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Putative Melatonin Receptors in the Blind Mole Rat Harderian Gland

E. GILAD,[1] U. SHANAS,[2] J. TERKEL,[2] AND N. ZISAPEL[1]*
[1]*Department of Neurobiochemistry, the George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, 69978, Israel*
[2]*Department of Zoology, the George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, 69978, Israel*

ABSTRACT The blind mole rat (*Spalax ehrenbergi*) displays daily and seasonal rhythms. Melatonin, secreted nocturnally by the pineal gland, is also produced in the harderian gland and affects its morphology in rodents. We report here on the presence of putative melatonin receptors in the blind mole rat harderian gland, located in the microsome-enriched fraction of the cells.

Equilibrium ^{125}I-melatonin binding studies indicated high- and low-affinity melatonin binding sites in the female (apparent Kd 10 pM and 2.4 nM, respectively) and low-affinity sites in the male (apparent Kd 2.6 nM) mole rat. The binding sites were not significantly affected by season. Castration increased the density of high-affinity binding sites in males and low-affinity binding in females.

^{125}I-melatonin binding to the gonadectomized mole rat preparation was inhibited by serotonin>2-iodomelatonin≥memelatonin>5-methoxytryptamine. The guanine nucleotide analogs, guanosine 5´-O-[3-thio-triphosphate] and guanosine 5´-O-[2-thio-diphosphate], inhibited specific ^{125}I-melatonin binding, whereas 5´-guanylyl imido-diphosphate was less potent.

These results indicate for the first time the presence of GTP-sensitive melatonin binding sites in the blind mole rat harderian gland, and suggest that their expression is under control of sex steroids. *J. Exp. Zool. 277:435–441, 1997.*

The mole rat (*Spalax ehrenbergi*) is a blind, solitary, highly aggressive subterranean rodent that shows striking behavioral and physiological adaptations to underground life (MacDonald, '85; Nevo, '91). Its rudimentary eye is atrophied, covered by a thick layer of skin and dark fur (Cei, '46; Sanyal et al., '90) and rests on a large harderian gland, which is the major organ in the orbital cavity.

The harderian gland, found in many vertebrates which possess a third eyelid, has been the subject of extensive studies. It has been postulated as a possible source of pheromones (Thiessen et al., '76; Payne, '79), a source of thermoregulatory lipids (Jost and Murawski, '77; Thiessen and Kittrell, '80), a photo-protective organ (Hugo et al., '87), and a part of the immune system (Burns, '79; Montgomery and Maslin, '92; for general reviews, see Sakai, '81; Payne, '94). In the golden hamster, the gland shows marked sexual dimorphism, especially regarding the histological (Hoffman, '71; Lin and Nadakavukaren, '79), and enzyme characteristics (Menendez-Pelaez et al., '88). Some of these characteristics have been shown to be under control of gonadal steroids (Payne et al., '77; Lin and Nadakavukaren, '79; Buzzell et al., '91). In some rodents, it has been suggested that the harderian gland serves as an extraretinal photoreceptor (Wetterberg et al., '70; Pevet et al., '84).

Melatonin, the principal hormone secreted by the pineal gland at night, mediates the effect of the photoperiod on reproduction and thermoregulation, presumably by acting on the hypothalamus–pituitary axis (see Reiter, '91, for review). In the golden hamster, melatonin has been suggested to play an important role in the metabolism and morphology of the harderian gland (McMaster and Hoffman, '84; Rodriguez-Colunga et al., '91). Binding sites for both melatonin (Menendez-Pelaez et al., '93), and gonadal steroids (Vilchis et al., '87) were found in the golden hamster harderian gland. The melatonin binding sites were characterized by sexual dimorphism in density (females

*Correspondence to: Prof. N. Zisapel, Department of Neurobiochemistry, the George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel.
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show more than males) but not in apparent dissociation constants. Castration of the males resulted in an increased density of the melatonin binding sites up to the levels of females (Menendez-Pelaez et al., '93). As a putative extraretinal photoreceptive organ, the harderian gland may be particularly important in blind animals (Wetterberg et al., '70), including the blind mole rat. If so, melatonin and its receptors in the harderian gland may participate in the circadian and seasonal activities of this blind and subterranean animal.

The mole rat exhibits sexual differences in harderian gland weight and acini star volume (i.e., average volume of the tubuli), although these are not as obvious as in the golden hamster (Shanas et al., '96). The male's gland weighs significantly more than the female's (Shanas et al., '96). This dimorphism is probably controlled by sex hormones, as an injection of GnRH caused a significant rise in the male gland weight (unpublished results). The harderian glands of a number of other species, including the Syrian hamster, contain high concentrations of porphyrins, and in some cases display sexual dimorphism in this respect (Payne, '94; Buzzell et al., '91). The blind mole rat harderian gland differs from those in that it does not contain detectable amounts of porphyrins (unpublished results). In addition, the mole rat harderian gland shows seasonal variations in mass, with peak mass during the breeding season, namely, in the winter. During the breeding season, the mass of the female gland increases and reaches that of the male gland (Shanas et al., '96).

Three roles have been proposed for the mole rat harderian gland. One study suggested that the gland is implicated in the detection of photoperiodic changes (Pevet et al., '84); a second study demonstrated the involvement of the gland in indole metabolism (Balemans et al., '80). Recently, we have suggested that the gland may be implicated in pheromone production (Shanas and Terkel, '95).

The purpose of the present study was to examine whether the mole rat harderian gland exhibits melatonin binding sites and whether these sites are under gonadal or seasonal regulation.

MATERIALS AND METHODS

Animals

All animals used in this study were adult mole rats trapped in the field around Tel Aviv during winter. After capture, the animals were maintained in the lab at 24±2°C under daily light dark cycles of 14L:10D for at least six months (cool white fluorescent illumination). They were kept in individual plastic cages (30 × 20 × 13 cm) with wood shavings for bedding, and fed with rat pellets, carrots, and apples. Although the above lighting regime resembles a long-day photoperiod, reproductive parameters such as females vaginal smears and body mass reveal that mole rats retain a circannual rhythm under these conditions (Shanas et al., '95; Shanas et al., '96).

Castrated animals: Ten animals (5 females and 5 males) were anesthetized with an i.m. injection of a Ketalar and Rumpon mixture. A cut was made through the skin and muscle on both sides of the body near the gonads. The blood vessels leading to the gonads were ligated and the gonads removed. The cuts were sutured and the animals were allowed to recover for two months.

Three groups of animals were studied (time of gland excision in parenthesis): 1) winter (February), 2) summer (August), and 3) gonadectomized (June). Each group consisted of two subgroups (5 females and 5 males).

The harderian glands were surgically removed after the animals were anesthetized with an i.m. injection of a Ketalar and Rumpon mixture. A one cm incision of the skin covering the orbit was made on both sides of the head, exposing the harderian glands. After ligating the blood vessels leading to the gland, the gland was removed, weighed, and immediately stored below –70°C until assay. All the glands were excised between 15:00–17:00 h.

Materials

Melatonin, 5-methoxytriptamine, serotonin, guanosine 5´-O-[3-thio-triphosphate] (GTPγS), guanosine 5´-O-[2-thio-diphosphate] (GDPβS), 5´-guanylyl imido-diphosphate (GppNHp), polyethylenimine (PEI), bovine serum albumin (BSA), and phenyl methane-sulfonyl fluoride (PMSF) were obtained from Sigma. $Na^{125}I$ was obtained from Amersham. ^{125}I-melatonin was prepared as described (Vakkuri et al., '84).

Equilibrium ^{125}I-melatonin binding

Harderian glands of each study group were trimmed of extraneous tissues, pooled together, homogenized with Teflon-glass homogenizer with 10 volumes/gram tissue of ice cold Tris-HCl buffer (50 mM Tris, 5 mM $CaCl_2$, pH = 7.4) containing 200 μM PMSF, and spun at 10,000g for 10 min. The pellets (P_1, particulate fraction), resuspended in the same buffer, and the supernatants (S_1) di-

luted (1:6) with the same buffer were collected. S_1 fractions were spun at 100,000g for 2 h to yield a microsome-enriched pellet (P_2, Laudon et al., '96), which was then suspended in the same buffer to 25% of the initial volume. All fractions were frozen and stored at –70°C until used.

Binding to the microsome-enriched fractions (P_2)

Aliquots of the microsome fractions were incubated in pentaplicates for 5–120 min. at 37°C with ^{125}I-melatonin (10 pM–1 nM), in assay buffer (Tris buffer containing 0.15% Triton X-100), in the absence (total binding) or presence (non-specific binding) of 50 mM melatonin. The binding reaction was terminated by the addition of 4 ml ice-cold Tris buffer. The bound ^{125}I-melatonin was collected by vaccum filtration using GF/F glass fiber filters, preincubated for 15 min in 0.3% PEI. Filters were washed with 3×4 ml ice-cold buffer and the bound ^{125}I-melatonin was determined in a γ counter as described (Laudon et al., '96). Specific binding was calculated from the difference between the total and non-specific binding and ranged from 40–50% of the total binding at 500 pM ^{125}I-melatonin. Scatchard analysis of the equilibrium binding data at this concentration range suggested a single- or two-site model in the various animal groups. Accordingly, the equilibrium binding parameters were obtained by nonlinear regression analyses (Sigmaplot), using a one- or two-site model.

Binding to particulate fraction (P_1)

Samples were incubated with ^{125}I-melatonin (10 pM–1 nM) in Tris-HCl buffer pH 7.4 containing 5 mM $CaCl_2$ in the absence or presence of 50 mM melatonin for 60 min at 37°C. Membranes were then collected by vacuum filtration using GF/C glass fiber filters as described (Laudon and Zisapel, '86).

Competition studies

Aliquots of microsome-enriched fractions, from gonadectomized animals, were incubated in pentaplicates with ^{125}I-melatonin (200 pM) for 1 h in the presence of diluent (0.01% ethanol) or of 1 nM–1 μM of various competitors (melatonin, 2-iodomelatonin, 5-methoxytryptamine and serotonin). The amount of protein-bound radioactivity was determined after filtration on GF/F filters.

Effects of nucleotides

Aliquots of the microsome-enriched fractions were incubated in pentaplicates for 10 min in the absence or presence of GTPγS, GppNHp, or GDPβS (50 and 200 mM) in assay buffer containing 5 mM $MgCl_2$. ^{125}I-melatonin (500 pM) was then added with or without melatonin (50 μM) and the incubation resumed for 60 min. Bound ^{125}I-melatonin was assessed as described above.

Protein determination

Protein content was determined as described (Markwell et al., '78) using bovine serum albumin (BSA) as a standard.

RESULTS

^{125}I-melatonin binding in harderian-derived preparations

Incubation of the microsome-enriched preparations with ^{125}I-melatonin (500 pM at 37°C) resulted in a time-dependent increase in specific binding, reaching equilibrium within 30 min. Following equilibration, excess melatonin (50 μM) was added and displacement of all specific binding of ^{125}I-melatonin occurred within 80 min, indicating that the binding was reversible (not shown). Incubation of the particulate fraction obtained from the harderian (P_1) with ^{125}I-melatonin under similar conditions did not reveal the presence of specific binding sites in this fraction (not shown).

The concentration dependencies of ^{125}I-melatonin binding to microsome-enriched preparations from the female harderian gland are shown in Figure 1a. Scatchard plots analysis of the equilibration binding data (Table 1) was comparable with a two-binding-sites model, with apparent dissociation constants (Kd) of 10 pM (high affinity) and 2.6 nM (low affinity) in the summer, and a single low-affinity site model in the winter. The apparent density of the low-affinity sites was similar in glands collected in the winter and summer.

In the males, the concentration dependency of ^{125}I-melatonin binding was compatible with a single low-affinity site model (apparent Kd 2.5 nM, Fig. 1b). Binding was not affected by season of gland excision.

In ovariectomized females, binding was compatible with a single-site model, displaying low affinity toward the ligand (Kd = 1.2 nM, Fig. 1c). The apparent density of these sites was significantly higher in ovariectomized than in intact females (Table 1).

In castrated males, the binding was compatible with a two-site model (Fig. 1c). The density of the high-affinity sites was apparently increased by

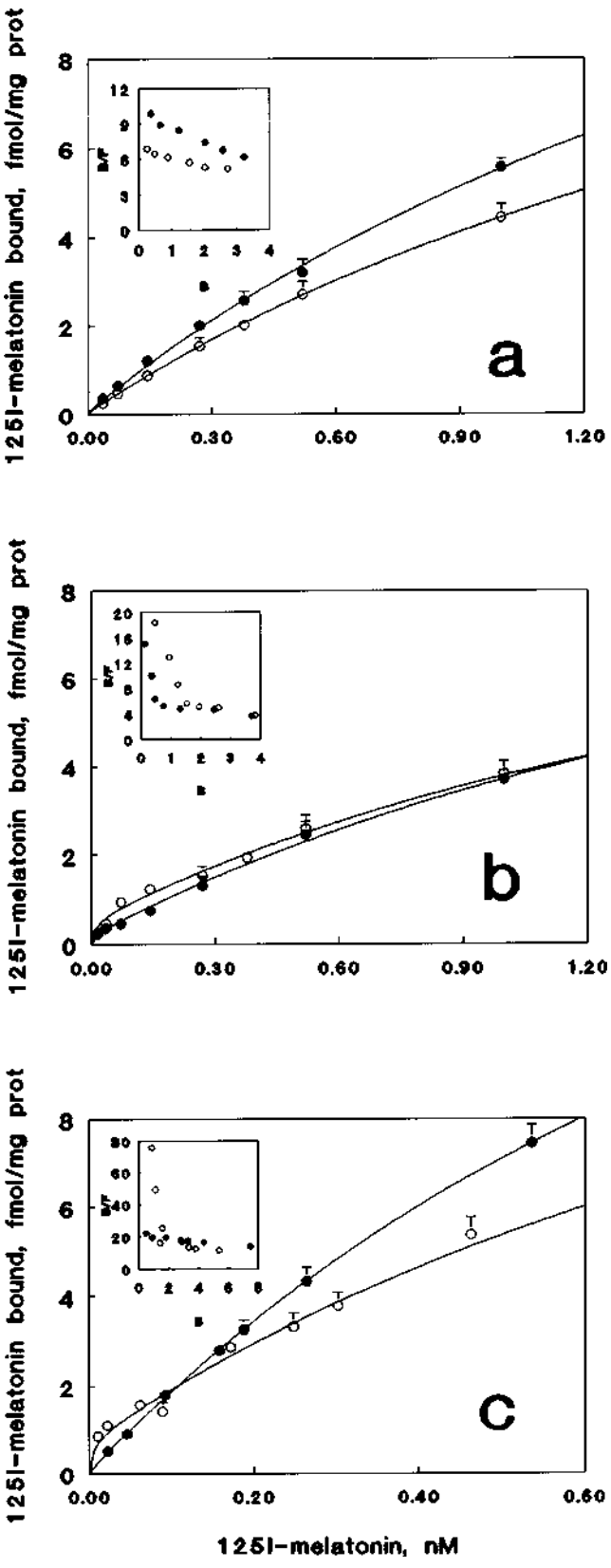

Figure 1.

castration, whereas that of the low-affinity sites was not significantly affected (Table 1).

The inhibition of specific ^{125}I-melatonin binding to microsome-enriched preparations from the harderian of gonadectomized blind mole rats by various indole derivatives is shown in Figure 2a–b. Specific ^{125}I-melatonin binding was inhibited by 2-iodomelatonin and melatonin (IC_{50} 10 and 50 pM, respectively), whereas 5-methoxytryptamine was less potent (Fig. 2). Serotonin competed for the specific- as well as the non-specific binding (i.e., binding which was not affected by either 2-iodomelatonin or melatonin). Data were similar for both male and female groups (Fig. 2, left and right panels).

The inhibition of ^{125}I-melatonin binding to the microsome-enriched preparations by nonhydrolizable guanine nucleotide analogs is shown in Figure 3. ^{125}I-melatonin binding was inhibited by GTPγS and GDPβS in a dose-dependent manner, whereas GppNHp was less effective. Data were similar for all study groups.

DISCUSSION

Our results show for the first time that the harderian gland of the blind mole rat contains specific melatonin binding sites, which bind ^{125}I-melatonin with high affinity in a reversible and saturable manner. The possibility that these sites are putative melatonin receptors is supported by several arguments: 1) Iodinated melatonin is a biologically active melatonin analog that mimics both the reproductive and circadian effects of melatonin (Laudon and Zisapel, '86; Dubocovich, '88). 2) The apparent affinities of these sites for ^{125}I-melatonin (around 10 pM and 2 nM) are within the range found by autoradiography for melatonin receptors in mammalian brain and pars-tuberalis (i.e., 10 pM–3 nM, see Dubocovich, '95, for review). 3) The inhibition by GTPγS and GDPβS of the specific ^{125}I-melatonin binding is compatible with a G-protein coupled receptors

Fig. 1. Equilibrium ^{125}I-melatonin binding to microsome-enriched preparations from the blind mole rat harderian as a function of ^{125}I-melatonin concentrations. Aliquots of the microsomal fractions (5 animals/group) were incubated with various concentrations of ^{125}I-melatonin in the absence and presence of melatonin (50 μM). Specific binding was determined at equilibrium. Mean ± SEM values (n = 5) are shown. The solid lines are theoretical curves reconstructed from the mean Kd and Bmax values depicted in Table 1. **a:** Males, winter (●) and summer (○). **b:** Females, winter (●) and summer (○). **c:** Castrated females (●) and males (○). The respective Scatchard plots of the data are shown in the insets.

TABLE 1. Apparent Kd and Bmax values (Mean ± SEM) calculated from the Scatchard plot analyses of specific ^{125}I-melatonin binding data presented in Fig 1. H = high affinigy site, l = low affinity site.

		Male		Female	
Group:		Kd (nM)	Bmax (fmol/ mg prot.)	Kd (nM)	Bmax (fmol/ mg prot.)
Summer:	H:	—	—	0.01 ± 0.007	0.54 ± 0.05
	L:	2.60 ± 0.56	15.80 ± 3.20	2.47 ± 0.55	11.30 ± 3.00
Winter:	H:	—	—	—	—
	L:	2.50 ± 0.44	19.10 ± 2.50	2.67 ± 0.80	12.90 ± 3.00
Castrated:	H:	0.01 ± 0.004	0.59 ± 0.09	—	—
	L:	1.20 ± 0.79	16.90 ± 8.70	1.26 ± 0.05	24.80 ± 0.82

(Birnbaumer, '91), as are the recently cloned melatonin receptors (Reppert et al., '96).

The association of these sites with the microsome-enriched fraction is compatible with our recent findings of microsome-associated ^{125}I-melatonin binding sites in the human benign prostate tissue and the rat prostate (Laudon et al., '96; Gilad et al., submitted).

Melatonin has been found to be produced in the mole rat harderian gland (Balemans et al., '80). In the rat it has been proposed that the melatonin binding sites in the harderian gland mediate a negative feedback control of melatonin on harderian gland melatonin production (Lopez-Gonzalez et al., '91). One possible function for melatonin binding sites found in this study in the harderian gland of the mole rat may thus be related to the regulation of melatonin production. Another possibility is of a hormonal feedback on the putative photoreceptive action of the gland. These possibilities deserve further investigation.

This study failed to find major seasonal effects on melatonin binding sites in the harderian gland. This could be due to the fact that the experimental animals were kept under a constant 14:10LD photoperiod regime. On the other hand, mole rats have been demonstrated to retain circannual rhythms even when they are kept under constant lighting regimes (Shanas et al., '95; Shanas et al., '96). It is thus possible that melatonin receptors in the mole rat harderian gland do not display seasonal variations, or that they depend on the photoperiod.

Castration-induced changes in ^{125}I-melatonin binding sites, in the male and female harderian gland, suggest that the expression of melatonin

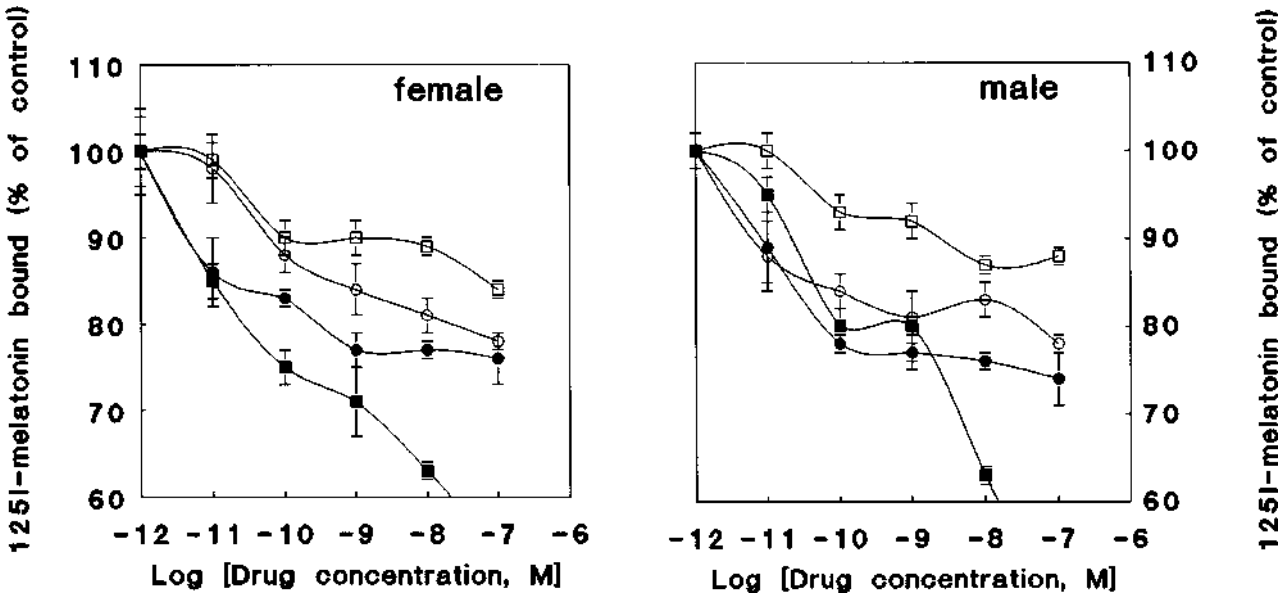

Fig. 2. Inhibition of ^{125}I-melatonin binding to harderian gland preparations by various indole derivatives. Microsome-enriched preparations from the harderian glands of gonadectomized male (right panel) and female (left panel) mole rats were incubated with ^{125}I-melatonin in the absence and presence of various concentrations of: melatonin (○), serotonin (■), 2-iodomelatonin (●) and 5 methoxytryptamine (□). Mean ± SEM of the binding data values (n = 5) are shown.

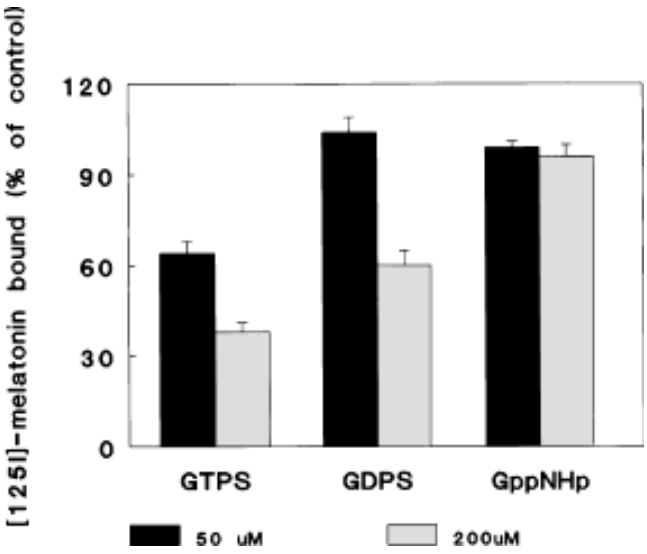

Fig. 3. Inhibition of ^{125}I-melatonin binding to mole rat harderian gland preparations by guanine nucleotide analogs. Microsome preparations were incubated for 10 min with GTPγS, GDPβS, or GppNHp or buffer. The specific binding of ^{125}I-melatonin was then assessed. Results are expressed as a percentage of the specific binding in the absence of guanine nucleotide analogs. Mean ± SEM values of all the study groups (n = 18) are shown.

receptors is influenced by steroids. Effects of sex steroids on melatonin binding sites have been demonstrated in rats and hamsters. In female rat hypothalamus and medulla-pons, ovariectomy produced a large estradiol-reversible decrease in low-affinity ^{125}I-melatonin binding sites (Laudon and Zisapel, '87). In male hamsters maintained on a long-day regime, castration produced a large testosterone-reversible decrease in ^{125}I-melatonin binding sites in the hypothalamus, medulla-pons, and hippocampus (Anis and Zisapel, '91). However, in aged male rat brain testosterone administration reduced ^{125}I-melatonin binding sites (Okanin-Bendahan et al., '92). In addition, castration has been shown to increase binding site density in the anterior pituitary of male rats (Vanecek et al., '90), and the harderian gland of male golden hamsters (Menendez-Pelaez et al., '93).

Castration of adult male hamsters results in harderian glands converting into the female type (Payne et al., '77), whereas administration of androgens to adult females results in male gland appearance (Sun and Nadakavukaren, '80; Spike et al., '85). In the present study, castration increased the density of high-affinity melatonin binding sites in the male, resulting in a binding pattern similar to that observed in the female harderian gland in the summer. Ovariectomy of females increased the density of the low-affinity binding sites so that the binding resembled that observed in intact males. The mechanisms that underlie these transformations have yet to be elucidated, and further studies will be needed to evaluate the significance of melatonin receptors in the harderian gland of the blind mole rat.

LITERATURE CITED

Anis, Y., and N. Zisapel (1991) Castration affects brain iodomelatonin binding in hamsters maintained in long but not short days. Molec. Cellul. Endocrinol., *76:*23–34.

Balemans, M.G.M., P. Pevet, W.C. Legerstee, and E. Nevo (1980) Preliminary investigations on Melatonin and 5-Methoxytryptophol synthesis in the pineal, retina, and Harderian gland of the mole rat and in the pineal of the mouse "eyeless." J. Neural Transm., *49:*247–255.

Birnbaumer, L., J. Abramowitz, and A.M. Brown (1990) Receptor-effector coupling by G proteins. Biochim. Biophys. Acta, *1031:*163–224.

Burns, R.B. (1979) Histological and immunological studies on the fowl lacrimal gland following surgical excision of Harder's gland. Research in Veterinary Science, *27:*69–75.

Buzzell, G.R., A. Menendez-Pelaez, V. Chlumecky, and R.J. Reiter (1991) Gender differences and time course of castration-induced changes in porphyrins, indoles, and proteins in the Harderian glands of the Syrian hamster. Can. J. Physiol. Pharmacol., *69:*1814–1818.

Cei, G. (1946) Ortogenesi parallela e degenerazione degli organi dello visto negli Spalacidi. Monitore. Zool Ital., *55:*69–88.

Dubocovich, M.L. (1988) Pharmacology and function of melatonin receptors. FASEB J., *2:*2765–2773.

Dubocovich, M.L. (1995) Melatonin receptors: Are there multiple subtypes? Trends. Pharmacol. Sci., *16:*50–56.

Heth, G., P. Pevet, E. Nevo, and A. Beiles (1986) The effect of melatonin administration and short exposures to cold on body temperature of the blind subterranean mole rat (Rodentia, *Spalax ehrenbergi,* Nehring). J. Exp. Zool., *238:*1–9.

Hoffman, R.A. (1971) Influence of some endocrine glands, hormones and blinding on the histology and porphyrins of the harderian glands of golden hamster. Am. J. Anat., *132:*463–478.

Hugo, J., J. Krijt, M. Vokurka, and V. Janousek (1987) Secretory response to light in rat Harderian gland: Possible photoprotective role of Harderian porphyrin. Gen. Physiol. Biophys., *6:*401–404.

Jost, U., and U. Murawski (1977) Adaptation to coldness in the synthesis of wax esters in the Harderian gland of the rat. Fette Seifen Anstrichm, *79:*82–83.

Laudon, M., and N. Zisapel (1986) Characterization of central melatonin receptors using 125I-melatonin. FEBS Lett., *197:*9–12.

Laudon, M., and N. Zisapel (1987) Impact of circulating estradiol on melatonin binding sites in discrete areas of the female rat brain. Brain Res., *402:*146–150.

Laudon, M., E. Gilad, H. Matzkin, Z. Braf, and N. Zisapel (1996) Putative melatonin receptors in benign human prostate tissue. J. Clin. Endocrinol. Metab., *81:*1336–1342.

Lin, W., and M.J. Nadakavukaren (1979) The androgenic effect on the fine structure of the Harderian gland in the male hamster. Cell Tissue Res., *198:*119–127.

Lopez-Gonzalez, M.A., J.R. Calvo, A. Rubio, R. Goberna, and J.M. Guerrero (1991) Characterization of melatonin binding sites in the harderian gland and median eminence of the rat. Life Sciences, *48:*1165–1171.

MacDonald, D. (1985) The Encyclopedia of Mammals. George Allen & Unwin, London, Sydney.

McMaster, K.M., and R.A. Hoffman (1984) Harderian gland: Regulation of sexual type by gonads and pineal gland. Biol. Reprod., *31:*579–585.

Markwell, M.A.K., S.M. Haas, L.C. Bieber, and N.E. Talbert (1978) A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Analyt. Biochem., *87:*206–210.

Menendez-Pelaez, A., R.J. Reiter, J.M. Guerrero, M. Puig-Domingo, and K.A. Howes (1988) Sexual dimorphism in N-acetyltransferase activity, Hydroxindole-O-methyltransferase activity, and melatonin content in the Harderian gland of Syrian hamsters: Changes following gonadectomy. Proc. Soc. Exp. Biol. Med., *187:*287–291.

Menendez-Pelaez, A., M.A. Lopez-Gonzalez, and J.M. Guerrero (1993) Melatonin binding sites in the Harderian gland of Syrian hamsters: Sexual differences and effect of castration. J. Pineal Res., *14:*34–38.

Montgomery, R.D., and W.R. Maslin (1992) A comparison of the gland of harder response and head-associated lymphoid tissue (HALT) morphology in chickens and turkeys. Avian Diseases, *36:*755–759.

Nevo, E. (1991) Evolutionary theory and processes of active speciation and adaptive radiation in subterranean mole rats, *Spalax ehrenbergi* superspecies in Israel. Evol. Biol., *25:*1–125.

Oaknin-Bendahan S., Y. Anis, I. Nir, and N. Zisapel (1992) Effect of melatonin and testosterone administration on iodomelatonin binding sites in the aged rat brain. Life Sci. Adv. (Neuroendocrinology) *11:*119–128.

Payne, A.P. (1979) Attractant properties of the Harderian gland and its products on male golden hamster of differing sexual experience. J. Endocr., *80:*69–70.

Payne, A.P. (1994) The harderian gland: A tercentennial review. J. Anat., *185:*1–49.

Payne, A.P., J. McGadey, M.R. Moore, and G. Thompson (1977) Androgenic control of the Harderian gland in the male golden hamster. J. Endocr., *75:*73–82.

Pevet, P., G. Heth, A. Haim, and E. Nevo (1984) Photoperiod perception in the blind Mole Rat (*Spalax ehrenbergi,* Nehring): Involvement of the Harderian gland, atrophied eyes, and Melatonin. J. Exp. Zool., *232:*41–50.

Reiter, R.J. (1991) Pineal melatonin: Cell biology of its synthesis and of its physiological interactions. Endocrine Rev., *12:*151–180.

Reppert, S.M., D.R. Weaver, and C. Godson (1996) Melatonin receptors steps into the light: Cloning and classification of subtypes. Trend Pharmacol. Sci., *17:*100–102.

Rodriguez-Colunga, M.I., C. Fernandez, I. Antolin, C. Rodriguez, D. Tolivia, and A. Menendez-Pelaez (1991) Chronic administration of melatonin induces changes in porphyrins and in the histology of male and female hamster Harderian gland: Interrelation with the gonadal status. J. Pineal Res., *11:*42–48.

Sakai, T. (1981) The mammalian Harderian gland: Morphology, biochemistry, function, and phylogeny. Arch. Histol. Jap. *44, No. 4:*299–333.

Sanyal, S., H.G. Jansen, W.J. De Grip, E. Nevo, and W.W. De Jong (1990) The eye of the blind mole rat, *Spalax ehrenbergi:* Rudiment with hidden function. Invest. Ophthalmol. Vis. Sci., *31:*1398–1404.

Shanas, U., G. Heth, E. Nevo, R. Shalgi, and J. Terkel (1995) Reproductive behavior in the female blind mole rat (*Spalax ehrenbergi*). J. Zool. Lond., *237:*195–210.

Shanas, U., B. Arensburg, I. Hammel, I. Hod, and J. Terkel (1996) Quantitative histomorphology of the blind mole rat Harderian gland. J. Anat., in press.

Shanas, U., and J. Terkel (1995) Grooming expresses Harderian gland materials in the blind mole rat. Aggress. Behav., *21:*137–146.

Sokal, R.R., and F.J. Rholf (1969) Biometry. Freeman, San Francisco.

Spike, R.C., H.S. Johnston, J. McGadey, M.R. Moore, G.G. Thompson, and A.P. Payne (1985) Quantitative studies on the effects of hormones on structure and porphyrin biosynthesis in the Harderian gland of the female golden hamster. I. The effects of ovariectomy and androgen administration. J. Anat., *142:*59–72.

Sun, C.Y., and M.J. Nadakavukaren (1980) Effect of testosterone on the female hamster Harderian gland pigmentation and ultrastructure. Cell Tissue Res., *207:*511–517.

Thiessen, D.D., A. Clancy, and M. Goodwin (1976) Harderian gland pheromone in the mongolian gerbil *Meriones unguiculatus.* J. Chem. Ecol., *2, No. 2:*231–238.

Thiessen, D.D., and M.W. Kittrell (1980) The Harderian gland and thermoregulation in the gerbil (*Meriones unguiculatus*). Physiol. Behav., *24:*417–424.

Vakkuri, O., J. Leppaluoto, and O. Vuolteenaho (1984) Development and validation of a melatonin radioimmunoassay using radioiodinated melatonin tracer. Acta Endocrinol (Copenhagen), *106:*152–157.

Vanecek, J., E. Kosar, and J. Vorlicek (1990) Daily changes in melatonin binding sites and the effect of castration. Mol. Cell Endocrinol., *73:*165–170.

Vilchis, F., A. Hernandez, A.E. Perez, and G. Perez-Palacios (1987) Hormone regulation of the rodent Harderian gland: Binding properties of the androgen receptor in the male golden hamster. J. Endocr., *112:*3–8.

Wetterberg, L., E. Geller, and A. Yuwiler (1970) Harderian gland: An extraretinal photoreceptor influencing the Pineal gland in neonatal rats. Science, *167:*880–881.