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Multiple domains of melatonin receptor are involved in the regulation of glucocorticoid receptor-induced gene expression

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Abstract

Melatonin, the principal hormone of the pineal gland, elicits potent anti-stress, anti-aging and oncostatic properties and influences various immunological and endocrinological functions. We have previously described the effects of melatonin on glucocorticoid receptors and suggested its potential influence on gene transcription. In the present study, the mechanistic basis for melatonin effects on glucocorticoid receptor (GR)-dependent gene expression was examined. Activation of the melatonin transduction pathway affects type I glucocorticoid receptor expression and reduces its transcriptional activity. Coexpression of the intact melatonin and glucocorticoid receptors with MMTV promoter construct reduced the GR transcriptional activity. N- and C-terminus deletions of melatonin receptor revealed the existence of regulatory sites mediating this process. These data identify for first time one of the molecular targets of melatonin action and suggest that melatonin signaling may involve relatively direct signal transmission from the cell surface to the nucleus.

Keywords: Melatonin; Glucocorticoids; Receptors; Transcription

1. Introduction

Melatonin (N-acethyl-5-methoxytriptamine) is the most investigated pineal neuroendocrine factor, and its pivotal roles in the regulation of a number of physiological and pathological processes are well documented [1,2]. Melatonin exerts its effects through pharmacologically specific, high affinity receptors [3,4]. These receptors (MR) are coupled to guanine nucleotide binding proteins (G proteins), and their activation consistently leads to the inhibition of adenylyl cyclase [3]. Recently, using an expression cloning strategy, melatonin receptor (MR) cDNAs were isolated from various species [3–5]. Functional analysis suggested that the cloned high affinity receptor likely mediates the major biological effects of melatonin in mammals [6].

Previously, it has been reported that the chronic melatonin treatment affects considerably the binding activity of glucocorticoid, estrogen and androgen receptors in various brain tissues and peripheral organs [7–11]. The glucocorticoid receptors are ligand-activated transcriptional regulators which bind to cis-acting response sequences and alter the rate of transcription of target genes. The molecular mechanism of GR action is a rather complex process, but it is apparent that the receptor interacts with the hormone and several non-receptor proteins to be transformed into an active form [12]. In the present studies, we present evidence for functional interactions between the melatonin transduction pathway and GR activation. Consequently, we proposed a working model that the

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MR either directly or indirectly regulates the activity of GR.

2. Materials and methods

2.1. In vivo studies

Primary thymus lymphocyte cell cultures were prepared from CD-1 mice by a conventional technique. Purified lymphocytes were obtained by centrifugation of crude cell preparations on a Percoll gradient as described [13]. Lymphocytes were grown in RPMI 1640 medium supplemented with 10% FBS in 96-well plates at a density of $1–2 \times 10^5$ cells per plate. The cells were incubated for 72 h, the medium was aspirated and fresh medium containing 2 nM melatonin was added for additional 2 h. At the end of an experiment the cells were harvested and stored at −70°C until analysis.

2.2. Plasmids

The human melatonin receptor expression vector pCMV-hMR containing the entire protein coding region inserted downstream of cytomegalovirus promoter of pcDNA1 (Invitrogene) has been previously described [3]. The mutated melatonin receptor constructs pCMVhMR-3′del and pCMVhMR-*Nde*I/*Bam*HI*de*I were generated by 3′-end deletion with *Bam*HI and an internal fragment elimination by *Nde*I–*Bam*HI restriction endonucleases respectively, followed by religation into pcDNA1 expression vector. The pActhMR-5′del and pActhMR-5′,3′del deletion constructs were generated by the same strategy as above using *Fok*I and *Bam*HI restriction site and were cloned downstream of the actin 5C promoter [14]. The mutations in various deletion constructs were confirmed by sequencing and restriction digestion. Sequence reactions were carried out by the dideoxynucleotide chain-termination method with Sequenase (United States Biochemical).

2.3. Radioreceptor analysis

The whole cell glucocorticoid receptor assay was performed as previously described [15]. Unoccupied nuclear glucocorticoid receptors were assayed by incubating 200 μl nuclear suspension with 50 μl ^{3}H-corticosterone or ^{3}H-dexamethasone in graduated concentrations (1.0–4.0 nM) for 2 h at 4°C. The nuclei were then separated at 800 g, washed three times with ice-cold 10 mM Tris–HCl buffer, pH 7.4, containing 0.25 M sucrose, 1 mM EDTA, 2 mM EGTA, 0.1 mM DTT, 1 mM $MgSO_4$, 1 mM $CaCl_2$ and 10% glycerol, and the pellet was extracted with ethanol. The crude nuclei preparation has been described [15]. The unoccupied cytosol receptors were determined by the methods previously described [16–18]. In summary, 100 μl high-speed cytosol was incubated with serial dilutions of ^{3}H-corticosterone or ^{3}H-dexametosone (0.5–20 nM) at 4°C for 2 h and then separated with 0.5 ml dextran-coated charcoal at 800 g. Nonspecific binding was determined in the presence of 500-fold excess of unlabeled steroids and 100-fold excess of RU 38486 (Roussel-UCLAF, France).

The melatonin receptor binding studies were carried out as previously described [19]. Crude thymocyte membranes were prepared by homogenization in ice-cold buffer (50 mM Tris-HCl, pH 7.5, 0.01% BSA, 1 mM EGTA) followed by centrifugation at 50,000 g for 10 min. Briefly, 100 μl crude membranes were incubated with 20 μl 2-(^{125}I)Iodomelatonin (0.03 nM-2 nM) for 6 h at 20°C. Nonspecific binding was defined by competition with 10 μM of melatonin.

EBDA/LIGAND program (version 2.0) was applied for initial data analysis and subsequent statistical processing of the results from saturation experiments [20,21].

2.4. RNA isolation and analysis

Total RNA was isolated from cultured mouse thymocytes by the guanidinium isothiocyanate-CsCl method [22]. Twenty micrograms of total RNA were separated on formaldehyde gels and transfered to GeneScreen Plus membranes (NEN Life Science Products). Membranes were hybridized with a fragment generated from the human GR cDNA by *Cla*I–*Sac*I endonucleases digestion [23]. Variation in the loading of total RNA was normalized by hybridization with human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA.

2.5. Gel shift assays

Binding reactions for gel shift assays were performed in a final volume of 14 μl, as previously described [24]. Whole cell extracts from mouse thymocytes were prepared as described [25]. Briefly, cells were homogenized in whole cell extraction buffer containing 20 mM Hepes, pH 7.9, 0.4 M NaCl, 1 mM EDTA, 10 mM DTT, 1 mM PMSF, 25% glycerol, and protease inhibitors, centrifuged for 10 min at 15,000 g and supernatant used for analysis. Competition with unlabeled oligonucleotides was performed with 100-fold excess of the cold competitor. DNA-protein complexes were resolved on 5% polyacrilamide gels.

The following synthetic oligonucleotide which contains the consensus primary glucocorticoid response element (GRE) was used:

Table 1
Effect of melatonin administration on type I glucocorticoid receptors in mouse thymocytes. Primary thymocyte cultures were treated for 2 h with 2 nM melatonin. Kd—dissociation constant; Bmax—receptor density. Data were generated from three independent experiments and are expressed as mean ± SEM

	Kd (nM)	Bmax (molecules/cell)
Control	102.4 ± 32.1	354,128 ± 84,216
Melatonin	52.2[a] ± 16.3	166,048[a] ± 32,412

[a] $P < 0.05$ vs control cells.

5′-AGAGATCTGTACAGGATGTTCTAGAT-3′

A double stranded oligonucleotide was labeled with [^{32}P]deoxy-CTP by fill-in reaction using Klenow.

2.6. Cell transfection and chloramphenicol Acetyltransferase (CAT) assay

The reporter plasmid MMTV–CAT and the expression vector RSV-h αGR containing the human glucocorticoid receptor cDNA inserted downstream of Raus sarcoma virus promoter have been previously described [23]. The human melatonin receptor expression vector and the construction of the various mutated forms were described above.

SL2 Drosophila cells were transfected by the calcium phosphate method and assayed for activity as described [14]. Briefly, cells were plated on 100-mm plates at a density of 1–2 × 10^7 cells per plate. Each plate received 5 μg of reporter plasmid, 2 ng of expression vectors, and pBluescript carrier DNA to bring the total concentration to 10 μg. CAT assay was performed using the organic-phase extraction method of Seed and Sheen [26].

3. Results

3.1. Regulation of GR binding activity by melatonin

In earlier studies we have found that glucocorticoid receptor systems in the brain and thymus gland responded to exogenous treatment with melatonin [19]. To investigate this phenomenon in more detail we prepared primary cultures of mouse thymocytes by Percoll gradients and analyzed glucocorticoid receptors gene expression in response to melatonin treatment. Table 1 summarizes the results of these studies. The incubation of thymocytes with 2 nM melatonin affected considerably the binding characteristics of corticosterone-preferable (type I) glucocorticoid receptors. The reduction of binding afinity (from 102 nM to 52 nM) and receptor density (from 354,128 to 166,048 molecules/cell) was apparent after 2 h in the presence of melatonin. The basis for the enhanced binding affinity of melatonin-treated cells is not certain at this time. Hypothetically, it may reflect a compensatory reaction to the decreased GR binding capacity, or alterations in the interactions between GR and one or more associated proteins in mouse thymocytes. Analysis of the cytosolic and nuclear corticosteroid receptor components confirmed the observed inhibition in corticosteroid receptor activity by melatonin (Table 2). Approximately a two fold decrease in GR concentration in the nucleus was observed following melatonin administration. Type II glucocorticoid receptors were not affected (data not shown). Putative melatonin binding sites were detected in crude thymocyte membranes in agreement with previously published data [27,28]. The binding capacity of these receptors decreased after melatonin treatment suggesting a down-regulatory mechanism (data not shown).

Table 2
Nuclear and cytosolic distribution of glucocorticoid receptors in mouse thymocytes following melatonin treatment. Kd—dissociation constant; Bmax—maximal number of binding sites. Standard errors are shown in parentheses

	Kd (nM)		Bmax (molecules/cell)	
	Nuclear	Cytosolic	Nuclear	Cytosolic
Control	48.2 (8.2)	12.3 (2.1)	484,240 (96,832)	62,886 (10,685)
Melatonin	29.9[a] (6.4)	9.4 (0.8)	240,184[a] (68,280)	38,320[a] (7560)

[a] $P < 0.05$ vs control cells.

3.2. Melatonin effect on GR expression is post-transcriptional

We next searched for changes in GR mRNA levels after melatonin administration. Northern blots did not

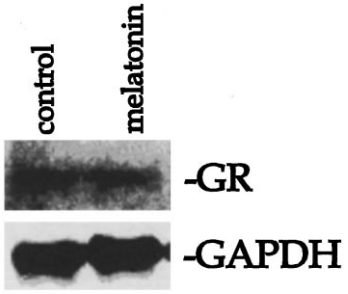

Fig. 1. Northern blot analysis of glucocorticoid receptor (GR) mRNA levels in mouse thymocytes following melatonin treatment. Total RNA (20 μg) was loaded per lane and probed with a human GR cDNA fragment. Membranes were subsequently stripped and rehybridized with a probe to human glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

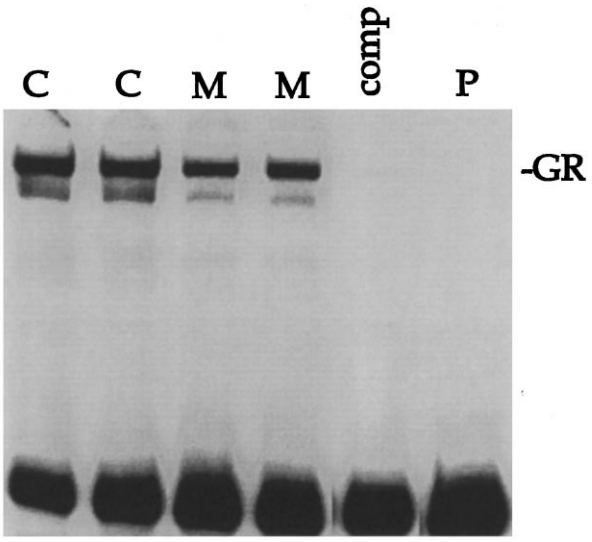

Fig. 2. Nuclear protein binding to GR consensus sequences. Retardation pattern using whole cell extracts (10 μg) from control (C) and melatinin treated (M) thymucytes. Competitor DNA (comp; 50-fold molar excess) consisted of unlabeled GR consensus oligonucleotide. P—probe alone. The position of the specific GR–DNA complexes is indicated.

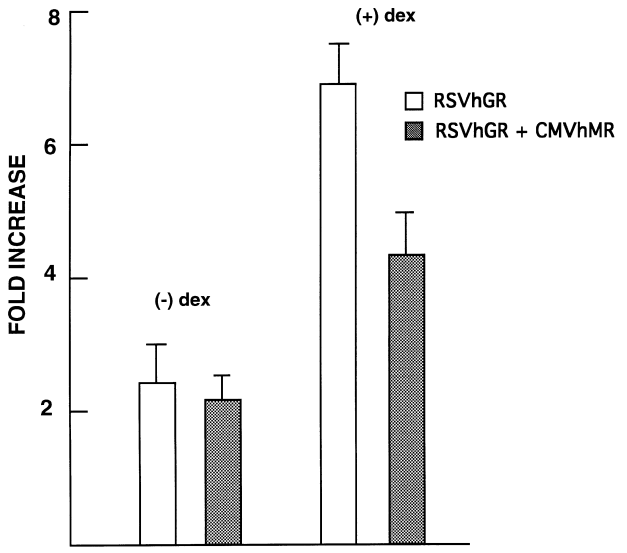

Fig. 3. Cotransfection analysis of hMR activity in SL2 cells. The expression vectors pCMVhMR and pRSVhGR were coexpressed with the reporter vector MMTV–CAT containing GR response element in the presence or absence of dexamethasone (dex). SL2 culture medium was supplemented with 2 nM melatonin. Data for CAT activity are presented as a fold increase versus basal activity of MMTV–CAT transfected cells, which was set at 1. Results were obtained from three independent experiments.

detect any significant alterations in GR mRNA expression in mouse thymocytes (Fig. 1) indicating a post-transcriptional level of melatonin regulation. Moreover, the preincubation of the cells with cyclohexiimide (10 μg/ml) abolished completely the melatonin effect on GR binding activity while actinomycin D was ineffective (data not shown).

To more directly assess whether the melatonin affects functional properties of the glucocorticoid receptor, DNA binding studies were performed. Gel shift analysis with GR consensus sequence of whole cell extracts from mouse thymocytes cultured in the presence or absence of melatonin revealed a reduction in GR binding activity following melatonin treatment (Fig. 2). This effect was observed consistently with several protein extract preparations and suggested a potential alteration in GR transcriptional activity. Also, the results are in agreement with the radioreceptor studies and imply a more direct mechanism of melatonin effect on GR receptor activity.

3.3. Melatonin receptor activation modulates GR-dependent gene expression

To study more precisely the cross-talk between the melatonin and glucocrticoid receptor systems we contransfected human glucocorticoid and melatonin receptors with mouse mamary tumor virus (MMTV) LTR promoter construct which contains the glucocorticoid receptor response element (GRE) [23]. Transfection studies were performed with Drosophila SL2 cells to eliminate any potential effect of endogenously expressed glucocorticoid or melatonin receptor forms. As noted above melatonin was capable of inhibiting glucocorticoid receptor binding activity and therefore was expected to inhibit glucocorticoid-induced gene transcription. In fact, the coexpression of melatonin expression vector reduced considerably the transcriptional activity of glucocorticoid receptor (Fig. 3). This effect was consistently observed in several experiments with different plasmid preparations. Therefore, these observations taken together with the above described binding studies strongly suggest a general inhibitory effect of the melatonin transduction pathway on glucocorticoid-dependent gene transcription.

It has been previously suggested based on the primary sequence information that the N- and C-termini could be of primary importance for melatonin receptor functioning [12]. Therefore, we constructed several mutant melatonin receptor forms to map the functional domains in the melatonin receptor molecules (Fig. 4). The results of the studies are summarized in Fig. 5. The deletion of the C-terminus which eliminated the potential protein kinase C sites, SH2 consensus sequence and the seventh transmembrane domain increased significantly the GR-induced gene transcription. In contrast, the deletion of the N-terminus which includes the two glycosylation sites and a considerable portion of the first transmembrane domain enhanced the generally observed inhibitory effect.

The analysis of amino acid sequence predicted seven

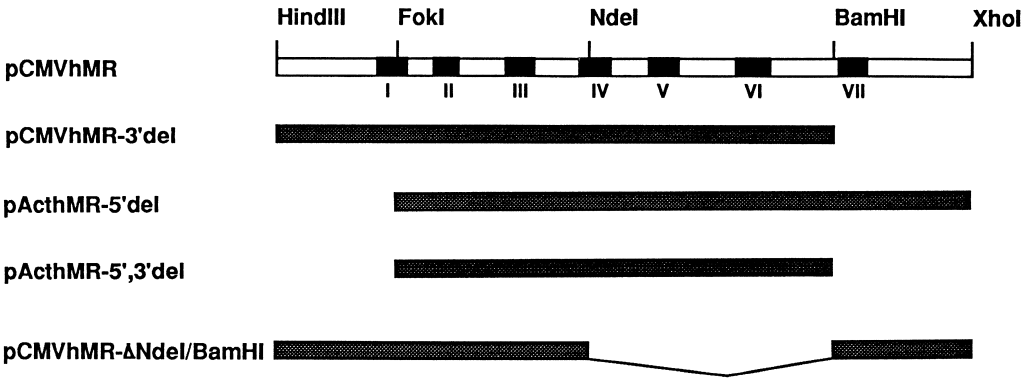

Fig. 4. Schematic diagram of human melatonin receptor (MR) cDNA sequences used to generate the expression constructs. The human MR cDNA is shown at the top with relevant restriction sites. cDNA fragments used in various pCMVhMR–CAT constructs are indicated below.

transmembrane domains in melatonin receptor molecule [1,2], which is chacracterististic for several transmembrane G-protein linked receptors [29–31] We deleted a part of this region to establish their role in the receptor activity. The outcome of these experiments revealed a complete inability of mutated melatonin receptor to modulate the GR-dependent transcription (Fig. 5). The functional significance of these data needs further experimentation but it indicates the existence of regulatory domains within melatonin receptor molecule which are critical for its activity.

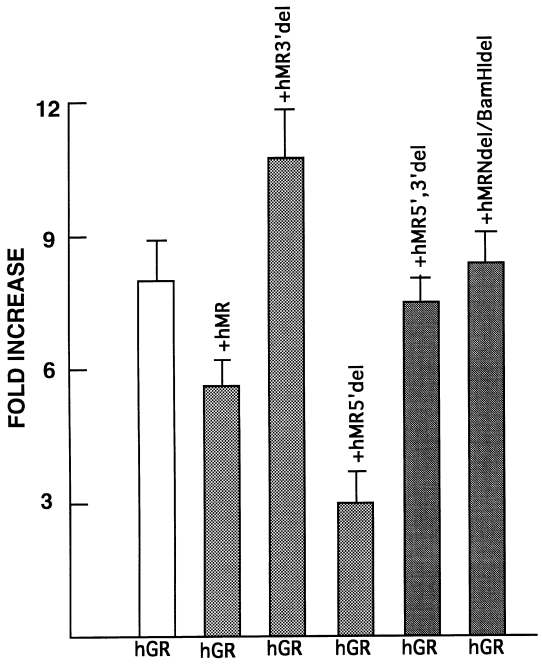

Fig. 5. Effect of various hMR expression constructs on MMVT–CAT activity in transient cotransfection experiments. SL2 cells were cotransfected with hMR deletion constructs and RSVhGR in the presence of dexamethasone and melatonin and CAT activity was analyzed. Data for CAT activity are presented as a fold increase versus basal activity of MMTV–CAT transfected cells, which was set at 1. Results were obtained from three independent experiments.

4. Discussion

The present results provide evidence for functional interactions between the glucocorticoid and melatonin transduction pathways. On the basis of our transfection studies it appears that the expression of glucocorticoid receptor-regulated genes is modulated by melatonin signaling. Thus, the rhythmic gene expression controlled by glucocorticoids could be modulated in a circadian fashion by melatonin.

Several structural motifs in the melatonin receptor molecule were found to be instrumental for the receptor activity. N- and C-termini which contain potential sites for protein kinase C, SH2 and protein glycosilation sites have opposite effects on receptor activity based on the in vivo transfection studies. The transmembrane region contains several structural motifs which are believed to stabilize the receptor structure and form the ligand binding pocket [4]. For instance, the conserved cysteine residues in the first two extracellular loops and the proline residues in transmembrane domains 4, 5 and 6 have been suggested to be essential for the proper receptor conformation [29,30]. The disruption of these structures obviously deteriorates the receptor stability and conformation and prevent receptor signaling.

Together, these data indicate that MR regulates GR transcriptional activity in a negative manner. This effect results in a reduced DNA binding activity of activated glucocorticoid receptor and subsequent decrease in mRNA accumulation of the target genes. A model of this process is depicted in Fig. 6. Inhibition of GR transcription activity may account for many of the anti-stress, immunological and endocrine activities of melatonin [32,33]. Glucocorticoid receptor plays a central role in the regulation of large

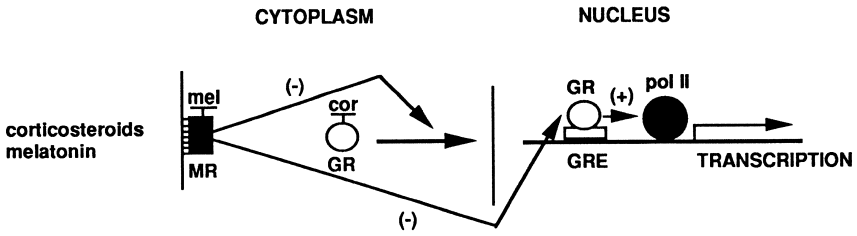

Fig. 6. Mechanisms by which melatonin may affect glucocorticoid receptor regulated gene expression. Melatonin may act by negatively regulating GR translocation from the cytoplasm into the nucleus, or by direct participation in the process of GR interactions with the *pol*II transcription complex.

number of genes including IkBa, cyclin D and AP1 [33–36]. These genes play a central role in the induction of important immunoregulatory and cel cycle regulatory genes including those for IL-1, IL-2, IL-3, IL-6, IL-8, interferon- gama and TNF-alfa. Since melatonin receptors decrease the glucocorticoid receptor binding capacity and tend to inhibit its transcription activity, it might be anticipated that some GR functions can be counteracted by melatonin. Thus, the cross-talk between GR and MR receptor system may serve as a general homeostatic mechanism to regulate the cellular response to environmental stimuli.

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References

[1] R.J. Reiter, Pineal melatonin: Cell biology of its synthesis and its physiological interactions, Endocr. Rev. 12 (1991) 151–180.
[2] D.C. Klein, Photoneural regulation of the mammalian pineal gland, in: (Ciba Foundation Symposium 117) Photoperiodism, melatonin and the pineal, Pitman, London, 1985, pp. 38–56.
[3] S.M. Reppert, D.R. Weaver, T. Ebisawa, Cloning and characterization of a mammalian melatonin receptor that mediates reproductive and circadian responses, Neuron 13 (1994) 1177–1185.
[4] T. Ebisawa, S. Karne, M.R. Lerner, S.M. Reppert, Expression cloning of a high-affinity melatonin receptor form from Xenopus dermal melanophores, Proc. Natl. Acad. Sci. USA 91 (1994) 6133–6137.
[5] S.M. Reppert, C. Godson, C.D. Mahle, D.R. Weaver, S.A. Slaugenhaupt, J.F. Gusella, Molecular characterization of a second melatonin receptor in human retina and brain: The *Mel*1b melatonin receptor, Proc. Natl. Acad. Sci. USA 92 (1995) 8734–8738.
[6] S.M. Reppert, D.R. Weaver, Melatonin madness, Cell 83 (1995) 1059–1062.
[7] S.P. Persengiev, C. Marinova, V. Patchev, Steroid hormone receptors in the thymus: A site of immunomodulatory action of melatonin, Int. J. Biochem. 23 (1991) 1483–1485.
[8] S.P. Persengiev, V. Patchev, B. Velev, Melatonin effects on thymus steroid receptors in the course of primary antibody responses: Significance of circulating glucocorticoid levels, Int. J. Biochem. 23 (1991) 1487–1489.
[9] S.P. Persengiev, I.I. Kondova, Tissue-specific modulation of rat glucocorticoid receptor binding activity by melatonin, Experientia 49 (1993) 332–334.
[10] D.N. Danforth, L. Tamarkin, M.E. Lippman, Melatonin increases oestrogen receptor binding activity of human breast cancer cells, Nature 305 (1983) 323–325.
[11] B. Stankov, V. Lucini, M. Snochowski, B. Gozzi, P. Fumagalli, G. Maccarineli, F. Fraschini, Cytosolic androgen receptors in the neuroendocrine tissues of the golden hamster: Influence of photoperiod and melatonin treatment, Endocr. 125 (1989) 1742–1744.
[12] R.M. Evans, The steroid and thyroid hormone receptor family, Science 240 (1988) 889–895.
[13] J.A. Salisbury, J.M. Graham, C.A. Pasternak, A rapid method for the separation of large and small thymucytes from rats and mice, J. Biochem. Biophys. Methods 1 (1979) 341–347.
[14] A.J. Corey, R. Tjian, Analysis of Sp1 in vivo reveals multiple transcription domains, including a novel glutamine-rich activation domain, Cell 55 (1988) 887–898.
[15] D. Bression, M. Snochowski, A. Belanger, A. Pousette, P. Ekman, B. Hogberg, J-A. Gustafsson, Cyclic uptake of steroids in cells and cell nuclei from prostate, liver and pituitary, FEBS Lett. 103 (1979) 355–361.
[16] M. Snochowski, E. Dahlberg, J-A. Gustafsson, Characterization and quantification of the androgen and glucocorticoid receptors in rat skeletal muscle cytosol, Europ. J. Biochem. 111 (1980) 603–610.
[17] J.M.H. Reul, F.R. van den Bosch, E.R. de Kloet, Relative occupation of type-I and type-II corticosteroid receptors in rat brain following stress and dexamethasone treatment: Functional implications, J. Endocr. 115 (1987) 459–467.
[18] S. Kyakuma, R. Kurokawa, M. Ota, Mechanism of replenishment of androgen receptors in cytosol of mouse submandibular gland, J. Endocr. 115 (1987) 411–418.
[19] S.P. Persengiev, 2-[^{125}I]iodomelatonin binding sites in rat adrenals: Pharmacological characteristics and subcellular distribution, Live Sci. 51 (1992) 647–651.
[20] P.J. Munson, D. Rodbard, A versative computerized approach for characterization of ligand-binding system, Anal. Biochem. 107 (1978) 220–239.
[21] G.A. McPherson, A practical computer-based approach to the analysis of radioligand binding experiments, Com. Prog. Biomed. 17 (1983) 107–117.
[22] J.M. Chirguin, A.E. Przbyla, R.J. MacDonald, W.J. Rutter, Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease, Biochemistry 18 (1979) 5294–5299.
[23] V. Giguere, S.M. Hollenberg, M.G. Rosenfeld, R.M. Evans, Functional domains of human glucocorticoid receptor, Cell 46 (1986) 645–652.

[24] Z. Galcheva-Gargova, J.P. Tokeson, L.K. Karagyosov, K.M. Ebert, D.L. Kilpatrick, The rat proenkephalin germ line promoter contains multiple binding sites for spermatogenic cell nuclear proteins, Mol Endocr. 7 (1993) 979–991.
[25] R.L. Beijersbergen, L. Carlel, R.M. Kerkhoven, R. Bernards, Regulation of the retinoblastoma protein-related p107 by G1 cyclin complex, Genes & Dev. 9 (1995) 1340–1353.
[26] B. Seed, J.Y. Sheen, A simple phase-extraction assay for chloramphenicol acyltransferase activity, Gene 67 (1988) 271–277.
[27] M.A. Lopez-Gonzalez, A. Martin-Cacao, J.R. Calvo, R.J. Reiter, C. Osuna, J.M. Guerrero, Specific binding of 2-[^{125}I]melatonin by partially purified membranes of rat thymus, J. Neuroimmunology 54 (1993) 121–126.
[28] A. Martin-Cacao, M.A. Lopez-Gonzalez, R.J. Reiter, J.R. Calvo, J.M. Guerrero, Binding of 2-[^{125}I]melatonin by rat thymus membranes during postnatal development, Immunol. Lett. 36 (1993) 59–64.
[29] L.F. Kolakowski Jr, GCRDb: aG-protein-coupled receptor database, Receptors & Channels 2 (1994) 1–7.
[30] S. Karnik, T.P. Sakmar, H.B. Chen, H.G. Khorana, Cysteine residues 110 and 187 are essential for the formation of correct structure in bovine rhodopsin, Proc. Natl. Acad. Sci. USA 85 (1988) 8459–8463.
[31] M.F. Hibert, S. Trumpp-Kallmeyer, A. Bruinvels, J. Hoflack, Three-dimensional models of neurotransmitter G-binding protein-coupled receptors, Mol. Pharmacology 40 (1991) 8–15.
[32] G.A.M. Maestroni, A. Conti, N. Pierpaoli, Role of the pineal gland in immunity: III. Melatonin antagonizes the immunosuppressive effect of acute stress via an opiatergic mechanism, Immunology 63 (1988) 465–469.
[33] R.I. Shheinman, P.C. Cogswell, A.K. Lofquist, A.C. Baldwin, Role of transcription activation of IkBa in mediation of immunosuppression by glucocorticoids, Science 270 (1995) 283–286.
[34] N. Auphan, J.A. DiDonato, C. Rosette, A. Helmberg, M. Karin, Immunosupression by glucocorticoids: Inhibition of NF-kB activity through induction of IkBa synthesis, Science 270 (1995) 286–290.
[35] D. Reisman, E.A. Tompson, Glucocorticoid regulation of cyclin D3 gene transcription and mRNA stability in lymphoid cells, Mol. Endocrinol. 9 (1995) 1500–1509.
[36] E.A. Tompson, Apoptosis and steroid hormones, Mol. Endocrinol. 8 (1994) 665–673.