The Journal of Experimental Biology 207, 437-441
Published by The Company of Biologists 2004
doi:10.1242/jeb.00786

Inhibition of the increased 17β-estradiol-induced mast cell number by melatonin in the testis of the frog *Rana esculenta*, *in vivo* and *in vitro*

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Accepted 7 November 2003

Summary

In the present study, we have utilized 17β-estradiol to induce the increase of mast cell number in order to verify the melatonin effect on mast cell accumulation in the frog testicular interstitium. Data obtained from *in vivo* experiments confirm that 17β-estradiol increases the mast cell number and indicate a melatonin-inhibitory role in their accumulation in the frog testis. In addition, melatonin interferes with the effects of estradiol on the increase of mast cell number in short-term cultured testes, and this result has also been obtained in a dose–response experiment at physiological concentration. The data suggest that melatonin acts on mast cell number directly *via* its local action in the frog gonads. In conclusion, our study shows, for the first time, that melatonin may interfere, probably *via* estrogen receptors, with the differentiation and/or proliferation of mast cells induced by estradiol treatment either *in vivo* or *in vitro* in the testis of the frog *Rana esculenta*.

Key words: melatonin, 17β-estradiol, mast cell, testis, frog, *Rana esculenta*.

Introduction

Rare mast cells of connective tissue type are generally present in the interstitial tissue of the testis of several mammalian (Nistal et al., 1984; Gaytan et al., 1986; Mayerhofer et al., 1989) and non-mammalian species (Di Matteo et al., 1992; Minucci et al., 1995, 1997). It has been demonstrated that estrogens affect mast cell activity in several vertebrate tissues. In fact, estradiol receptors have been identified in mast cells (Vliagoftis et al., 1992; Nicovani and Rudolph, 2002), and estradiol induces histamine and serotonin secretion, in a dose-dependent manner, in purified rat mast cells when stimulated by the secretagogue compound 48/80 or substance P (Vliagoftis et al., 1992). These effects are counteracted by tamoxifen, an antiestrogen (Vliagoftis et al., 1992). In addition, after either estradiol administration or ethane dimethane sulphonate (EDS) treatment (a toxicant that specifically destroys Leydig cells; see Palmiero et al., 2003), accumulation of mast cells occurs in the testicular interstitium of neonatal rats (Gaytan et al., 1990), adult frogs (Di Matteo et al., 1992, 2000) and adult lizards (Minucci et al., 1995).

It is well established that MCF7 cells, a cell line derived from a human mammary adenocarcinoma (Hill and Blask, 1988), have high levels of estrogen receptors and that estradiol stimulates their growth, while melatonin, a pineal hormone that plays a key role in a variety of important physiological responses including reproduction (Reiter, 1991; Morgan et al., 1994), inhibits their growth. In addition, melatonin acts as an antiestrogen (Molis et al., 1994) since it inhibits the expression of estrogen-regulated genes (Molis et al., 1995), potentiates the sensitivity of MCF7 to tamoxifen (Wilson et al., 1992) and modulates the transcription of estrogen receptor in this cell line.

Recently, we have demonstrated that melatonin has a direct inhibitory effect on the basal and estradiol-stimulated mitotic activity of primary spermatogonia in the testis of the frog *Rana esculenta* (d'Istria et al., 2003). As melatonin interferes with the activation of the estrogen receptors and destabilizes the binding of the estradiol–estrogen receptor complex to the estrogen-responsive element (Rato et al., 1999), we attempt in the present paper to correlate the effects of the administration of melatonin alone or in combination with estradiol to mast cell number in the frog *Rana esculenta* testis.

Materials and methods

Adult male frogs of *Rana esculenta*, collected from the area surrounding Naples by a local dealer during the months of March, October and December 2002, were used. The frogs were maintained in plastic tanks (23 cm×16 cm×11 cm), with mealworms and water available *ad libitum*. Soon after the capture or at the end of the experimental treatments, the frogs were killed by decapitation following anesthesia with 1 g l^{-1} MS222 (Sigma, St Louis, MO, USA).

Experiment 1: in vivo *(24 h and 48 h)*

Adult males (*N*=30), collected in March (10 h:14 h L:D), were injected with either vehicle alone [100 μl of Krebs Ringer bicarbonate buffer (KRB), pH 7.4] or with 1 μg of melatonin (dissolved in 100 μl KRB). Twenty-four hours after the treatment, each group of animals (*N*=15) was further divided into three experimental groups: (1) five animals were used as a control; five animals were injected with 2 μg of estradiol (E_2; dissolved in 100 μl KRB) 24 h later and (3) five animals received two injections of E_2 (2 μg per 100 μl KRB) 24 h and 48 h later. The injections were given into the dorsal lymph sac during the scotophase.

Experiment 2: in vitro

Adult males (*N*=10), collected in October (12 h:12 h L:D), were killed during the scotophase and the excised testes were cut in half. Half-testes of five animals were incubated in tubes containing either KRB alone or KRB + E_2 (10^{-6} mol l^{-1}) for 0 h, 6 h or 12 h. Half-testes of the remaining five animals were incubated in KRB + melatonin (10^{-6} mol l^{-1}) alone or in KRB + melatonin (10^{-6} mol l^{-1}) + E_2 (10^{-6} mol l^{-1}) for the same time periods. All the tubes were placed at room temperature (20°C) in a shaking water bath.

Experiment 3: in vivo *(8 days)*

Adult males (*N*=32), collected in October (12 h:12 h L:D), were divided as follows: five animals were immediately sacrificed as an initial control; nine frogs were injected with vehicle alone (KRB) and nine frogs were injected with melatonin (1 μg per 100 μl KRB; Day 0). All animals were injected with E_2 (2 μg per 100 μl KRB) 24 h before being sacrificed and were decapitated 48 h, 72 h or 8 days after the injection of melatonin or KRB solution. An additional nine frogs that had been injected with KRB alone were used as controls at each time point (3 animals per time point). The injections were given into the dorsal lymph sac during the scotophase.

Experiment 4: in vitro *(dose–response)*

Adult males (*N*=8), collected in October (12 h:12 h L:D), were killed during the scotophase and their testes were excised. Testes of three animals were used as an initial control and were then incubated in KRB alone; the testes of the remaining five frogs were cut in half. Half-testes of each animal were incubated for 6 h in one of the following: (1) KRB + E_2 (10^{-6} mol l^{-1}), (2) KRB + melatonin (10^{-6} mol l^{-1}) + E_2 (10^{-6} mol l^{-1}), (3) KRB + melatonin (10^{-9} mol l^{-1}) + E_2 (10^{-6} mol l^{-1}) or (4) KRB + melatonin (10^{-12} mol l^{-1}) + E_2 (10^{-6} mol l^{-1}). All the incubations were carried out at room temperature (20°C) in a shaking water bath.

Histology and ultrastructure

For histological studies, the testes of each animal were fixed in Bouin's fluid, dehydrated in a graded ethanol series and cleared in xylene. Serial paraffin sections (5 μm) were stained with hematoxylin–eosin and 0.2% Toluidine Blue in Walpole buffer at pH 4.2 (Gabe, 1968) for the recognition and evaluation of mast cell number (MCN).

For the electron microscopic study, small pieces (1 mm^3) of testis were fixed for 2 h at 4°C in Karnovsky's fluid (Karnovsky, 1968) in 0.1 mol l^{-1} phosphate buffer at pH 7.4 and post-fixed in 1% osmium tetroxide in the same buffer at 4°C. Samples were dehydrated and embedded in TAAB 812 (epoxy resin–araldite 812; TAAB Lab., Berkshire, UK). Ultrathin sections were counterstained with 4% uranyl acetate followed by 1% lead citrate and observed with a Philips 301 transmission electron microscope.

Numerical and statistical analysis

For each experiment, three randomly chosen sections from each animal/experimental group were viewed under a light microscope at a magnification of 400×. The mast cell number (MCN) within interstitial tissue and the tubule numbers of the testes were counted to give a value of MCN per 100 tubules per animal. For each experiment, the groups were compared by one-way analysis of variance (ANOVA) followed by Duncan's test (at $P<0.05$ and $P<0.01$) for multi-group comparisons.

Results

Morphological observations

In the testes of control frogs of each experimental group, rare mast cells of connective tissue type were observed in the interstitial compartment. By contrast, in the interstitial tissue of the testes of frogs injected with estradiol alone, an accumulation of mast cells was observed at all time points (not shown) both *in vivo* and *in vitro*. No differences in MCN were observed at any time in the testes of animals injected with melatonin followed by E_2 *in vivo* and *in vitro* as compared with the control testes (not shown).

At the ultrastructural level, mast cells filled with many mature and non-homogeneous granules can be seen in the testis of E_2-injected animals at 24 h. The granules contain regularly arranged lamellae, which form parallel straight or curved structures. The nuclei are irregular and contain large masses of heterochromatin, particularly near the nuclear envelope. The endoplasmic reticulum and the Golgi apparatus were not visible in the cytoplasm due to the abundance of secretory granules (Minucci et al., 1997).

Experiment 1: in vivo *(24 h and 48 h)*

Testes of March frogs injected with E_2 showed a significant increase ($P<0.01$) in MCN either at 24 h or 48 h compared with the untreated control testes (Fig. 1). No significant differences were found in the testes of animals injected with melatonin alone and melatonin plus E_2 at either 24 h or 48 h compared with those of untreated control testes (Fig. 1).

Experiment 2: in vitro

Testes of October frogs incubated for 6 h or 12 h in KRB containing E_2 alone showed a significant increase in MCN

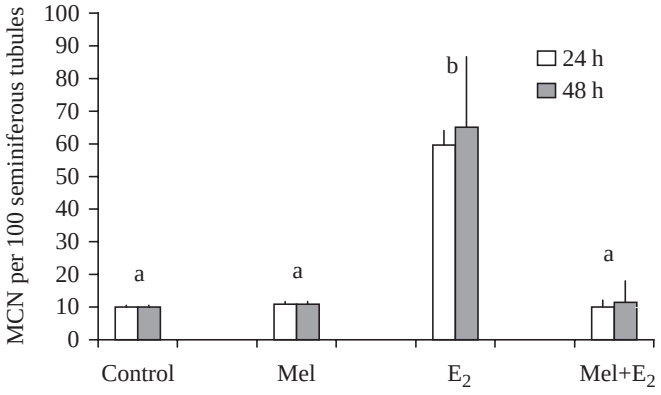

Fig. 1. *In vivo* effect of estradiol (E_2), E_2 administered in combination with melatonin, and melatonin (mel) alone on mast cell number (MCN) after 24 h or 48 h of treatment in testis of frogs captured during March. Values are expressed as means ± S.D. Different letters (a *versus* b) indicate significance at $P<0.01$.

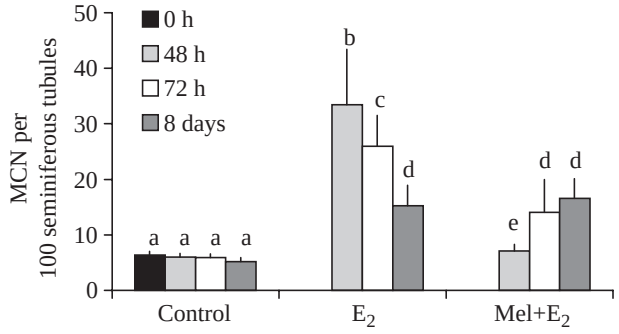

Fig. 3. *In vivo* effect of estradiol (E_2) and E_2 administered in combination with melatonin (mel) on mast cell number (MCN) in the frog *Rana esculenta*. All the animals were injected with E_2 24 h before being sacrificed and were killed at 48 h, 72 h and on day 8 after the injection of melatonin or Krebs Ringer bicarbonate (KRB) buffer (control). Values are expressed as means ± S.D. Different letters indicate significance (a *versus* b, c, d, $P<0.01$; b *versus* d, e, $P<0.01$; b *versus* c, $P<0.05$; c *versus* d, e, $P<0.01$).

compared with untreated control testes (Fig. 2; $P<0.01$). No differences were found between the MCN of the testes incubated in melatonin alone or in melatonin plus E_2 and the untreated control group either at 6 h or 12 h (Fig. 2).

Experiment 3: in vivo *(8 days)*

Testes of frogs treated with E_2 during October showed a significant increase ($P<0.01$) in MCN compared with the untreated initial control testes at all time points (Fig. 3). In addition, testes of animals sacrificed at 48 h showed a higher MCN compared with those observed in the testes of animals sacrificed at 72 h ($P<0.05$) and on day 8 ($P<0.01$).

Testes of animals injected on day 0 with melatonin and at 24 h before the sacrifice with E_2 showed a significant increase in MCN at all time points. The increase was more pronounced at 72 h and on day 8 (Fig. 3; $P<0.01$) compared with the value observed in the testes of untreated animals.

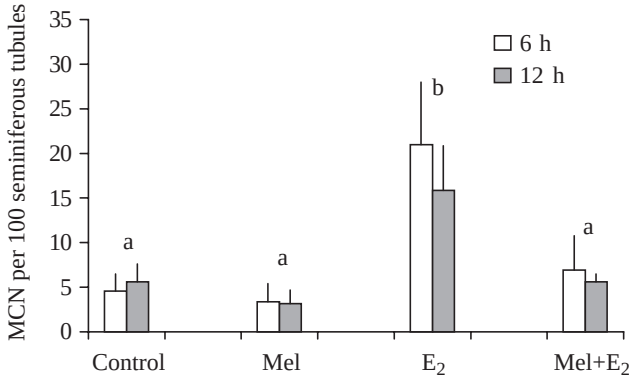

Fig. 2. *In vitro* effect of estradiol (E_2; 10^{-6} mol l^{-1}), E_2 given in combination with melatonin (mel; 10^{-6} mol l^{-1}), and melatonin alone (10^{-6} mol l^{-1}) on mast cell number (MCN) after 6 h or 12 h of incubation in testis of frogs captured in October. Values are expressed as means ± S.D. Different letters (a *versus* b) indicate significance at $P<0.01$.

Interestingly, in the testes of frogs injected on day 0 with melatonin and then with E_2 at 48 h and 72 h (24 h before the sacrifice), MCN is lower than that observed in the testes of frogs injected with E_2 alone at the same time points (Fig. 3; $P<0.01$). No differences in MCN were observed in the testes of frogs injected on day 0 with melatonin and then with E_2 and sacrificed on day 8, as compared with MCN observed in the testes of frogs injected with E_2 alone at the same time point (Fig. 3).

Experiment 4: in vitro *(dose–response)*

Testes of October frogs incubated for 6 h in KRB containing E_2 alone showed a significant increase in MCN compared with that observed in the control testes ($P<0.01$; Fig. 4), while no significant differences were found in the testes of animals incubated in E_2 plus melatonin at any of the concentrations used (10^{-6} mol l^{-1}, 10^{-9} mol l^{-1} and 10^{-12} mol l^{-1}) compared with the values observed in the untreated control testes (Fig. 4).

Discussion

Mast cells occur in all classes of vertebrates (Galli, 1990) and are the only cells that possess high-affinity receptors for IgE and synthesize histamine (Galli, 1990; Scharenberg and Kinet, 1995). It has been demonstrated that estrogen treatment is effective in inducing mast cell proliferation in different vertebrate tissues (Gibbons and Chang, 1972; Gaytan et al., 1986; Mohanty and Chainy, 1992). Although the mechanism controlling changes in mast cell number (MCN) is still unclear, it is generally accepted that estradiol acts on mast cells (Modat et al., 1982; Krishna and Terranova, 1985). Indeed, estrogens have been shown to affect mast cell secretion (Shelesnyak, 1959; Conrad and Feigen, 1974; Krishna and Terranova, 1985; Slater and Kaliner, 1987); estradiol increases histamine and serotonin secretion and this effect is counteracted by tamoxifen (Vliagoftis et al., 1992).

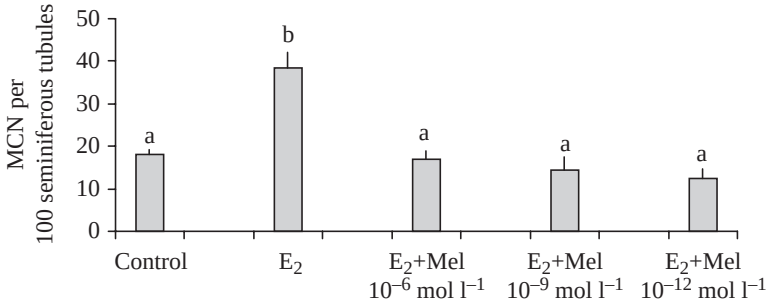

Fig. 4. *In vitro* effect of estradiol (E_2; 10^{-6} mol l^{-1}) and E_2 given in combination with melatonin (mel; 10^{-6} mol l^{-1}, 10^{-9} mol l^{-1} or 10^{-12} mol l^{-1}) on the mast cell number after 6 h of incubation in testis of frogs captured in October. Vertical bars indicate means ± S.D. Different letters (a *versus* b) indicate significance at $P<0.01$.

In amphibians, mast cells of a connective tissue type have been characterized at the histochemical, biochemical and ultrastructural levels in different tissues of the frog *Rana esculenta* (Chieffi Baccari et al., 1998). In particular, the ultrastructure of their cytoplasmic granules is unique and totally unlike any other previously described granules in other animal species (Chieffi Baccari et al., 1998). It was previously shown that, in the testis of *Rana esculenta*, 17β-estradiol increases MCN *in vivo* and *in vitro*, while testosterone has no effect (Minucci et al., 1997; Di Matteo et al., 2000). Accumulation of mast cells after estradiol treatment was also observed in the testis of the lizard *Podarcis s. sicula* Raf (Minucci et al., 1995) and in the testicular interstitium of neonatal rats (Gaytan et al., 1990). Estradiol probably acts on the multiplication and/or differentiation of mast cells through its direct action on the putative precursor of testicular mast cells and/or by stimulating growth factors (Minucci et al., 1997).

In the present study, we confirm that estradiol treatment increases MCN in the frog testis and, for the first time, we found that melatonin has an inhibitory effect on estradiol-stimulated MCN *in vivo* and *in vitro*. Several studies have demonstrated the antiproliferative role of melatonin using an MCF7 cell model to study the anti-estrogenic effect of this hormone (Hill and Blask, 1988; Wilson et al., 1992; Molis et al., 1994, 1995; Lissoni et al., 1995). Estradiol was used in the present study to induce MCN increase in the testis to verify the effect(s) of melatonin on mast cell accumulation. The results obtained from the *in vivo* experiments confirm that administration of estradiol increases the MCN in the frog testis within both 24 h and 48 h of treatment and indicates an inhibitory role for melatonin on mast cell accumulation induced by estradiol. Interestingly, the inhibitory effect almost disappears at 72 h and is not present on day 8 after melatonin injection, indicating that it is reversible. In addition, testis pieces incubated with estradiol for 6 h showed increased MCN, and this effect is counteracted by melatonin at 10^{-6} mol l^{-1} and was confirmed at a physiological concentration (10^{-12} mol l^{-1}).

The inhibitory effect exerted by melatonin on mast cell accumulation could be explained either through a mechanism involving the hypothalamus–pituitary–gonadal axis or directly *via* the local action of indoleamine on the frog gonads. Although it is now obvious that melatonin prevents the estrogen-induced MCN increase, more data are needed to verify the mechanism of its action. Bearing in mind that melatonin interferes with the activation of the estrogen receptor, destabilizing the binding of the estradiol–estrogen receptor complex to the estrogen-responsive element (Rato et al., 1999), and that the expression of estradiol receptors have been demonstrated in mast cells (Zhao et al., 2002; Nicovani and Rudolph, 2002), we suggest that melatonin may be involved in mast cell differentiation and/or proliferation induced by estradiol treatment *via* estrogen receptors. An alternative hypothesis of melatonin action on mast cells comes from the identification of a melatonin receptor transcript in the RBL-2H3 line of rat mast cells (Chen et al., 1998). This result strongly suggests a direct action of melatonin on mast cells *via* its own receptor. This is consistent with a recent report demonstrating a direct inhibitory effect of melatonin on the basal and estradiol-induced mitotic activity of primary spermatogonia in the frog testis (d'Istria et al., 2003); the results support the hypothesis that melatonin exerts the inhibitory effect *via* its local action on the frog gonads (d'Istria et al., 2003).

In conclusion, the results of the present study validate the accumulation of mast cells following estradiol treatment and show, for the first time, that melatonin interacts with the proliferation and/or differentiation of mast cells induced by estradiol in the testis of the frog *Rana esculenta* either *in vivo* or *in vitro*.

The authors thank F. Iamunno of the Stazione Zoologica 'A. Dohrn' (Naples, Italy) for his technical assistance and J. Gates for the revision of the manuscript. This work was supported by the MIUR (ex 60% "Second University of Naples") grant.

References

Chen, H., Centola, M., Altschul, S. F. and Metzger, H. (1998). Characterization of gene expression in resting and activated mast cells. *J. Exp. Med.* **188**, 1657-1668. (Erratum: *J. Exp. Med.* **188**, 2387.)

Chieffi Baccari, G., de Paulis, A., Di Matteo, L., Gentile, M., Marone, G. and Minucci, S. (1998). In situ characterization of mast cells in the frog (*Rana esculenta*). *Cell. Tissue Res*. **292**, 151-162.

Conrad, M. J. and Feigen, G. A. (1974). Sex hormones and kinetics of anaphylactic histamine release. *Physiol. Chem. Phys*. **6**, 11-16.

d'Istria, M., Palmiero, C., Serino, I., Izzo, G. and Minucci, S. (2003). Inhibition of the basal and oestradiol-stimulated mitotic activity of primary spermatogonia by melatonin in the testis of the frog, (*Rana esculenta*), *in vivo* and *in vitro*. *Reproduction* **126**, 83-90.

Di Matteo, L., Chieffi Baccari, G., Marmorino, C., Minucci, S. and Pierantoni, R. (1992). Leydig–mast cell communication in the testis of the frog (*Rana esculenta*). *Anim. Biol.* **1**, 163-168.

Di Matteo, L., Izzo Vitiello, I. and Minucci, S. (2000). Effects of sex steroid

hormones and their antagonists on mast cell number in the testis of the frog (*Rana esculenta*). *Zygote* **8**, 225-234.

Gabe, M. (1968). Detection de la basophilie dans un but histochemique. In *Tecniques Histologiques* (ed. M. Gaba), pp. 351-353. Paris: Masson.

Galli, S. J. (1990). New insights into the riddle of the mast cells: microenvironmental regulation of mast cell development and phenotypic heterogeneity. *Lab. Invest*. **62**, 5-33.

Gaytan, F., Bellido, C., Carrera, G. and Aguilar, E. (1990). Differentiation of mast cells during postnatal development of neonatally estrogen-treated rats. *Cell Tissue Res.* **259**, 25-31.

Gaytan, F., Bellido, C., Lucena, M. C. and Paniagua, R. (1986). Increased mast cell number in the testis of neonatally estrogenized rats. *Arch. Androl.* **16**, 175-181.

Gibbons, A. F. E. and Chang, M. C. (1972). Number of mast cells in the rat uterus with special reference to its relation to hormonal treatment and decidual response. *Biol. Reprod*. **6**, 193-203.

Hill, S. M. and Blask, D. E. (1988). Effects of the pineal gland hormone melatonin on the proliferation and morphological characteristics of human breast cancer cells (MCF-7) in culture. *Cancer Res.* **48**, 6121-6126.

Karnovsky, M. J. (1968). Ultrastructural basis of transcapillar exchanges. *J. Gen. Physiol.* **52**, Suppl. 95s.

Krishna, A. and Terranova, P. F. (1985). Alterations in mast cell degranulation and ovarian histamine in the proestrus hamster. *Biol. Reprod*. **32**, 1211-1217.

Lissoni, P., Barni, S., Meregalli, S., Fossati, V., Cazzaniga, M., Esposti, D. and Tancini, G. (1995). Modulation of cancer endocrine therapy by melatonin: a phase II study of tamoxifen plus melatonin in metastatic breast cancer patients progressing under tamoxifen alone. *Br. J. Cancer*. **71**, 854-856.

Mayerhofer, A., Bartke, A., Amador, A. G. and Began, T. (1989). Histamine affects testicular steroid production in the golden hamster. *Endocrinology* **125**, 560-562.

Minucci, S., Di Matteo, L., Chieffi, P., Pierantoni, R. and Fasano, S. (1997). 17β-Estradiol effects on mast cell number and spermatogonial mitotic index in the testis of the frog (*Rana esculenta*). *J. Exp. Zool*. **278**, 93-100.

Minucci, S., Izzo Vitiello, I., Marmorino, C., Di Matteo, L. and Chieffi Baccari, G. (1995). Mast cell–Leydig cell relationships in the testis of the lizard (*Podarcis s. sicula* Raf): thermal manipulation, ethane 1,2-dimethane sulphonate (EDS) and sex hormone treatment. *Zygote* **3**, 259-264.

Modat, G., Bombarek, A. and Lanaure, M. (1982). Variations quantitatives du nombre et de la taille des mastocytes peritoneaux chez la ratte au cours du cycle sexual normal, apres ovariectomie et administration d'oestrogenes. *C. R. Soc. Biol*. **176**, 675-681.

Mohanty, N. and Chainy, G. B. N. (1992). Effects of estradiol valerate on the uterus of the musk shrew (*Suncus murinus* L). *Gen. Comp. Endocrinol*. **88**, 91-99.

Molis, T. M., Spriggs, L. L. and Hill, S. M. (1994). Modulation of estrogen receptor mRNA expression by melatonin in MCF-7 human breast cancer cells. *Mol. Endocrinol.* **8**, 1681-1690.

Molis, T. M., Spriggs, L. L., Jupiter, Y. and Hill, S. M. (1995). Melatonin modulation of estrogen-regulated protein growth factors and proto-oncogenes in human breast cancer. *J. Pineal Res.* **18**, 93-103.

Morgan, P. J., Barrett, P., Howell, H. E. and Helliwell, R. (1994). Melatonin receptors: localization, molecular pharmacology and physiological significance. *Neurochem. Int.* **24**, 101-146.

Nicovani, S. and Rudolph, M. I. (2002). Estrogen receptors in mast cells from arterial walls. *Biocell* **26**, 15-24.

Nistal, M., Santamaria, L. and Paniagua, R. (1984). Mast cells in the human testis and epididymis from birth to adulthood. *Acta Anat.* **119**, 155-160.

Palmiero, C., Ferrara, D., De Rienzo, G., d'Istria, M. and Minucci, S. (2003). Ethane 1,2-dimethane sulphonate is a useful tool for studying cell-to-cell interactions in the testis of the frog, *Rana esculenta. Gen. Comp. Endocrinol.* **131**, 38-47.

Rato, A. G., Pedrero, J. G., Martinez, M. A., del Rio, B., Lazo, P. S. and Ramos, S. (1999). Melatonin blocks the activation of estrogen receptor for DNA binding. *FASEB J.* **13**, 857-868.

Reiter, R. J. (1991). Pineal melatonin: cell biology of its synthesis and of its physiological interaction. *Endocrine Rev*. **12**, 151-180.

Scharenberg, A. M. and Kinet, J. P. (1995). Early events in mast cell signal transduction. In *Human Basophils and Mast Cells: Biological Aspects* (ed. G. Marone), pp. 72-87. Basel: Karger.

Shelesnyak, M. C. (1959). Histamine releasing activity of natural estrogens. *Proc. Soc. Exp. Biol. Med.* **100**, 739-741.

Slater, J. E. and Kaliner, M. (1987). Effects of sex hormones on basophil histamine release in recurrent idiopathic anaphylaxis. *J. Allergy Clin. Immunol*. **80**, 285-290.

Vliagoftis, H., Dimitriadou, V., Boucher, W. M., Rozniecki, J. J., Correia, I., Raam, S. and Theoharides, T. C. (1992). Estradiol augments while tamoxifen inhibits rat mast cell secretion. *Int. Arch. Allergy Immunol.* **98**, 398-409.

Wilson, S. T., Blask, D. E. and Lemus-Wilson, A. M. (1992). Melatonin augments the sensitivity of MCF-7 human breast cancer cells to tamoxifen *in vitro*. *J. Clin. Endocrinol. Metab*. **75**, 669-670.

Zhao, H., Pang, S. F. and Poon, A. M. S. (2002). MT1 receptor-mediated antiproliferative effects of melatonin on the rat uterine antimesometrial stromal cells. *Mol. Reprod. Dev*. **61**, 192-199.