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Functional role of melatonin involved in the mechanism of androgen action in LNCaP cells

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Abstract

The present experiments were performed to verify whether the melatonin nuclear receptor (RORα/RZRα) might be expressed and whether melatonin might modulate the production of prostate-specific antigen (PSA) in androgen-dependent prostate cancer cells (LNCaP cells), and to obtain information on the possible mechanisms of melatonin action. The melatonin nuclear receptor RORα/RZRα was expressed in LNCaP cells. Dihydrotestosterone (DHT) significantly enhanced PSA production, and DHT-induced PSA increase was completely abolished by melatonin. However, melatonin at doses used did not influence cell proliferation. The effect of melatonin on the expression of androgen receptor (AR) was less pronounced, though insulin-like growth factor-1 receptor was significant. The present results indicated that melatonin inhibits a mechanism involving PSA production rather than AR expression and cell proliferation. Thus, it seems very likely that melatonin at higher doses is involved in the reduction of androgen-response gene expression via its nuclear receptors, while at lower doses it is involved in the inhibition of cell proliferation via membrane receptors.

Introduction

Melatonin, secreted mainly by the pineal gland, is known to be involved in the regulation of many physiological functions, including circadian and seasonal rhythms, sleep, sexual maturation, and reproduction[1)2)3)]. In addition to well-defined neurobiological effects of melatonin on the brain and the hypothalamo-pituitary axis, radio-receptor binding studies performed in the past decade have demonstrated specific functional melatonin receptors in many peripheral mammalian tissues and cells, suggesting some as yet unidentified, but possibly significant actions of melatonin on the cell biology of these tissues outside the central nervous system[4)5)]. In the last 10 years, it has been observed that melatonin may also exert direct regulatory influences on neoplastic growth in a number of experimental models, particularly of prostate cancer[6)7)8)9)]. The signal transduction pathways which convey the oncostatic message of melatonin on the tumor cells are still unclear. At least two affinity cell surface receptors (Mella, Mellb)[10)11)] and a putative nuclear receptor[12)] have been identified for this hormone. The putative nuclear receptors, RORα/RZRα and RORβ/RZRβ, belong to the orphan receptor family and have been shown to share sequence homology with the retinoid acid receptor RXR[12)13)14)]. RORβ/RZRβ is known to be a brain specific isoform, while RORα/RZRα is expressed in many peripheral tissues and cells[12)13)15)16)].

Prostate specific antigen (PSA) is a 33-kDa serine protease produced at high concentrations by prostate epithelial cells and secreted into the seminal fluids. It is well known as a prostate tumor maker[17)]. PSA production in the prostate is regulated by androgen through the action of AR. The human prostate cancer cell line LNCaP cells also produced PSA in response to adminis-

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tered androgens[18]. PSA cleaves a trimetric complex composed of insulin-like growth factor 1 (IGF-1), IGF binding protein-3 (IGBP-3) and liver-derived glycoprotein, and leads to liberation of more IGF-1 from the complex. IGF-1, mediated through its receptor (IGF-1 R), acting as a mitogenic peptide in the regulation of cell proliferation, differentiation and apoptosis both in normal and cancer prostate cells[19][20]. Tennant et al[21] found that IGBP-3 decreased in prostate cancer tissue. This change at the tissue level helps to enhance the IGF-1 action. IGF-1 stimulates DNA synthesis in LNCaP cells, either alone or in conjunction with androgen[22]. LNCaP cells are target cells for androgen and IGF-1, but the interaction with their factors, and the mechanisms involved in PSA synthesis and cell proliferation are still incompletely understood.

In the first experiment, we attempted to determine whether the RORα/RZRα receptor is expressed in LNCaP cells, using reverse transcription-polymerase chain reaction method (RT-PCR), since RT-PCR offers a potentially more sensitive assay for the detection of cells expressing RORα/RZRα mRNA in prostate cancer cells. The second experiment was performed to clarify the effects of melatonin on the production of PSA in LNCaP cells subjected to androgen. An androgen action in androgen-response cells is known to be mediated through the AR, which regulates the expression of androgen-response genes, leading to morphological and physiological changes including PSA synthesis. Therefore, it is conceivable that melatonin plays important roles in controlling the expression of AR. The third experiment was designed to investigate the influence of exposure to melatonin on the expression of AR and IGF-1 receptor (IGF-1R) proteins, using Western-blotting and immuno-histochemical methods.

Materials and Methods

Chemicals

Melatonin (N-Acetyl-5-methoxytryptamine) and dihydrotestosterone (5α-Androstane-17β-OL-one= DHT) were obtained from Sigma Chemical Co (St. Louis, MO, USA), and dissolved in absolute ethanol as a 1.0 mM stock.

Cell culture

The human prostate cartinoma cell line, LNCaP cells (ATCC No : CRL-1740), which express AR and produces prostate-specific antigen (PSA) was obtained from the American Type Culture Collection (Rockville, MD, USA). They were maintained in RPMI-1640 medium (Gibco BRL, Grand Island, NY, USA) supplemented with 5% heat-inactivated fetal bovine serum (FBS) (Gibco BRL), 2 ml L-glutamine, antibiotics (100 μg/ml kanamycin, 10 μg/ml gentamicin ; Sigma Chemical). Cells were maintained in humidified air : CO_2 atmosphere (95% : 5%) at 37°C, and were subcultured twice weekly.

RORα_1/RZRα_1 gene expression analysis

In the first part of our molecular analysis of the RORα_1/RZRα_1 gene regulation, we used the RT-PCR technique to verify whether RORα_1/RZRα_1 gene was expressed in LNCaP cells.

Cells were cultured under standard conditions and, when subconfluent, detached by trypsinization from the culture plates. They were washed with phosphate buffered saline (PBS) and cell plates were immediately frozen in liquid nitrogen and stored at −80°C. Total RNA was extracted using TRI zol RNA isolation solvent (Gibco BRL) according to the manufacturer's recommendations. The quality of the extracted RNA was checked by spectrophotometric measurements at 260 and 280 nm.

An aliquot of 1 μg of total RNA was reverse transcripted to cDNA using First-Strand-Beads (Amersham Pharmacia Biotech, Washington, DC, USA). Polymerase chain reaction with RORα_1/RZRα_1 primer yield a 430 bp (1117-1606) fragment which is specific for the human RORα_1/RZRα_1 cDNA coding region[23]. The sequence of primers was as follows.

Forward primer : 5′-GTTCTCTAGAGGTGGTGTTT-3′

Reverse primer : 5′-CCAGACATTGTGCGACTTCA-3′

Polymerase chain reaction with the above primers was performed using RTG PCR Beads (Amersham Pharmacia Biotech). The RT product was used for amplification with PCR. PCR was performed for a total of 30 cycles and each cycle lasted for 30 sec at 95°C, 30 sec at 37°C, and 1 min at 72°C. Aliquots of the reaction product were electrophoresed on ethidium bromide-stained 1.8% agarose gel and viewed under ultraviolet light. The nucleotide sequence of RT-PCR products was confirmed by dideoxy chain termination sequencing.

Cell growth

Growth of LNCaP cells was tested by plating out the cells at an initial density of 10^5 cells/ml of medium supplemented with FBS at concentrations ranging from 0% to 10%. Afterward, the cells were incubated in medium containing 0.1% FBS in the presence of DHT (from 10^{-11} to 10^{-6} M) and melatonin (10^{-7} to 10^{-3} M) for 4 days. Melatonin was added daily to the medium. In all the culture plates, the final ethanol concentration never exceeded 0.1%. At the end of the treatment, cells were harvested and DNA levels of these cells were determined by CYQUANT Cell Proliferation Assay Kit (Molecular Probes, OR, USA).

PSA determination

LNCaP cells were pre-incubated into steroid-free medium supplemented with 5% FBS prior to the start of all experiments. Cells were then plated in 12-well culture plates (2×10^5 cells/well) in 2 ml of the medium consisting of 0.1 % FBS. After a 72-h attachment period the vehicle (ethanol) or DHT at concentration of 10^{-9} M and /or melatonin at concentrations of 10^{-7}–10^{-3} M were added to triplicate wells. Parallel control plates (without drugs) were run in each experiment. Medium and treatments were changed every day. After 4 days treatment, the cells were removed. The PSA concentrations in these mediums were measured using an E-Plate PSA Kit (Eiken Chem., Tokyo, Japan) according to the manufacturer's recommendations. The lowest limit of detection was 0.1 ng/ml. Nuclear DNA levels in each well were determined as described above.

Western blotting

Western blot analysis of LNCaP cells was performed on nuclear proteins. Cells were harvested with strong pipetting and homogenized in RIPA buffer (50 mM Tris-HCl, 150 mM NaCl, 1% NP-40, 1% DCA-Na, 0.05% SDS) containing protein inhibitors (Complete mini ; Roche, Basel, Switzerland). The homogenates were centrifuged for 10 min at 15,000 rpm at room temperature. Nuclear proteins were extracted from nuclei in warm (95°C) Laemmli sample buffer (Bio-Rad, Hercules, CA, USA) containing 5% βME for 3 min immediately before loading. Tubes were then centrifuged for 10 min at 12,000 rpm at room temperature. The supernatant, containing nuclear proteins was stored at −80°C until use.

Nuclear proteins in the supernatant were assayed by Protein Assay Reagent Concentrate (Bio-Rad). Samples (50 μg nuclear protein per lane) were loaded on a SDS-polysuccharide gel (Ready Gels J ; Bio-Rad) and run for 30 min at 150 mA. After electrophoresis, gels were blotted onto Immobilon-P membranes (Millipore, MA, USA) for immunoblot analysis.

Non-specific binding sites of membranes were blocked with 5% skimmed milk (Gibco BRL) in TBST buffer (50 mM Tris pH 7.5, 150 mM NaCl, 1% Tween 20) overnight at 4°C. Membranes were incubated with the primary AR and IGF-1 Rβ antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 1 : 500–2,000 dilutions in blocking solution overnight at 4°C, then with the peroxidase-labeled second antibody (Amersham Life Science) in TBST buffer for 30 min at room temperature. Each of these two steps was followed by a 15 min wash in TBST containing 0.1% Tween 20. Detection was performed with the Lumi-Light^{PLUS} (Roche) and exposed to Polaroid T667 film for times ranging from 10 min to 4 hours.

Immunohistochemical studies of androgen receptor protein

Treated or untreated control cells were cultured on 2 well chamber slides (Lab-Tek, Nalge Nunc International, Vernon Hill, IL, USA). The slides were fixed in 4.0% formaldehyde in phosphate buffered saline for 20 min and permeabilized in 0.2% Triton X-100 (Sigma) for 4 min. The slides were then incubated with 0.6% H_2O_2 (Wako Pure Chemical Industries Ltd. Osaka, Japan) in 80% methanol for 15 min to eliminate endogenous peroxidase and blocked with 10% bovine serum albumin (Sigma) in 80% methanol for 30 min. The slide were soaked in rabbit polyclonal AR antibody (Santa Cruz Biotechnology) overnight at 4°C. In negative controls, the primary antibody was omitted and replaced by 4% BSA in PBS. The slides were then covered with second antibody, horseradish peroxidase linked anti-rabbit Ig (Amersham Life Science) for 30 min. After washing, cell areas were covered with 0.7 mg/ml diamino-benzidine-1.6 mg/ml H_2O_2 (Fast DAB tablet sets ; Sigma) as chromogen for 10 min in the dark. After sequential dehydration with ethanol and cleaning with xyrol, the slides were mounted with MP 500 (Matsunami, Tokyo, Japan) and photographed at a 400-fold magnification.

Statistical analysis

The statistical analysis of differences between groups was assessed by one-factor ANOVA.

Results

Cell morphology

Analysis on cell morphology by phase-contrast microscopy showed that after 4 days in culture, the treatment of DHT and melatonin caused some changes in cell shape. Compared to controls, 10^{-9} M DHT-treated LNCaP cells displayed mainly small processes. In 10^{-3} M melatonin-treated cells subjected to 10^{-9} M DHT, a large number of cells became rounded and eventually detached from culture flasks. Loss of adherence of cells that normally attach to tissue-culture dishes has been shown to reflect the onset of apoptosis[24]. After 4 days in culture, the percentage of floating cells was increased in both control and hormone-treated cells. No cytotoxicity could be demonstrated at concentrations up to 10^{-3} M melatonin (data not shown).

Cell proliferation

10^{-9} M of DHT slightly increased in DNA levels (120%), when cells were incubated in medium subjected to 0.1% FBS for 4 days. Melatonin at concentrations of 10^{-11}–10^{-5} M had no effects on DNA concentrations in LNCaP cells incubated in medium containing 0.1% FBS for 4 days. When the cells were cultured at the phar-

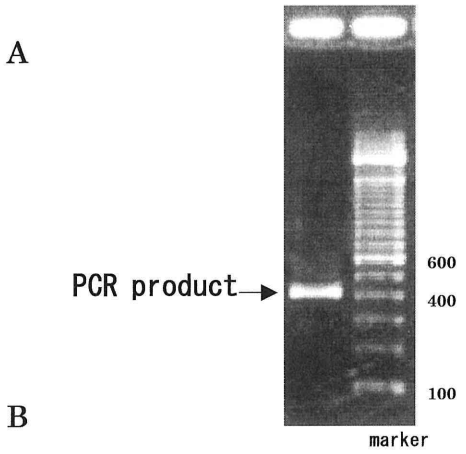
macological concentrations of melatonin (10^{-4} and 10^{-3} M), DNA levels were slightly reduced to about 90% of that in the vehicle-treated control group. However, significant differences between control and hormone-treated groups could not be observed in the present experimental conditions (data not shown).

Detection of human RORα_1/RZRα_1 expression by RT-PCR

Reverse transcription-PCR of DNA extract from LNCaP cells yielded a product of approximately 430 bases which was of predicted size based on the published cDNA sequence for human RORα_1/RZRα_1 (1177-1606)[23]. Sequence analysis of the LNCaP cell product of the generated partial sequence of 430 bases was 88% homologue to human RORα_1/RZRα_1 in the region from bases 1177-1606, from which we conclude that a homologous of RORα_1/RZRα_1 is most likely expressed at the RNA level in the prostate cancer LNCaP cell (Fig. 1).

PSA concentrations

The effects of DHT on the production of PSA in LNCaP cells are presented in Fig. 2. DHT increased in PSA concentrations in a dose-dependent manner up to a concentration of 10^{-9} M. The levels reached a plateau of 300% at the concentrations between 10^{-9} M and 10^{-7} M. Inhibitory effects of melatonin on PSA concentrations in LNCaP cells cultured in medium containing 0.1% FBS for 4 days are shown in Fig. 3. Melatonin inhibited PSA concentration in a dose-dependent manner up to a concentration of 10^{-3} M, but significant differences were observed in cells treated with higher doses of melatonin (10^{-4} M and 10^{-3} M) as compared with vehicle-treated controls. PSA concentrations in cells incubated for 4 days with increasing doses of melatonin (10^{-7} M-10^{-3} M) in the presence of a fixed dose of DHT (10^{-9} M) are presented in Fig. 4. Melatonin markedly inhibited PSA production from LNCaP cells grown with DHT in dose-dependent

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hROR α  1177: gttctctaga ggtggtgttt atcaaatgtg  ccgtgccttt tgactctcag aacaacaccg
LNCaP      1 : gtnctcnang gtaggtgtnn nnnnnnnnnn  nnnggntttc tgactctcag ancancaccg

           1237: tgtactttga tgggaagtat gccagccccg acgtcttcaa tccttaggtt gtgaagactt
             61: tgtantctga tgggaagtat gccacccccg angtcttcaa tccttaggtt gtgaaaactn

           1297: tattagcttt gtgtttgaaa tttggaaaga gtttatgttc tatgcacctg actgaagatg
            121: tattancttt gtgtntgaaa tntggaaana gtttatgttc tatgcnnctg actgaagatg

           1357: aaattgcatt attttctgca tttgtactga tgtcagcaga tcgctcatgg ctgcaagaaa
            181: aaattgcatt attttctgca titgtactga tgtcagcaaa tcnctcatgg ctgcaacaaa

           1417: aggtaaaaat tgaaaaactg caacagaaaa ttcagctagc tcttcaacac gtcctacaga
            241: aggtaaaaat ngaaaaactg caacagaaaa ttcagctanc tcttctgcac gtcctacaga

           1477: agaatcaccg agaagatgga atactaacaa agttaatatg caaggtgtct acattaagag
            301: agaatcnccn anaagatgga atacnaacaa ngttcatntg ccaggtgtct acattaagag

           1537: ccttatgtgg acgacataca gaaaagctaa tggcatttaa agcaatatac ccagacattg
            361: ccttaagtng acgacataca gaaaagctca tggcatttcc ancaatatac ccngacattg

           1597: tgcgacttca
            421: tgcgacttca
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Fig. 1 (A) Agarose gel electrophoresis of RT-PCR products from LNCaP cells generated with the primers hRORα/RZRα (1177-1196) and hRORα/RZRα (1606-1585) (sequences given below). (B) Aligment of the partial cDNA sequence obtained from the LNCaP cell PCR product with that for hRORα/RZRα (1177-1606).

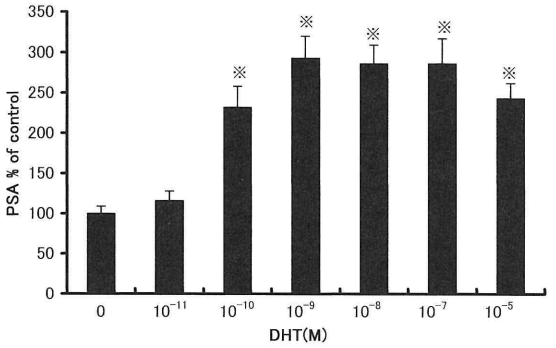

Fig. 2 Effects of DHT on PSA production. Cells were incubated in medium containing 0.1% FBS in the presence of DHT for 4 days. The columns represent the mean of the PSA levels in triplicate wells from two separate experiments. The PSA levels are expressed as percentages of the mean concentration in the control wells. Values are expressed as mean±S.E.M. *$p<0.01$ compared with the nontreated control group.

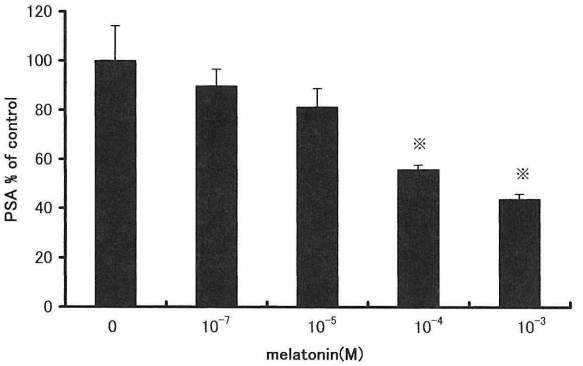

Fig. 3 Effects of melatonin on PSA production. Cells were incubated in medium containing 0.1% FBS in the presence of melatonin for 4 days. The columns represent the mean of the PSA levels in triplicate wells from two separate experiments. The PSA levels are expressed as percentages of the mean concentration in the control wells. Values are expressed as mean±S.E.M. *$p<0.01$ compared with nontreated control group.

manner; production decreased by 50% and 80% at 10^{-4} M and 10^{-3} M, respectively. At the other doses, the inhibition by melatonin was less pronounced.

Western blot analysis

Influences of DHT and/or melatonin on the expression of AR and IGF-1 R proteins are shown in Figs. 5 and 6. LNCaP cells were cultured in medium containing 0.1% FBS in the presence or absence of DHT (10^{-9} M) and/or melatonin (10^{-3} M) for 4 days. The expression of AR in cells treated with DHT increased by about 300% as compared with that in vehicle-treated controls. Melatonin had no effect on the AR expression. Melatonin also did not affect the AR expression induced by DHT administration. The expression in

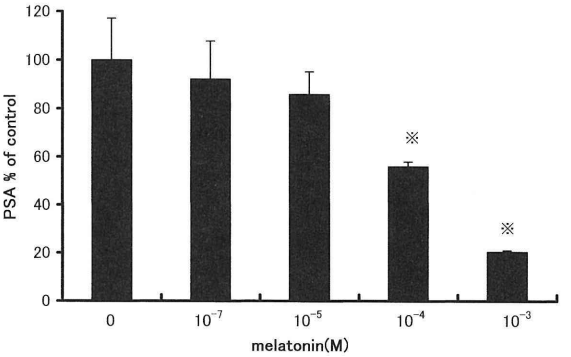

Fig. 4 Effects of melatonin on PSA production in LNCaP cells treated with 10^{-9} M of DHT. Cells were incubated in medium containing 0.1 % FBS in the presence of various doses of melatonin for 4 days. The columns represent the mean of the PSA levels in triplicate wells from two separated experiments. The PSA levels are expressed as percentages of the mean concentration in the control wells. Values are expressed as mean±S.E.M. *$p<0.01$ compared with DHT (10^{-9} M)-treated group.

cells cultured in 5% FBS was higher than that in 0.1% FBS, suggesting that starvation inhibits the expression of AR. As shown in Fig. 6, the expression of IGF-1 R in cells cultured in 0.1% was double that in 5%. This result showed that starvation results in the over expression of IGF-1 R as opposed to that of AR. DHT stimulated the expression of IGF-1 R in cells cultured in 0.1% FBS. Melatonin had more effect on its expression than that of DHT alone.

Immunohistochemical study of AR

Immunohistochemical studies of androgen receptor protein in LNCaP cells are shown in Fig. 7. In untreated cells, about 75% of cells were negative (Fig. 7a). About 50% of cells were positive in cells subjected to melatonin (Fig. 7b). In cells treated with DHT for 4 days, about 75 % of cells were positive and nuclei were firmly stained (Fig. 7c). In DHT and melatonin-treated cells, most cells were positive and the intensity of staining in the nucleus was strong (Fig. 7d).

Discussion

Lupowits and Zisapel[25] have demonstrated that melatonin may inhibit the growth of LNCaP prostate cancer cells in a dose-dependent manner. However, its activity seems to be transient, disappearing after 24 h of treatment. At partial variance with those data, Moretti et al[26] have reported that melatonin added for 9 consecutive days inhibits the proliferation of this cell line, only when utilized at physiological concentrations. These phenomena were also observed in a variety of neoplasms, most notably breast cancer. It has been shown that melatonin inhibits the proliferation of MCF-7

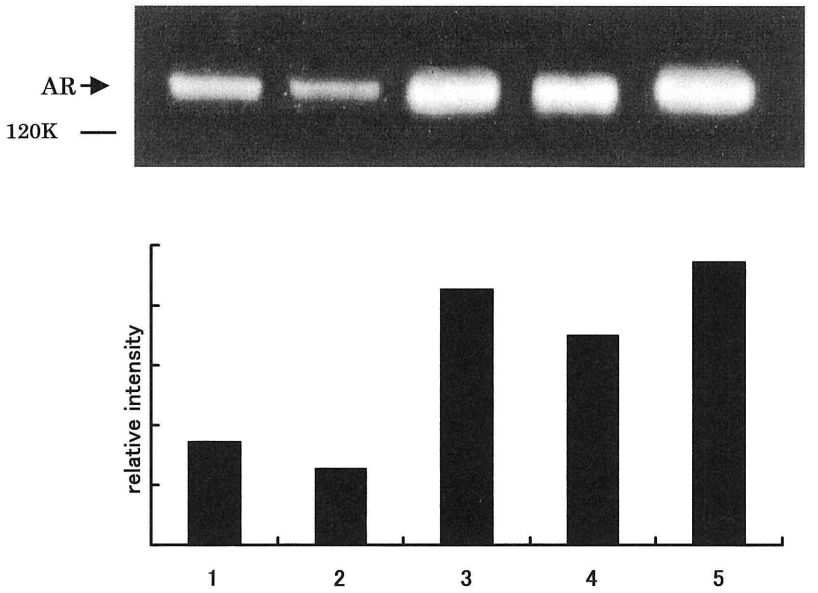

Fig. 5 Western blot analysis of AR of LNCaP cells. Cells were incubated in medium containing 0.1 % FBS in the presence of vehicle (lane 1), melatonin (lane 2), DHT (lane 3) and melatonin+DHT (lane 4), and containing 5 % FBS in the absence of hormones (lane 5) for 4 days. 50 μg proteins of cell extract were separated by electrophoresis in SDS-polyacrylamide gel. Androgen receptors were detected by a double antibody method. Molecular weight marker expressed in kDa.

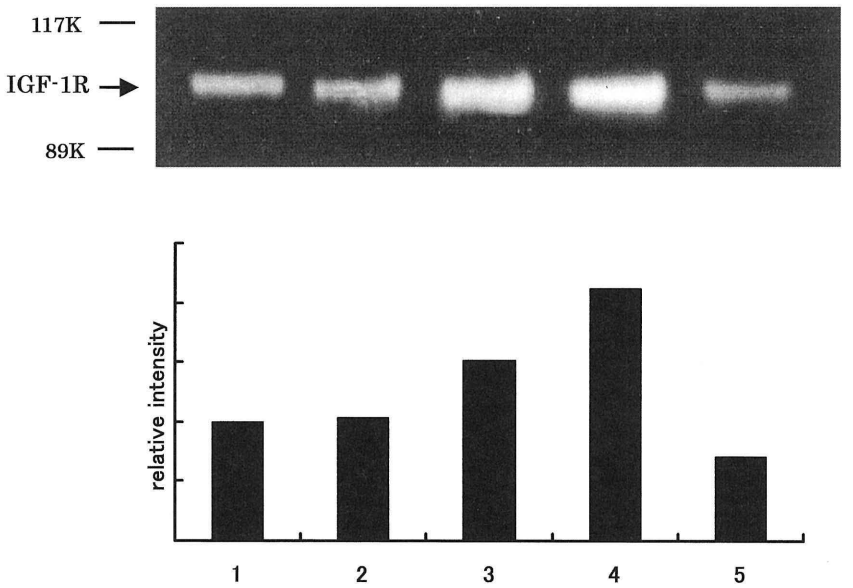

Fig. 6 Western blot analysis of IGF-1 receptor protein of LNCaP cells. Cells were incubated in medium containing 0.1 % FBS in the presence of vehicle (lane 1), melatonin (lane 2), DHT (lane 3) and melatonin+DHT (lane 4), and containing 5 % FBS in the absence of hormones (lane 5) for 4 days. 50 μg proteins of cell extract were separated by electrophoresis in SDS-polyacrylamide gel. IGF-1 Rs were detected by a double antibody method. Molecular weight marker expressed in kDa.

breast cancer cells only in very narrow windows of physiological concentrations[27]. Slominski and Pruski[28] have demonstrated that low dose melatonin can prolong the cycle of rodent melanoma cells, whereas higher concentrations stimulate cell division. The present results showed that the effects of melatonin (both physiological and pharmacological doses) on the DNA levels associated with cell numbers are less remarkable in LNCaP cells. The reasons for the discrepancy concerning the melatonin effects on tumor cell proliferation are not yet known. However, they may be related to the experimental conditions employed (cell culture conditions, proliferation assay methods etc).

Knowledge concerning the physiological actions of melatonin preceded by many years information on how melatonin carries out these effects. In the last few years,

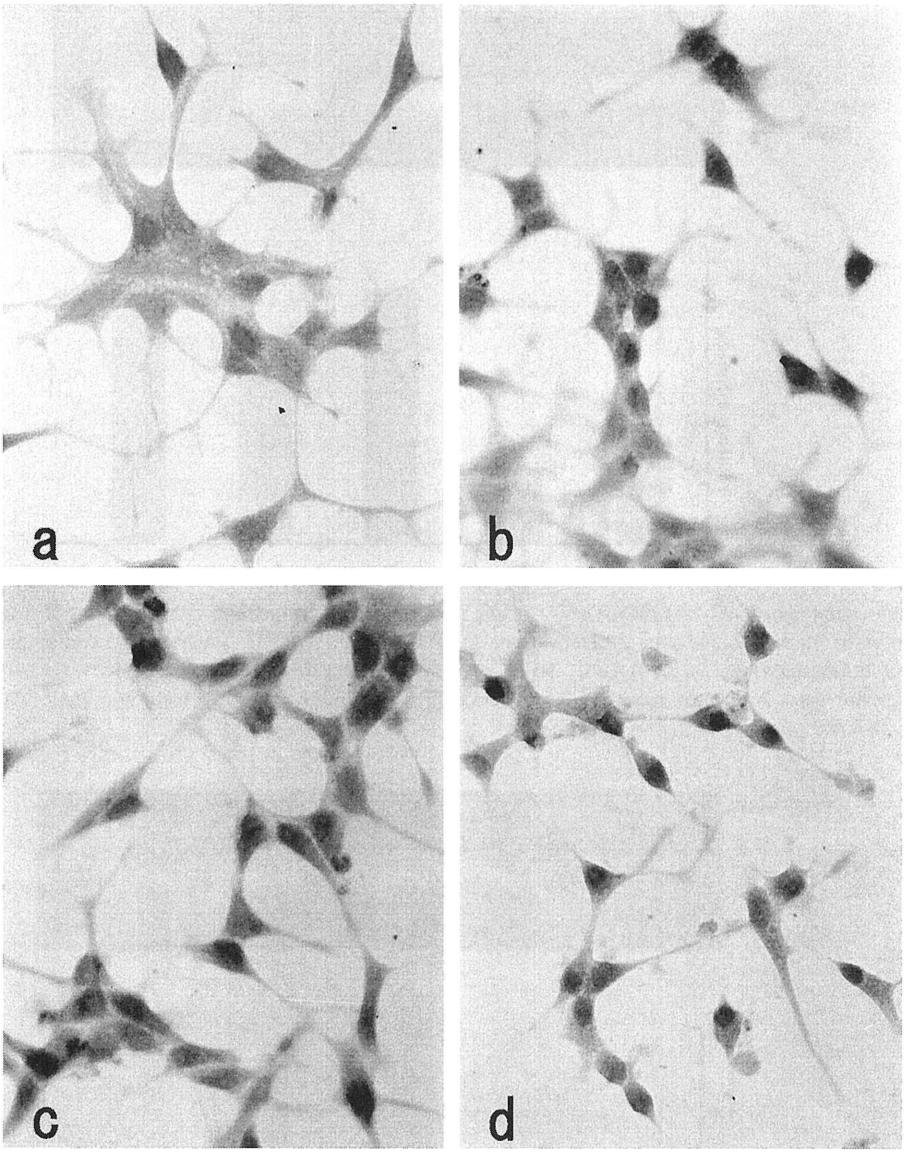

Fig. 7 Immunohistochemical study of androgen receptor protein in LNCaP cells. After 48 h in 0.1% FBS medium, cells were treated with hormone for 4 days. This figure illustrates typical results obtained reproducibly in repeated experiments. (a) non-treated (b) melatonin-treated (c) DHT-treated (d) melatonin+DHT-treated groups.

multiple putative melatonin receptors were cloned. These receptors were found in both the membrane and nuclear fractions of cells. Melatonin membrane receptor (Mell) protein was expressed in LNCaP cells, suggesting that the antiproliferative action of melatonin on LNCaP cells is associated with Mell[29]. In the present studies, we demonstrated that the nuclear receptor RORα/RZRα is also expressed in LNCaP cells. However, it is not yet known whether the receptor associated with melatonin has effects on LNCaP cells. In the immune system, both Mell and RORα/RZRα melatonin receptors were also expressed in lymphocytes[30)31)32)33]. From these observations, the mechanisms involved in these two receptors may be different between melatonin effects on lymphocytes. Melatonin is able to activate human lymphocytes by increasing cytokines (ILs and IFN-γ)[34)35]. Guerrero et al[33] have demonstrated that the expression of nuclear receptors but not membrane receptors is sufficient for melatonin to activate IL-6 production in human lymphocytic and monocytic cell lines. However, no clear association between signaling through the receptors or a biological effect of melatonin on prostate cartinoma cell has been shown. IL-6, which is implicated in modulation of growth and differentiation in many malignant tumors, induces the activation of AR in a synergistic manner with androgen and increases the expression of PSA mRNA, while it inhibits LNCaP cell proliferation[36]. These results suggested that IL-6 exerts effects on an androgen-response gene expression rather than cell proliferation in prostate cartinoma cells. Since higher doses of melatonin induced the reduction

of PSA production but not cell proliferation or AR expression in the present studies, it seems very likely that melatonin at higher doses (pharmacological doses) is involved in the reduction of PSA production but not AR gene expressions via its nuclear receptor, while at low doses (physiological doses) it is involved in the inhibition of cell proliferation via membrane receptor.

PSA is one of the most important IGFBP-specific protease. PSA leaves IGFBP-3, and isolates IGF-1. Tennant et al.[21] have found that IGFBP-3 is decreased in prostate tissue. This change at the tissue level helps to enhance the action of IGF-1. IGF-1 has direct effects on the proliferation of many tissues and cell types, both *in vitro* and *in vivo*, and is thought to be a significant autocrine or paracrine factor in normal and malignant cellular proliferation[37]. The proliferative actions of IGF-1 have been known to be mediated through the IGF-1 R. In the present study we found more expression of IGF-1 R protein in DHT-treated LNCaP cells than in non-treated controls. Melatonin in conjunction with DHT was more effective on IGF-1 R expression than DHT alone, suggesting that the pineal hormone, in addition to DHT, has the ability to regulate the expression of IGF-1 R in LNCaP cells. IGF-1 mediated through its receptor is able to stimulate or activate the androgen receptors, resulting in PSA production, which has been shown *in vitro* in LNCaP cells[38]. From the fact that androgen could stimulate the IGF-1 R expression in the present results, it is suggested that both androgen and IGF-1 may cause the accumulation of their receptors to each other, and that probably androgens stimulate the cell transition from the G_0 to G_1 phase, making them into IGF-1, which then facilitates the progression from the G_1 into the S phase.

The present results also showed that the concentration of FBS in culture medium significantly affects the expression of IGF-1 R, its effect being dose-dependent. Some authors have demonstrated that the nutrition status can profoundly affect serum IGF-1 and IGF binding protein levels[39][40]. Melatonin might affect IGF-1 R expression involving the ability of IGF-1 action in LNCaP cells, as indicated by starvation status. Although it is not clear whether LNCaP cells produce their own IGF-1, melatonin may act as an analogue mediated through IGF-1 R. Moreover, it is of interest that melatonin may not be able to show an antiproliferative action under the low nutrition status, even if the hormone increased in the IGF-1 R expression.

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LNCaP細胞におけるアンドロジェンの作用発現機序に関わるメラトニン

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【要旨】 今回の研究では前立腺癌細胞 (LNCaP 細胞) にメラトニンの核受容体があるかどうか、メラトニンが前立腺特異抗原 (PSA) 産生を調節しうるか否か、更にはこの腫瘍細胞に対するメラトニンの作用機序を明らかにしようとしたものである。メラトニンの核受容体の検索にはRT-PCR法を、PSAの測定にはELISA法を、アンドロジェン受容体 (AR) およびインスリン様成長因子受容体 (IGF-1R) の発現の検索にはWestern blotting法を、またARの発現には免疫組織化学的方法を用いた。

LNCaP細胞にはメラトニンの核受容体であるオーファンファミリーに属するレチノイド-O受容体 (ROR/RZR) が発現することが明らかになった。このことは以前より知られているメラトニンの膜受容体と共に核受容体ももつことを意味する。ディヒドロテストステロンの投与によりPSA産生は有意に増加するが、その増加はメラトニン投与により完全に阻害された。しかし、今回用いたメラトニン量ではARの発現および細胞増殖には大きな影響をおよぼすことはなかった。以上の結果は、メラトニンがARに影響を与えずに自らの核受容体を介してアンドロジェン依存性遺伝子の発現を抑制することを示すものであった。以前より低濃度メラトニンは膜受容体を介して腫瘍細胞の増殖を抑制することが知られていることから、メラトニンの作用機序は用いる量によって異なることが示唆された。

〈**Key words**〉メラトニン、アンドロジェン、LNCaP 細胞、RORα/RZRα、PSA