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Effects of melatonin, testosterone and the two hormones administered in parallel on epididymis of the rat estrogenized with stilbestrol in the first day of life

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Summary. Effect of melatonin, testosterone and of both hormones given in parallel on rat epididymis was tested in rats given a single dose of 1 mg stilbestrol on the first day of the life. The hormones were given daily for 39 days, beginning from the 20th or 28th day of life. The single dose of estrogen treatment resulted in epididymis atrophy, accompanied by changes in glandular epithelium and in its stroma, when the rats reached mature age (59 or 67 days of life). In such rats, LH gonadotropin level was elevated and testosterone level was decreased. Administration of melatonin failed to affect the changes induced by estrogen treatment. Administration of testosterone alone or of testosterone in parallel with melatonin caused the epididymis status to resemble more closely that seen in control animals. Efferent ductules of the testis (head of epididymis) were also demonstrated to be more sensitive to the performed experimental procedures than the duct of the epididymis (body and tail of the epididymis).

Key words: Neonatal estrogenization, Epididymis, Melatonin, Testosterone, LH

Introduction

Melatonin represents a hormone whose effect on the hypothalamo-hypophyseal-gonadal system depends upon multiple factors, such as dose, timing and route of administration, season and many others. Some authors stress in their studies the antigonadotropic effect of melatonin (Masson-Pevet et al., 1986; Glass et al., 1988), while other authors have detected its stimulatory effect on the hypothalamus-hypophysis-gonadal system (Reiter et al., 1978; Amador et al., 1986; Lukaszyk, 1990). Our own studies have shown that a single dose of stilbestrol, introduced into male rats in the first day of life, results in extensive alterations in the reproductive system after the rats reach maturity. The rats have manifested inhibition of spermatogenesis and pronounced morphological and functional alterations in the accessory sexual glands, accompanied by high serum LH and low serum testosterone levels (Limanowski et al., 1994a,b). Melatonin introduced into rats at various times after the perinatal estrogen treatment accentuated the above described lesions in gonads and seminal vesicles (Limanowski et al., 1991). A particularly strong inhibitory action of melatonin on the structure and function was noted in prostates of animals treated with estrogens on the first day of life (Limanowski et al., 1995).

The epididymis represents male accessory sexual glands which are relatively seldom investigated. Considering this, we decided to examine the effect of melatonin on epididymis of the rat, treated on the first day of life with a single dose of stilbestrol.

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Materials and methods

The experiments were performed on Wistar strain male rats, given a single subcutaneous dose of 1 mg Stilboestrolum dipropionicum (Polfa) on the first day of life. After 20 and 28 days, groups of the rats were given daily subcutaneous injections of melatonin (Sigma 50 μg/day), Testosteronum (Polfa, 3 mg/day) or both hormones in doses given above for 39 days.

Untreated rats and rats treated only with estrogen on the first day of life served as controls. Both experimental and control rats were kept throughout the experiment in constant conditions of temperature (20±2 °C) and illumination (10L-14D) and were given free access to chow and water. At the age of 59 or 67 days animals were weighed and sacrificed in ether anesthesia by exsanguination from the left ventricle of the heart. In the serum, LH gonadotropin and testosterone levels were estimated using RIA techniques. RIA assays were performed in the radioisotope lab of the Department of Endocrinology, Institute of Internal Diseases, Karol Marcinkowski University of Medical Sciences in Poznan using antibodies and antigens of their own production, labeled with ^{131}I.

For histological studies, epididymis was weighed

and fixed in Bouin's solution and sections were stained with hematoxylin and eosin.

The data pertaining to body weight, relative weight of epididymis, LH and testosterone levels were subjected to statistical analysis using Duncan's test.

Results

Data on body weight, relative weight of epididymis, LH gonadotropin and testosterone levels are shown in Tables 1A,B, 2A,B. Single-dose estrogen treatment on the first day of life resulted in a slight increase in body weight of animals in each age group, when compared to control rats of the corresponding age. On the other hand, body weights of all rats in the remaining experimental groups exhibited a decrease when compared with the appropriate controls. Single-dose estrogen treatment on the first day of life resulted in a marked decrease in the relative weight of the epididymis: in 67-day-old rats the weight reached only 19% of the values for epididymis of the control group rats. Melatonin administration to neonatally estrogen-treated rats exerted no significant effect on the relative weight of the epididymis in either age group.

However, administration of testosterone in parallel with melatonin beginning from the 20th day after single-dose estrogen treatment augmented the relative weight of the epididymis to, respectively, 106% and 122% of the control values. Administration of the hormones beginning from the 28th day of single-dose estrogen

Table 1A

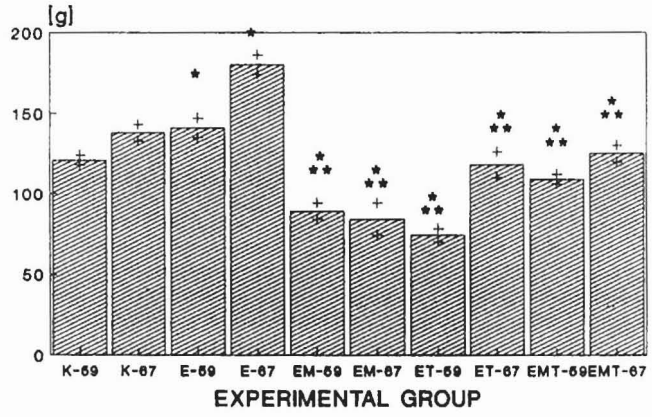

Table 1B

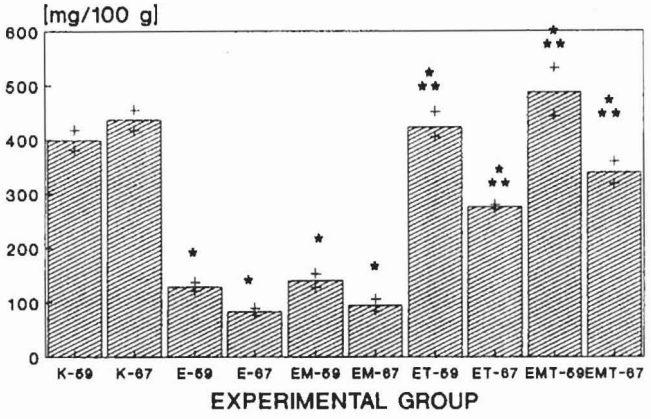

Table 1. Body weight (Table 1A), relative weight of epididymis (Table 1B) of control rats (K), rats treated with a single dose of stilbestrol on the first day of life (E) and pretreated with stilbestrol as above but stimulated with melatonin (EM), testosterone (ET) or melatonin plus testosterone (EMT). The numbers indicate days of rats' life. *: difference significant when compared to the control ($p<0.05$); **: difference significant when compared to the group of rats treated with estrogens on the first day of life ($p<0.05$).

Table 2A

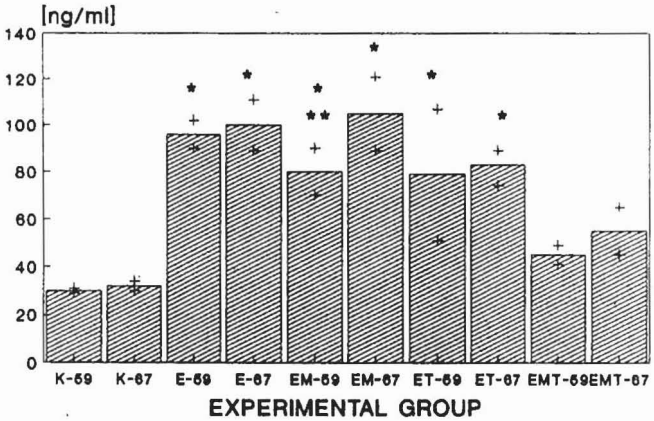

Table 2B

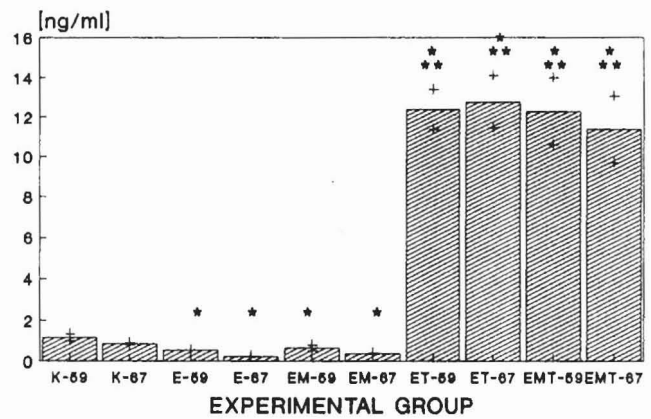

Table 2. Level of LH gonadotropin (Table 2A) and level of testosterone (Table 2B) in sera of control rats (K), rats treated with a single dose of stilbestrol on the first day of life (E) and pretreated with stilbestrol as above but stimulated with melatonin (EM), testosterone (ET) or melatonin plus testosterone (EMT). The numbers indicate days of rats' life. *: difference significant when compared to the control ($p<0.05$); **: difference significant when compared to the group of rats treated with estrogens on the first day of life ($p<0.05$).

treatment led to a relative epididymis weight of 63% in the case of testosterone administration and of 77.5% in the case of parallel administration of testosterone and melatonin. Estrogen treatment on the first day of life clearly augmented serum LH gonadotropin levels in all studied experimental groups of rats. The most pronounced, more than a triple increase, was observed in rats subjected to estrogen treatment only. Similar observations were made in the case of LH level in rats treated with estrogen on the first day of life and, beginning from the 28th day, treated with melatonin. After stimulation with testosterone, LH gonadotropin level in estrogen-pretreated rats approximated 250% and after testosterone plus melatonin treatment - 150 to 170% of control values. Testosterone level in the serum of rats treated on the first day of life with estrogen only or in such rats treated subsequently with melatonin, decreased to 25-50% of control values. Administration of testosterone or testosterone plus melatonin to neonatally estrogen-treated rats augmented approximately tenfold the testosterone level, when compared to the control. As far as epididymis histology is concerned, single-dose estrogen treatment led to metaplasia of glandular epithelium toward the stratified columnar epithelium, thickening of the basement membrane of the epithelium as well as to marked hyperplasia of connective tissue and smooth muscle tissue of the stroma (Fig. 2A,B). The above-described alterations were particularly well expressed in the efferent ductules (head of epididymis). Subsequent administration of melatonin to the estrogen-pretreated animals failed to affect significantly the histological pattern of the epididymis (Fig. 3A,B). However, in estrogen-pretreated rats administration of testosterone alone or testosterone plus melatonin restored the normal histological pattern of the epididymis within the glandular epithelium, although a moderate level of stromal hyperplasia persisted (Figs. 4A,B, 5A,B). For comparison, the histological pattern of normal rat epididymis is illustrated in Fig. 1A,B.

Fig. 1. Sixty-seven-day-old control rat. **A.** Head of epididymis. **B.** Duct of epididymis. H&E. x 200

Discussion

Experimental studies on the hypothalamic-hypophyseal-gonadal system in the perinatal period have

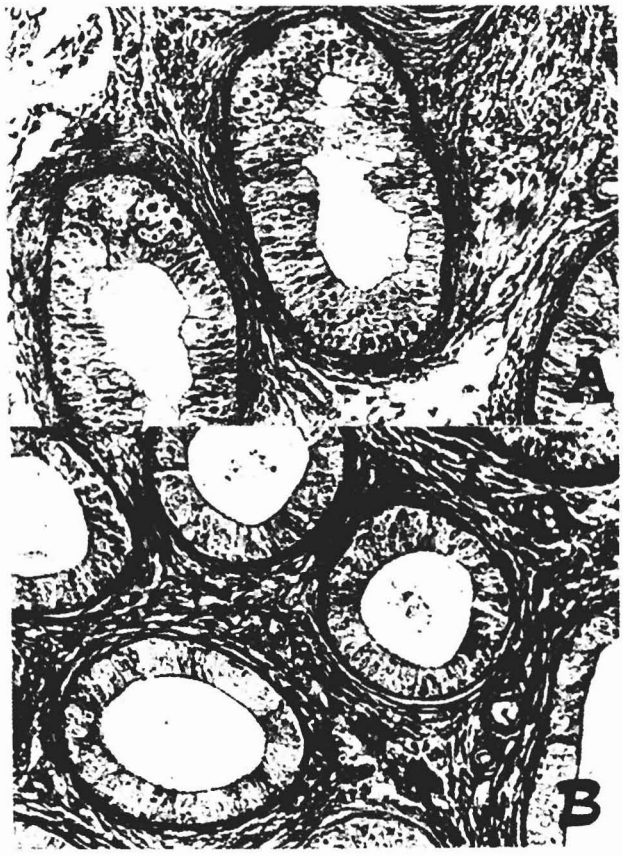

Fig. 2. Sixty-seven-day-old rat treated on the first day of life with a single dose of stilbestrol. **A.** Head of epididymis. Note metaplasia of glandular epithelium toward stratified columnar epithelium, thickening of the basement membrane of the epithelium, and marked hyperplasia of connective and smooth muscle tissues of the stroma. **B.** Duct of epididymis. Pattern of glandular epithelium resembles the control. Slight thickening of epithelium basement membrane and moderate hyperplasia of stroma. H&E. x 200

supplied several interesting data on function of the system (Corbier, 1985). Perinatal estrogen treatment clearly decreases weights of gonads and of accessory sexual glands (Limanowski et al., 1991). In 67-day-old rats, weight of the epididymis amounted only to 19% of the weight of the epididymis in control rats of the same age. A similar decrease in gonad weight and in weight of accessory sexual glands including epididymis after perinatal estrogen treatment was described by Pinilla et al. (1989). The alterations have been associated with marked hyperplasia of connective and smooth muscle tissue of the stroma, particularly evident in the head of the epididymis (efferent ductules) and with metaplasia of glandular epithelium toward the stratified columnar epithelium. Gayton et al. (1990) administered estrogens to newborn rats and after 15 days demonstrated hyperplasia of the connective tissue-muscular stroma and slowed down proliferation of glandular epithelium, accompanied by an increased number of eosinophils in the epididymis. The authors have suggested that eosinophils mediate the estrogen treatment-induced alterations in the epididymis. Effects of estrogens on epididymis morphology and function has been studied by several investigators. Toney and Danzo (1989) demonstrated that estradiol and testosterone represent physiological regulators of epididymis development and function in the rabbit. Hatier et al. (1990) showed that ^{3}H-estradiol binding to fetal epididymis of guinea pig was 100-fold more pronounced in tissues of mesenchymal origin than in other tissues, while Cooke et al. (1991) detected the presence of estrogen receptors in mouse epididymis; more pronounced in fibroblasts than in smooth muscle cells.

In our earlier studies we also noted hyperplasia of connective tissue and muscular tissue stroma in the prostate of rats treated with estrogen on the first day of life (Limanowski et al., 1994). Administration of melatonin to such estrogen-treated rats failed to affect relative weight of the epididymis and its morphological pattern, when compared to rats treated only with neonatal administration of estrogens. Studies of other authors have shown that melatonin unfavourably affects the male reproductive system (Tekpetey and Amann, 1988). Bartness and Goldman (1988), after 5 weeks of intravenous infusion of melatonin to hamsters, observed a decrease weight of gonads and epididymis, accompanied by a decreased activity of lipoprotein

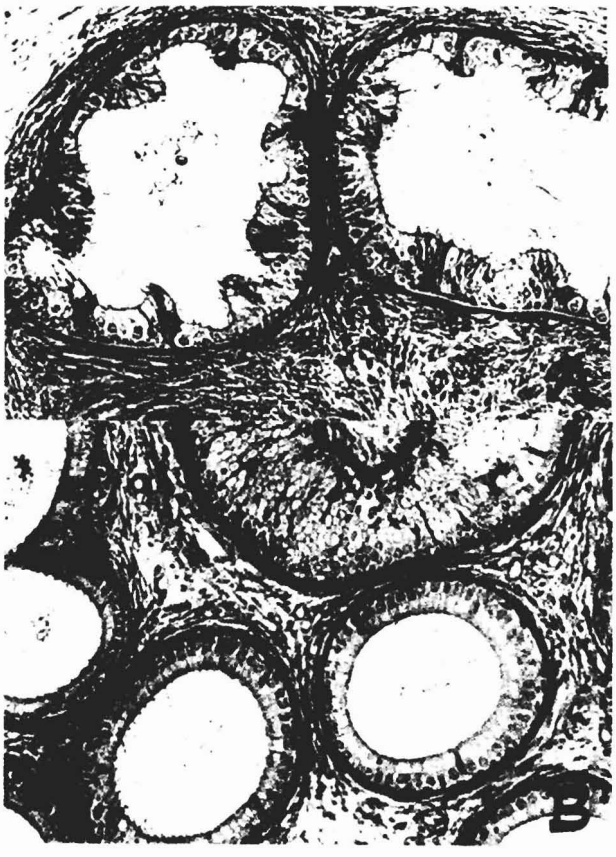

Fig. 3. Epididymis of a rat treated on the first day of life with estrogens and, beginning from the 28th day of life, treated for 39 days with melatonin. Patterns of epididymis and duct. Resemble those presented in Figs **A** and **B**. H&E. x 299

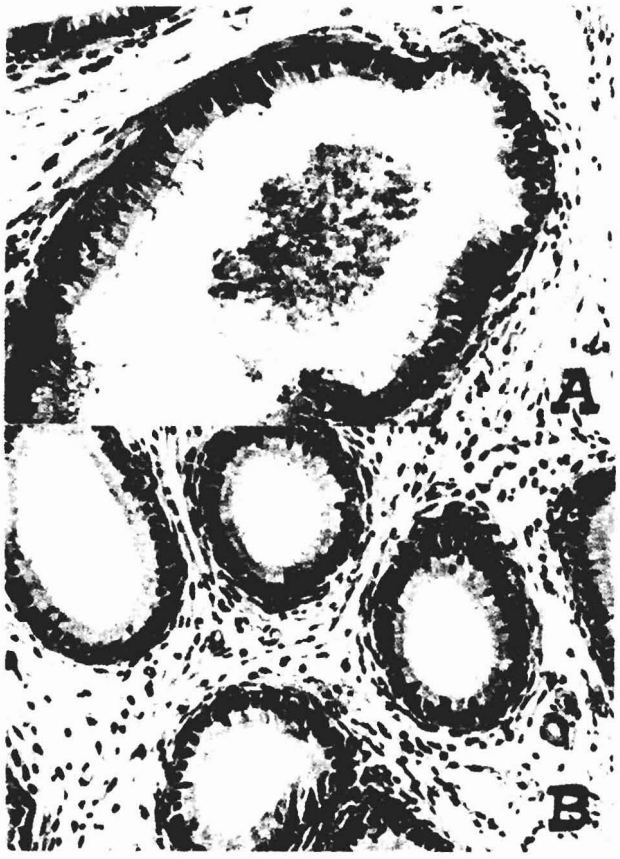

Fig. 4. Epididymis of a rat treated with estrogens on the first day of life and, beginning from the 20th day on, treated for 39 days with testosterone. **A**. Head of epididymis. **B.** Duct of epididymis. Apart from the still present stroma hyperplasia, glandular epithelium resembles the control. H&E. x 200

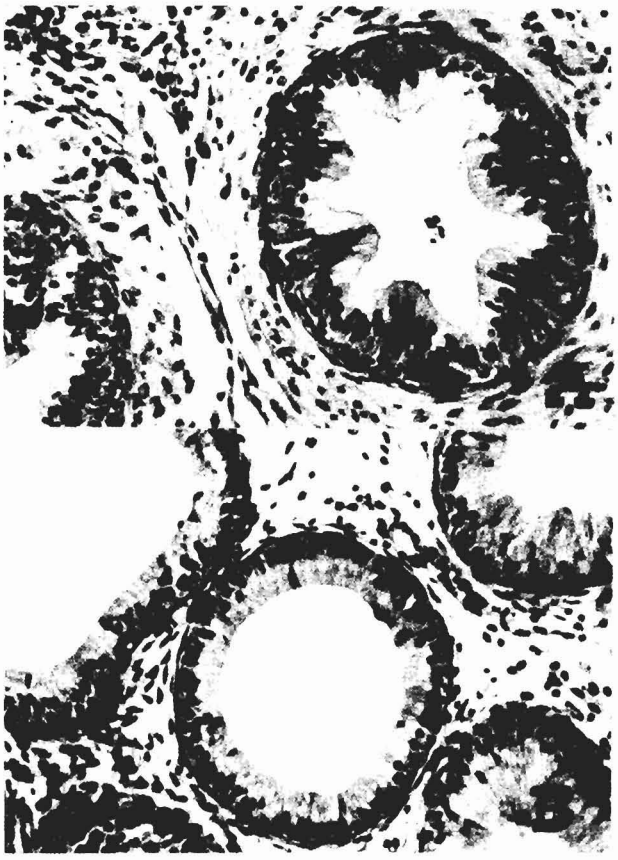

Fig. 5. Epididymis of a rat treated with estrogens on the first day of life and, beginning from the 28th day on, treated for 39 days with melatonin and testosterone in parallel. **A.** Head of epididymis. **B.** Duct of epididymis. Alterations comparable with those illustrated in Fig. 4A,B. H&E. x 200

lipase in the epididymis. Chow and Pang (1989) observed ultrastructural changes in glandular epithelium of accessory sexual organs of melatonin-treated hamsters. The lesions were manifested by atrophied Golgi apparatus, endoplasmic reticulum and by a decreased number of secretory vacuoles. The lesions resulted in an inhibited secretory function of epithelial cells and even in their necrosis. In studies of our own (Limanowski, 1994b) we have indicated that melatonin accentuates rat prostate lesions induced by estrogen treatment on the first day of life, acting most probably through its receptors. The receptors were described in the epididymis by Withyschumnarukul et al. (1986). The absence of evident effects of melatonin on epididymis of rats treated perinatally with estrogen, documented in this study, may suggest absence or a low number of melatonin receptors in the epididymis. Administration of testosterone or testosterone plus melatonin to rats treated perinatally with estrogen has exerted an evident stimulatory effect on the epididymis affected by the estrogen treatment and the effect has been more pronounced in animals in which the stimulation started earlier (on the 20th day of life). Stimulatory effect of testosterone on male sexual accessory glands in generally recognized and, thus, requires no discussion.

In the studies presented heretofore, greater sensitivity to neonatal estrogen treatment of the epididymis head (efferent ductules) has been noted when compared to the duct of epididymis. The phenomenon may be explained by the data of Cooke et al. (1991) who have shown that estrogen receptors appear first in the epididymis head in fetal mice. The authors have also described differences in smooth muscle differentiation processes after introducing 17-beta estradiol to the head of the duct of the epididymis (Cooke and Eroschenko, 1990). Morphological alterations in the epididymis presented in this work and reflecting perinatal estrogen treatment have been accompanied by high LH gonadotropin levels when compared to the control, which have also been maintained in animals treated subsequently with melatonin, testosterone and the two hormones in parallel. Serum testosterone level decreased compared to the control in estrogen-pretreated rats, whether or not treated subsequently with melatonin. Stimulation by testosterone or by testosterone plus melatonin augmented testosterone level serveralfold in the serum. An explanation of the above-described changes in studied hormones has been presented in our earlier studies (Limanowski et al., 1994a,b, 1995).

The present results allow us to conclude that melatonin does not exert perceptible effects on the epididymis of rats treated with estrogen on the first day of life. It is also worth noting that the epididymis head (efferent ductules) shows greater sensitivity to the applied experimental procedure than does the epididymis duct.

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