected on the 1st day of life with a single dose of 1 mg stilbestrol. Note hypertrophy of stromal connective tissue and atrophy of glandular epithelium. H+E, x 200

(1989) has shown in humans that hypertrophy of stromal connective tissue reflects estrogen-stimulated collagen type I and type III production by smooth muscle cells. According to Aumuller (1989) and Suzuki et al. (1992), hypertrophy of prostate stroma reflects a disarranged relation between testosterone and estrogens. A single dose of estrogens in the first day of life has undoubtedly resulted in disturbances in the relations.

Administration of melatonin to estrogen treated rats has resulted in a further decrease in relative prostate weight, which in 84-day-old rats amounted to only 2% weight observed in control rats. Amador et al. (1986) have explained a decrease in relative weight of the prostate in hamsters treated with melatonin between the 8th and 12th week of life by a decreased number of testicular receptors for LH which has been supposed to decrease testosterone secretion by Leydig cells. A similar result has been obtained by other authors after administering melatonin to anterior hypothalamus (Dowell and Lynch, 1987; Glass and Knotts, 1987). In microscopic studies, melatonin administered to rats treated with estrogens in the first day of life has resulted in a significant hypertrophy of stromal connective tissue and of smooth muscle cells and to atrophy of glandular epithelium. Chow and Pang (1989) studied ultrastructure of Golgi apparatus, endoplasmic reticulum and secretory vacuoles in glandular epithelium of the prostate and other accessory sexual glands of melatonin-treated hamsters. The authors have shown that melatonin is capable of inducing atrophy of the organelle, inhibits secretory function of glandular epithelium and may even lead to necrosis of glandular cells, thus inhibiting function on the reproductive system. We have drawn a similar conclusion from our earlier studies (Limanowski et al., 1991). Attention should also be paid to results of Srivilai et al. (1989) who have related melatonin-induced alterations in the prostate to the dose of the hormone. Low doses (50 to 400 µg per day) and high doses (2000 µg per day) have inhibited structure and function of the prostate, while average doses (800 µg per day) have exerted effect. The above quoted literature on the subject indicates that melatonin may exert direct effect on the prostate, mediated through prostate receptors for melatonin, described by Withyschumnarukul et al. (1986).

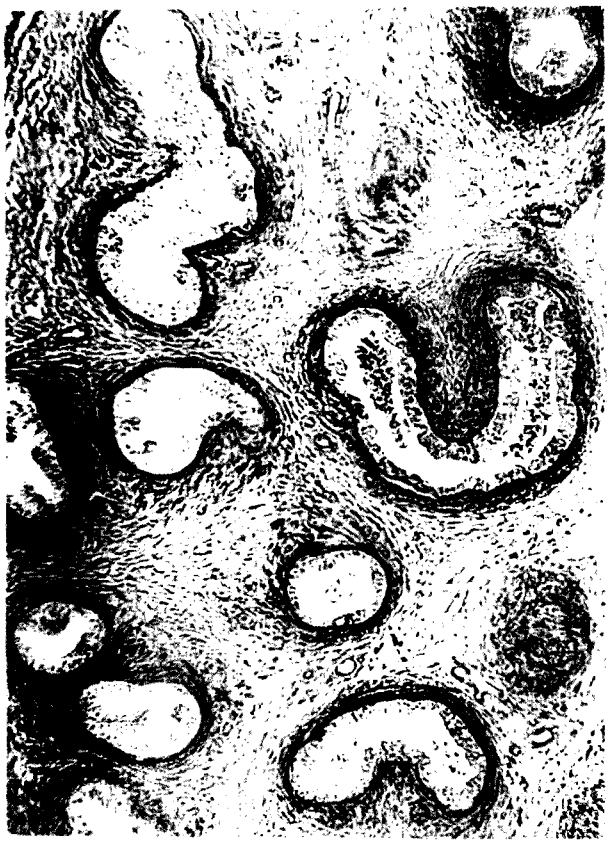

Fig. 3. Ventral prostate of a rat injected on the 1st day of life with a single dose of 1 mg stilbestrol and subsequently injected with melatonin between the 35th and 74th days of life. Note ongoing proliferation of stromal connective tissue and, in addition, of smooth muscle cells. Alterations in glandular epithelium correspond to those described in Fig. 2. H+E, x 200

Fig. 4. Ventral prostate of a rat injected on the 1st day of life with a single dose of 1 mg stilbestrol and subsequently injected with testosterone between the 45th and 84th day of life. The microscopic pattern resembles that of the control. H+E, x 200

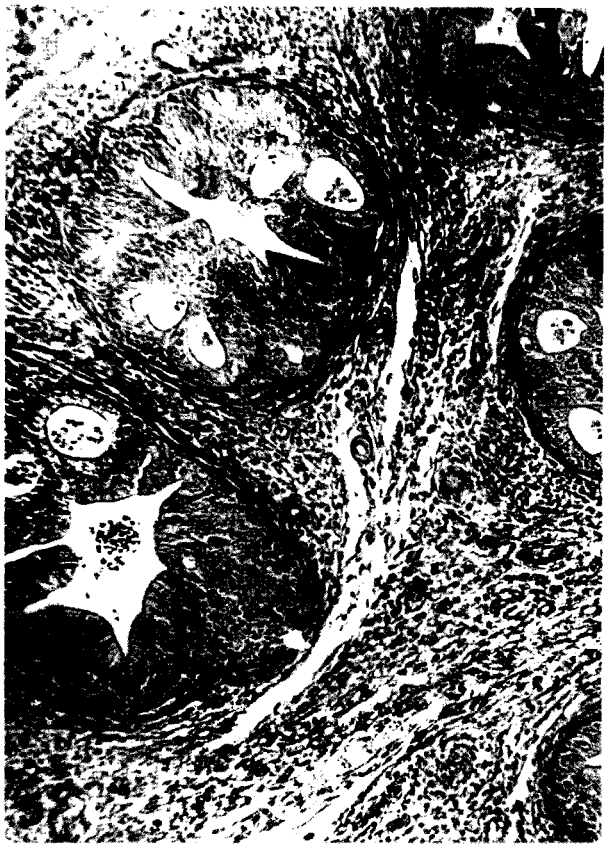

Fig. 5. Ventral prostate of a rat injected on the 1st day of life with a single dose of 1 mg stilbestrol and subsequently injected with testosterone plus melatonin between the 45th and 48th day of life. Note hypertrophy of glandular epithelium and presence of intraepithelial cysts, stromal hypertrophy more pronounced than in the normal control. H+E, x 200

Testosterone administered to estrogen-treated animals restored normal structure and secretory function of the prostate. The stimulatory role of testosterone toward male accessory sexual glands has been recognized for a long time. Testosterone administered together with melatonin-stimulated prostate similarly to testosterone alone, but in the former case glandular epithelium exhibits hypertrophy and presence of intraepithelial cysts, accompanied by a certain degree of stromal tissue proliferation. In the experimental conditions, the stimulating role of testosterone is evident. As far as LH gonadotropin levels in all examined rats are concerned, high levels of the hormone are noted, as compared to the controls, particularly in animals injected with estrogens in the first day of life and untreated thereafter. The phenomenon reflects estrogen-induced injury to Leydig cells, leading to decreased testosterone levels in the serum. In such experimental conditions, the animals exemplify a type of «pharmacological castration» (Limanowski et al., 1994a).

The obtained results may be summed up as indicating that melatonin in the presented experimental system not only fails to stimulate rat prostate, but, on the contrary, accentuates the gland lesions induced by a single dose of estrogens given in the first day of life and, thus, is capable of inhibiting development and secretory function of the gland.

References

Amador A.G., Richardson B.A., Klemcke H.G., Vanghan M.K., Steger R.W., Bartke A. and Reiter R.J. (1986). Effects of longterm treatment with melatonin or melatonin plus ectopic pituitary transplants on testicular LH/hCG and prolactin receptors in juvenile Syrian hamsters (*Mesocricetus auratus*). J. Reprod. Fertil. 78, 557-564.

Aumuller G. (1989). Functional morphology of the prostate. Urologe 28, 306-310.

Bartsch G., Brungger A., Schweikert U., Hintner H., Hopfl R. and Rohr H.P. (1989). Benign prostatic hyperplasia: a stromal disease. Urologe 28, 321-328.

Chow P.H. and Pang S.F. (1989). Ultrastructural of secretory cells of male accessory sex glands of golden hamster (*Mesocricetus auratus*) and effect of melatonin. Acta Anat. 134, 327-340.

Corbier P. (1985). Sexual differentiation of positive feedback: effect of hour of castration at birth on estradiol induced luteinizing hormone secretion in immature male rats. Endocrinology 116, 142-147.

Dalterio S., Bartke A., Steger R. and Mayfield D. (1985). Neonatal exposure to DES in BALB-c male mice: effect on pituitary-gonadal function. Biochem. Behav. 22, 1019-1024.

Dowell S.F. and Lynch G.R. (1987). Duration of the melatonin pulse in the hypothalamus controls testicular function in pinealectomized mice (*Peromyscus leucopus*). Biol. Reprod. 36, 1095-1101.

Glass G.D. and Knotts L.K. (1987). A brain site for the antigonadal action of melatonin in the white-footed mouse (*Peromyscus leucopus*): involvement of the immunoreactive GnRH neuronal system. Neuroendocrinology 46, 48-55.

Glass J.D. and Dolan P.L. (1988). Melatonin acts in the brain to mediate seasonal steroid inhibition of luteinizing hormone secretion in the white-footed mouse (*Peromyscus leucopus*). Proc. Soc. Exp. Biol. Med. 188, 375-380.

Handa J.R., Corbier P., Shryne J.E., Schoomnaker J.N. and Gorski R.A. (1985). Differential effect of perinatal steroid environment on three dimorphic parameters on the rat brain. Biol. Reprod. 32, 855-870.

Jansson J.D., Ekberg S. and Isaksson O.G. (1985). Sexual dimorphism in the control of growth hormone secretion. Endocrinol. Rev. 6, 128-143.

Limanowski A. (1978). Age-dependent effects of hormonal stimulation of gonad of male rat estrogenized on the first day of life. Pol. Endocrinol. 29, 257-268.

Limanowski A., Miśkowiak B. and Filipiak B. (1990). Perinatal effects of sex hormones on the reproductive system in male and female rats. Pol. Endocrinol. 41, 49-55.

Limanowski A., Otulakowski B. and Miśkowiak B. (1991). Effect of melatonin on reproductive system in male rat, treated with estrogen in the first day of life. Folia Histochem. Cytochem. 29, 71-74.

Limanowski A., Miśkowiak B. and Otulakowski B. (1994a). Effect of estrogenization in the first day of life on the reproductive system in male rats. Histol. Histopathol. 9, 59-63.

Limanowski A., Otulakowski B., Miśkowiak B. and Filipiak B. (1994b). Wplyw jednorazowego wprowadzania stilbestrolu w 1 dniu na

morfologie i czynnosc gruczolu krokowego szczura. Urol. Pol. 47, 131-138.

Lukaszyk A. (1990). Melatonin as a modulator of testosterone secretion in vivo and in vitro. Advances in pineal research. John Libbey and Co. Ltd. pp 217-224.

Masson-Pevet M., Pevet P. and Vivien-Roels B. (1986). Pinealectomy and constant release of melatonin or 5-metoxytryptamine induce testicular atrophy in the European hamster (*Cricetus cricetus*, L). J. Neurol. Transm. 67, 147-162.

Reiter R.J., Rolang M.D., Panke E.S. and Banks A.F. (1978). Melatonin: reproductive effects. J. Neurol. Transm. Suppl 13, 209-223.

Ricciardelli C., Horsfall D.J., Skinner J.M., Henderson D.W., Marshall V.R. and Tilley W.D. (1989). Development and characterization of primary cultures of smooth muscle cells from the fibromuscular stroma of the guinea pig prostate. In Vitro Cell Dev. Biol. 25, 1016-1024.

Srivilai W., Withyachumnarukul B. and Trakulrunngsi W. (1989). Stereological changes in rat ventral prostate induced by melatonin. J. Pineal Res. 6, 111-119.

Suzuki K., Inaba S., Takauschi H., Takezawa Y., Fukabori Y., Suzuki T., Imai K., Yamanaka H. and Honma S. (1992). Endocrine environment of benign prostatic hyperplasia- relationships of sex steorid hormone levels with age and size of the prostate. Nippon Hinyokika Gakkai Zasshi. 83, 664-671.

Withyschumnarukul B., Limpanawattanakul M. and Trakulrungsi W. (1986). Retention of radioactive substances in the hypothalamus, anterior pituitary and reproductive organs of male rats after ^{3}H-melatonin administration. Life Sci. 12, 1757-1765.

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