different final concentrations (1 or 10 μM) and their stimulatory effect on phospholipid methylation was tested. As shown in table 2, the order of stimulatory potency was histamine > 4-methylhistamine > 3-methylhistamine. This is in accord with the known $H_2$-receptor agonist potency order of these analogues[6], and suggests the involvement of an $H_2$-receptor in the methylation of mastocyte phospholipid by histamine.

*Discussion.* The results of the present investigation indicate that exposure to histamine analogues causes a transient increase of methylated phospholipids in mast cells. The same metabolic event has been reported to occur in rat erythrocyte ghosts following β-adrenoreceptor activation[4], in human monocytes stimulated by chemotactic factors[7], in murine T lymphocytes exposed to mitogenic lectins[8], and in rat mastocytes stimulated by antibody raised against IgE receptors[9]. It seems, therefore, that methylation of phospholipids is an essential step in the recognition and transduction of regulatory signals by eukaryotic cells. It can be supposed that the insertion of methyl groups into the hydrocarbon chains of the fatty acid residues of phospholipids may favor signal transduction by loosening the packing of the lipid molecules in the bilayer thereby increasing membrane fluidity and permeability. Support for this view comes from the finding that phospholipid methylation and demethylation are apparently coupled in rat erythrocytes[10] and in *Dictyostelium discoideum*[11] to the flux of calcium ions across the membrane. The stimulatory effect of histamine on adenylate cyclase activity could thus be generated by localized changes in lipid structure or by changes in binding of $Ca^{2+}$ to the regulatory unit of adenylate cyclase system. Unfortunately, our attempts to demonstrate changes in viscosity or $Ca^{2+}$ binding associated with phospholipid methylation in membranes isolated from mast cells were unsuccessfull, due to the low yield of material available for study, and no final conclusion could be reached.

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## Orally administered melatonin stimulates the 3α/β-hydroxysteroid oxidoreductase but not the 5α-reductase in the ventral prostate and seminal vesicles of pinealectomized rats[1]

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*Summary.* Pinealectomized rats were treated orally with melatonin (MEL) for 14 days. Prostates and seminal vesicles were investigated for the activity of the Δ 4-3-ketosteroid-5α-oxidoreductase (5α-R) and of the 3α/β-hydroxysteroid-oxidoreductase (3α-HSO). The activity of the 3a-HSO was significantly stimulated ($p < 0.01$, Friedman test) when compared to controls. The activity of the 5α-R did not change significantly.

Melatonin (MEL) is primarily considered to be an antigonadotropic hormone[2] which exerts its action by a blockade of the hypothalamic LHRH secretion[3] and by stimulatory or inhibitory influences on the secretion of prolactin[4]. However, some results have reinforced the opinion that it may also have a direct influence on the metabolism of steroids in peripheral organs such as the ovary[5], testes[6,7] and adrenals[8]. Recently we have demonstrated that MEL affects the activity of the reduced NADP: 3α-hydroxysteroid-oxidoreductase (E.C. No. 1.1.1.50) in human hyperplastic prostatic tissue when added in vitro[9].

The aim of this study is to demonstrate whether or not MEL is capable of causing enzymatic changes in the rat prostate and seminal vesicles if administered orally for 14 days to animals in which the endogenous MEL rhythm has been interrupted by pinealectomy and constant illumination.

*Material and methods.* Adult male Wistar rats weighing 320–370 g were used in this study. Water and laboratory chow were available ad libitum. The intestinal absorption of 125 μCi $^3$H-MEL (NEN) plus 1 mg unlabeled MEL (Sigma) was investigated in 4 rats. Blood samples of 20 μl were taken from the retroorbital vein plexus 30, 45, 60, 90, 120, 150, 210, 270 and 720 min after force-feeding and measured for total radioactivity content. The presence of $^3$H-MEL and its metabolites was determined 12 h after force-feeding by TLC in the system chloroform: methanol = 9:1.

16 rats were pinealectomized using a technique similar to that suggested by Kuszak and Rodin[10]. Eight of them received 2 mg MEL (Sigma) in 1 ml of triolein (Sigma) per day orally for 14 days via a bulb headed feeding tube fitted to a syringe (group A) while the remaining 8 rats (group B) and 8 sham-operated animals (group C) were treated with only 1 ml of triolein. All rats were further maintained under constant light in order to suppress the pineal gland in the sham-operated controls and to lower extrapineal MEL secretion.

All hormone and control treatments started 1 day after pinealectomy or after sham-operation and were carried out at 19.00 h. On the 14th day of treatment the rats were bilaterally orchidectomized at 19.00 h and sacrificed 24–27 h later[11]. After exsanguination the ventral lobes of the prostates were removed, dissected free of connective

tissue and fat, blotted, weighed and immediately transferred into ice-cold saline (0.9% NaCl). $NADPH_2$-containing phosphate buffer, pH 6.9 was added to give a dilution of 1:10. Seminal vesicles were treated in a similar manner and both organs were separately homogenized in a Wheaton homogenizer. Basically, for the determination of enzyme activities the method of Moore and Wilson[12] was employed with minor modifications; the concentrations of labeled steroids in the incubation medium were $3.2\times10^{-11}$ moles ($2.11\times10^{-7}$ M) $^3$H-testosterone ($^3$H-T, sp. act. 98.8 Ci/mmoles, NEN) and $1.2\times10^{-11}$ moles ($0.81\times10^{-7}$ M) $^3$H-5$\alpha$-dihydrotestosterone ($^3$H-DHT, sp. act. 50.6 Ci/mmoles, NEN) per 150 µl. 3 rats, 1 of each group, were investigated in 1 experiment. The procedure for each individual rat was as follows: homogenization, 2 separate incubations (1 with $^3$H-T and 1 with $^3$H-DHT), extraction and TLC. This procedure was carried out in 8 separate experiments. All samples and blanks, the latter containing NADPH buffer instead of homogenate, were prepared in duplicate. In deviation from the method of Moore and Wilson the 3$\alpha$-HSO was investigated by the TLC technique of Burić et al.[13] which effectively separates 3$\alpha$- from 3$\beta$-5$\alpha$-androstane-compounds of which only the former was considered for calculation of the enzyme activity. The activity was defined as pmole/g tissue per 30 min. Conversion of androstanediols back to 5$\alpha$-DHT was not considered.

Radiogaschromatography using a Pye/Unicam 104 gas chromatograph interfaced with a Panax/Pye radiogas detector unit was performed in order to characterize further the metabolites of the labeled compounds and to confirm the TLC-data. The separation of steroids was achieved in a $2700\times4$ mm glass column filled with gaschrom Q coated with 3% QF 1, with linear temperature increase from 220 to 245 °C at a rate of 1 °C/min.

The analysis of rank variance according to Friedman[14] was used for statistical calculations. It refers to the block character of the groups and does not utilize SD of the mean.

*Results.* The 4 animals given 125 µCi $^3$H-MEL and 1 mg unlabeled MEL already showed a quick rise in plasma radioactivity after 45 min. An additional increase was seen after 12 h (fig. 1). TLC of the blood samples taken after 12 h showed the major radioactivity (73%) in the fraction of MEL which was clearly separated from 5-hydroxytryptamine, 5-methoxyindole and N-acetyl-5-hydroxytryptamine. Due to the lack of a standard, 6-hydroxymelatonin was not tested, but could be expected to be separated from MEL[15].

The weight of the testes and of the accessory sex organs was not significantly reduced in the animals treated with melatonin for 14 days (table).

Likewise the 5$\alpha$-R did not change significantly between the 3 groups, neither in the prostate nor in the seminal vesicles (fig. 2). The 3$\alpha$-HSO, however, was significantly elevated in the prostate and seminal vesicles of animals treated for the same time period with MEL when compared to group B and C (fig. 2). Radiogaschromatography did not confirm the existence of 5$\alpha$-androstane-3$\beta$-17$\beta$-diol. Therefore this fraction was not considered when calculating enzyme activities.

*Discussion.* The finding that oral administration of MEL under constant illumination affects the activity of the prostatic 3$\alpha$-HSO in pinealectomized rats has several important aspects:

1. It can be confirmed that the mode of action of exogenous MEL on the prostate includes a peripheral effect on the steroid metabolism of the organ itself.
2. The slight decrease in the weight of the prostate after treatment with MEL can be related to changes in the intracellular androgen metabolism.
3. The equilibrium between T, DHT and 3$\alpha$-androstanediol in our system has changed towards more androstanediol and less DHT because of the constant 5$\alpha$-R and increasing 3$\alpha$-HSO.

Earlier results on the human hyperplastic prostate and on the rat prostate in vitro[9,16] show that the changes in the activity of the 3$\alpha$-HSO can be induced even without participation of pituitary and gonadal hormones.

It is known that the androstanediols are metabolized into androstanetriols[17] by the 6$\alpha$- or 7$\beta$-hydroxylase by a 1-way reaction. This again would point to an enhanced androgen catabolism under the influence of MEL which could be

Weight of prostate and seminal vesicles per 100 g b.wt in pinealectomized rats treated daily with 2 mg MEL (A) or with the vehicle only (B) orally for 14 days (mean ± SD). Group C was sham operated and treated with the vehicle

| | Prostate (g/100 g b.wt) | Seminal vesicles (g/100 g b.wt) |
|---|---|---|
| A | 0.072 ± 0.020 | 0.066 ± 0.002 |
| B | 0.080 ± 0.023 | 0.067 ± 0.017 |
| C | 0.076 ± 0.023 | 0.067 ± 0.017 |

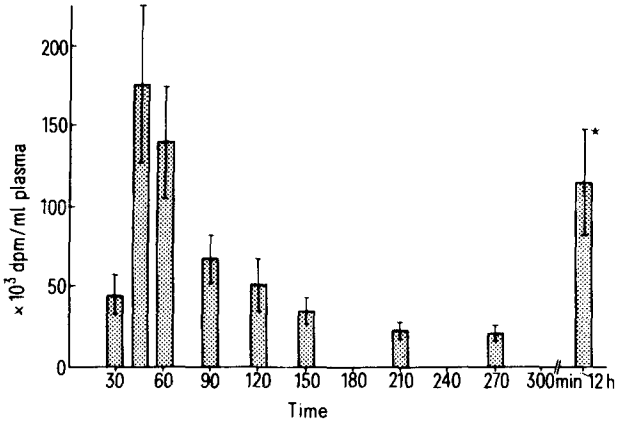


Figure 1. Uptake of radioactivity into blood after oral administration of 125 µCi $^3$H-MEL plus 1 mg unlabeled MEL to adult male rats (mean ± SD). * $p \leq 0.01$ when compared to 270 min (t-test).

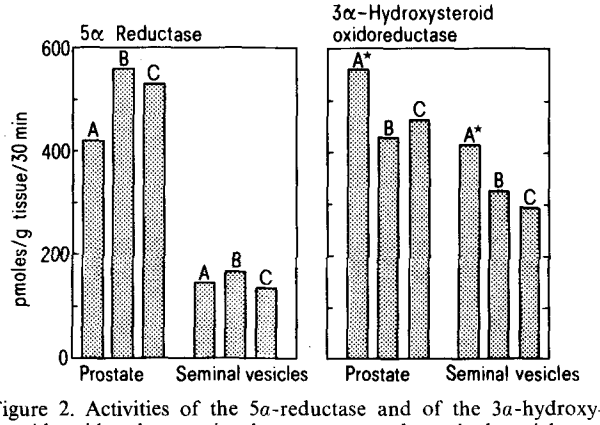


Figure 2. Activities of the 5$\alpha$-reductase and of the 3$\alpha$-hydroxysteroid oxidoreductase in the prostate and seminal vesicles of pinealectomized rats treated daily with 2 mg MEL (A) or with the vehicle only (B and C, see legend to the table) orally for 14 days. Significance was calculated by the Friedman-test and therefore no SD is given. * Significantly different from B and C at $p \leq 0.01$.

important for the process of prostatic involution which occurs periodically in several animal species[18]. The enzymatic changes in our study became evident before changes in the weight of the prostate were noticed. This may be considered as an indication that lowering of the prostatic weight may be secondary to the increase in the activity of 3α-HSO. The mechanism by which MEL is capable of changing the activity of 3α-HSO is at present unknown. However, results from our laboratory[16] on 6 ventral prostates of rats to which MEL was added in vitro in increasing concentrations and which show the same increase of 3α-HSO suggest that it does not involve an increase in protein biosynthesis, since initiation of protein synthesis requires considerably more time.

The existence of a direct stimulatory action of MEL on the prostatic 3α-HSO could also be of interest for the accumulation of DHT in the prostatic tissue which has been observed in the hyperplastic prostate of man[19]. It has also been shown that this process seems to be associated with a fall in the 3α-HSO and a change in the 3α-androstanediol/DHT ratio.

The steroid-biochemical changes seen in this study are comparable with changes in steroid metabolism observed in rodents with intact pineal glands kept under short photoperiod, in constant darkness or blindness because under these conditions MEL is elevated[18,20].

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## Hormonal levels and protein variations during sexual maturation of *Schistocerca gregaria;* effect of rearing temperature

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*Summary.* As a result of the decrease of diurnal rearing temperature from 33 to 28 °C the following phenomena were induced in adults of *Schistocerca gregaria:* a) In females, a delay in the appearance of maximal levels of JH III and ecdysteroids in the hemolymph, a slowing down of oocyte growth, and an accumulation of hemolymph proteins; b) in males, a decrease of hemolymphatic JH III levels without changes in protein levels.

The decrease of diurnal rearing temperature from 30 to 28 °C seriously disturbs the reproduction of *Schistocerca gregaria.* The results of such a change during the 5th larval instar are a decrease of hemolymph JH III levels in the larva[1], and of the number of functional ovarioles in the adult[2]. Rearing of adults at 28 °C suppresses reproduction: the spermatogenesis is disturbed and the males are sterile[3,4]; in the females, oocyte resorptions are numerous and laid eggs do not develop; under these conditions, neurosecretory products accumulate in the pars intercerebralis and the secretory activity of the corpora allata and corpora cardiaca slows down[5]. The following study describes the effects of a low temperature on the levels of proteins and hormones during sexual maturation.

*Material and methods.* The insects were reared in groups under following conditions: 12 h light/day; diurnal temperature 33 or 28 °C; nocturnal temperature 20 °C; 30-35% relative humidity. Assay for proteins was performed by the biuret method on hemolymph cleared of hemocytes by centrifugation (9600 × g; 20 min; 4 °C).

Radioimmunoassay of JH III was carried out on 100-200-µl samples of hemolymph obtained from 2-3 females at the same stage of oocyte development. Extraction and RIA procedures were previously described[1,6].

Radioimmunoassay of ecdysteroids was performed on hemolymph and ovaries. Ovaries and eggs were crushed in methanol and ecdysteroids were then extracted by a series of butanol-water partitions[7,8]. Butanol extracts were evaporated to dryness and used in RIA as well as hemolymph extracts, according to the procedure previously described[7-9].

*Results.* Whatever the rearing temperature was, vitellogenesis started in females when oocytes achieved the length of 1.4-1.6 mm. When the diurnal temperature was lowered from 33 to 28 °C the following was observed: a 5-6-day delay in the onset of vitellogenesis, a 20-day delay in the laying of the 1st ootheca and a reduction of the size of the oocytes at the time of laying (fig. 1, A).

1. *Hemolymph protein levels.* Immediately after the imaginal moult of females, until the 6th day at 33 °C and the 10th day at 28 °C, the protein levels diminished and the oocytes were smaller than 1 mm. This was followed by a rise in protein level. At 33 °C, the blood protein level quickly increased during the 14th and 15th day (oocyte length: 1.6-2.2 mm); until the ovulation, 6 or 7 days later on, the protein concentrations were almost stable (70 mg/ml) (fig. 1, B). At 28 °C, the increase of protein level was accentuated near the 19th day, at the onset of vitellogenesis, and continued for 10 days, the mean values reaching 157 mg/ml (oocyte length 4-5 mm). In spite of a slight decrease during the end of previtellogenesis of penultimate oocytes, the level of proteins remained very high until ovulation (fig. 1, B).