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Transcriptional activation of the nuclear receptor RZRα by the pineal gland hormone melatonin and identification of CGP 52608 as a synthetic ligand

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ABSTRACT

Many important physiological functions are controlled by hormones via binding and activating members of the nuclear receptor superfamily. This group of structurally related transcription factors also includes a still growing number of orphan receptors for which no ligand is known so far. The identification of ligands for orphan receptors is a key to understanding their physiological role, as has been successfully shown for retinoid X receptors and the discovery of 9-*cis* retinoic acid as a specific ligand. We have discovered very recently that the pineal gland hormone melatonin is a specific ligand for the brain-specific nuclear receptor RZRβ. Here we report that the α-subtype of RZR, RZRα and its splicing variant RORα1, is also a nuclear receptor for melatonin with binding specificities in the low nanomolar range. In contrast to RZRβ, RZR/RORα is expressed in many tissues and cells outside the brain. We found that RZRα and RORα1 vary in their constitutive transactivational activity and are activated to a different extent by melatonin. Furthermore, we identified a synthetic RZR-ligand, the thiazolidine dione CGP 52608. This compound is a functional analogue of melatonin at its nuclear receptor, but does not bind to the high affinity membrane receptor for melatonin. Therefore, this specific RZR-ligand may help to differentiate between nuclear and membrane signalling of melatonin.

INTRODUCTION

Various physiological processes are controlled by nuclear receptors that are activated by specific ligands, classically known as hormones. Steroid hormones (glucocorticoid, mineralocorticoid, estrogen, progesterone, androgen) and non-steroid hormones [1,25 dihydroxyvitamin D_3 (VD), 3,5,3′-triiodothyronine (T_3) and retinoids] bind with high affinity to their specific members of the nuclear receptor superfamily (1,2). Like many transcription factors, nuclear receptors generally consist of three major structural and functional domains (3): a variable amino-terminal domain, containing transactivation functions, a highly conserved DNA-binding domain and a regulatory carboxy-terminal region, which binds hormone and has been termed the ligand-binding domain (LBD). The LBD is conserved among members of the superfamily and also carries transactivation, silencing, dimerization and nuclear localization functions.

Over the past years an ever growing number of novel members of the nuclear receptor superfamily has been identified, either by low stringency cross-hybridization using cDNA probes of previously identified receptors (e.g. EAR1, EAR2, ERR1 and ERR2) or by biochemical purification methods (e.g. EAR3/COUP-TF I, ARP1/COUP-TF II and HNF4). Consequently, when initially identified, these receptors have no known ligand and usually no known function; therefore they are called orphan receptors. Since there is significant homology between the C-terminal domains of orphan receptors and the LBDs of classical receptors and a high degree of interspecies conservation of the amino acid sequences of the putative LBD of a particular orphan receptor, it has been suggested that at least some of them are ligand-inducible transcription factors. The identification of specific ligands is therefore a crucial step toward understanding the physiological role of orphan receptors.

The first example of successful ligand identification for an orphan nuclear receptor was 9-*cis* retinoic acid (RA) (4,5), which has been shown to be the specific ligand of retinoid X receptors (RXRs) (6). The K_d and EC_{50} values for binding and activation of RXRs by 9-*cis* RA were determined as 11.7 and 18 nM, respectively (4,5). RXRs are the major heterodimeric partners of the nuclear receptors for VD, T_3 and all-*trans* RA (VDR, T_3R and RAR) (7–10). 9-*cis* RA has been shown to induce homodimerization of RXRs (11), which appears to squelch the nuclear signalling of T_3 (12), VD (13) and all-*trans* RA. These findings revolutionized the understanding of the nuclear signalling pathways of these three hormones and gave 9-*cis* RA a key regulatory role therein.

We found very recently that the pineal gland hormone melatonin specifically binds and activates the brain-specific orphan nuclear receptor RZRβ at low nanomolar concentrations

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(14). Melatonin plays an important role as a transmitter of photoperiodic information and regulator of seasonal reproductive cycles (15). We assume that these brain-specific functions of melatonin may be, at least in part, mediated by RZRβ (14). The existence of a nuclear receptor for melatonin has been proposed also in peripheral tissues (16,17). However, first binding sites for melatonin with K_d values in the picomolar range have been identified in membrane preparations of various tissues and cell lines (18–20) and a seven transmembrane receptor for melatonin with a K_d value of 63 pM has been recently cloned from *Xenopus laevis* (21).

RZRβ is a member of the RZR/ROR family of orphan receptors that also comprises RZRα (22) and the three splicing variants of RORα1, α2 and α3 (23). Sequence comparison showed that RZRα is a further splicing variant of RORα, i.e. the DNA and ligand binding domains of all four receptors are identical and they vary only in their amino-terminal domain. RZR/RORα has been shown to be expressed in various tissues, e.g. in peripheral blood, liver, smooth muscle and testes (22,24,25). RZRs have sequence homology with retinoid receptors, but bind to their response elements as a monomer (26).

Here we show that RZRα and RORα1 are also nuclear receptors for melatonin and we propose that they are involved in melatonin-modulated transcriptional regulation in peripheral tissues. Furthermore, we have identified a synthetic RZR-ligand, the thiazolidine dione CGP 52608. This compound is a functional melatonin agonist that mediates nuclear signalling without binding to the membrane receptor.

MATERIALS AND METHODS

Compounds

CGP 52608 (1-[3-allyl-4-oxo-thiazolidine-2ylidene]-4-methyl-thiosemi-carbazone) was synthesized in the Department of Chemical Research (Ciba-Geigy AG, Basel) and melatonin (N-acetyl-5-methoxytryptamine) was obtained from Fluka. Both compounds were dissolved in dimethylsulfoxide (DMSO) at 10 mM. Dilutions were performed prior to use in DMSO (final concentration of DMSO in the medium: 0.1%). 2-[^{125}I]-iodo-melatonin was obtained from NEN-DuPont and [^{3}H]-CGP 52608 was kindly provided by N. Wigger, Ciba-Geigy AG, Basel.

DNA constructs

The pBLCAT2 (27) derived chloramphenicol acetyl transferase (CAT) reporter constructs, containing at their *Xba*I site the response elements for RZR (26), VDR (28), T_3R (29) and RAR (30), have been described recently; their sequences are given in Figure 2. The cDNAs of human RARα, human RORα1, human VDR, chicken T_3Rα, human RARα and human RXRα have been subcloned into the expression vector pSG5 (Stratagene).

Cell culture, transfection and CAT assays

Drosophila SL-3 cells (2×10^6 cells per well in a 6-well plate) were grown overnight in Schneider's medium (Life Technologies) without fetal calf serum (FCS). Liposomes were formed by incubating 2 μg of the reporter plasmid, 1 μg of receptor-expression vector and 1 μg of the reference plasmid pCH110 (Pharmacia) with 11 μg N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylammonium methylsulfate (DOTAP, Boehringer Mannheim) for 15 min at room temperature in a total volume of 100 μl. After dilution with 0.9 ml Schneider's medium the liposomes were added to the cells. 4–8 h after transfection 500 μl Schneider's medium supplemented with the indicated ligand was added. After a further 16 h the cells were harvested and CAT-assays were performed as described (31). The CAT activities were normalized to β-galactosidase activity and induction factors were calculated as the ratio of CAT activity of ligand-stimulated cells to that of mock-induced controls. Each condition was analyzed at least in triplicates and data are shown as means with standard deviation.

Ligand binding assay

SL-3 cells were transfected with the expression vector for human RZRα or the parental vector pSG5. Forty hours after transfection nuclear extracts were prepared as described (32). Twenty μl of nuclear extract were mixed, on ice, with binding buffer (25 mM Tris–acetate, pH 7.8, 100 mM K-acetate, 10 mM Mg-acetate, 1 mM DTT) in a total volume of 100 μl, and 10 000 Bq of radiolabelled ligand (either 2-[^{125}I]-iodomelatonin, 814 GBq/mmol or [^{3}H]-CGP 52608, 990 GBq/mmol) were added (with or without 500-fold molar excess of 'cold' competitor ligand). The mixture was incubated on ice for 3–4 h. 500 μl of cold 5% charcoal, 0.5% dextran T40 in binding buffer was then added to absorb unbound ligand and the incubation was continued for 30 min on ice. Following a centrifugation at 10 000 *g* for 15 min at 4°C the supernatant was transferred and, in the case of melatonin, measured directly in a γ-counter or, in the case of CGP 52608, added to scintillant and counted in a scintillation counter. Specific ligand binding was determined by subtraction of the counts bound in the presence of competitor, from the counts bound in the absence of competitor. Competion experiments with 2-[^{125}I]-iodomelatonin bound to membrane fractions from chicken brain have been performed using NovaScreen® (Hanover, MD) as described (33).

In vitro translation and DNA binding assays

Linearized cDNAs encoding RZRα and RORα1 were used for *in vitro* transcription as recommended by the supplier (Promega). For *in vitro* translations, 5 μg of each RNA were mixed with 175 μl rabbit reticulocyte lysate, 100 U RNasin and 20 μM complete amino acid mixture (all from Promega) in a total volume of 250 μl and incubated at 30°C for 180 min. The response element probe was prepared by *Xba*I digestion of the respective plasmid DNA, purified and quantified by gel electrophoresis and labelled by a fill-in reaction using [α-^{32}P]dCTP and the Klenow fragment of DNA polymerase I (Promega). Per reaction, 5 μl of *in vitro* translated receptors were pre-incubated with ligand (final concentration 1 μM) for 10 min at room temperature in a total volume of 20 μl binding buffer (10 mM Hepes [pH 7.9], 80 mM KCl, 1 mM DTT, 0.2 μg/μl poly(dI/dC) and 5% glycerol). About 1 ng of labelled probe (50 000 c.p.m.) was added and the incubation was continued for 20 min. Protein–DNA complexes were resolved on a 5% non-denaturing polyacrylamide gel (at room temperature) in 0.5 × TBE (45 mM Tris, 45 mM boric acid, 1 mM EDTA [pH 8.3]).

RESULTS

The structures of the pineal gland hormone melatonin and of the thiazolidine dione CGP 52608 are given in Figure 1. To

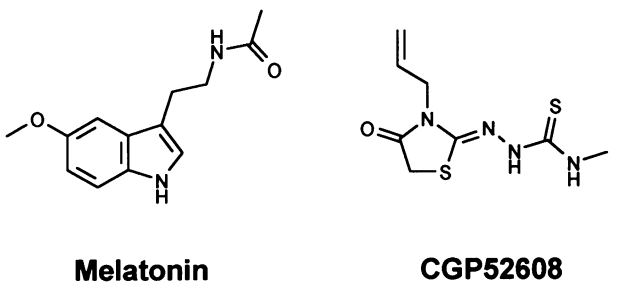

Figure 1. The structure of CGP 52608 in comparison to melatonin.

investigate the specificity of these two substances to induce RZRα-mediated transcriptional activity, we used reporter constructs containing response elements for RZR monomers (26), VDR-RXR heterodimers [from the mouse osteopontin gene (34)], T_3R-RXR heterodimers [from the Moloney murine leukemia virus (35)] and RAR-RXR heterodimers [from the RARβ gene (36)], respectively, fused to the heterologous thymidine kinase (*tk*) promoter driving the CAT gene. We transfected the four CAT reporter constructs together with the expression vectors for human RZRα, human VDR, chicken T_3Rα and human RARα (the last three heterodimer-forming receptors in combination with an expression vector for human RXRα) into the *Drosophila* cell line SL-3 (Fig. 2). This cell line is devoid of mammalian nuclear receptors by definition and has been shown to be a suitable model system to analyze their isolated activity (29,30,37–41). We stimulated each of the four receptor/response element combinations with melatonin, CGP 52608, VD, T_3 and all-*trans* RA, but did not add any FCS. Figure 2 shows that both melatonin and CGP 52608 stimulated RZRα-mediated gene activity about 6-fold, but were inactive on VDR, T_3R and RAR. The latter three receptors showed only activity in response to their specific ligands.

We wished then to determine the sensitivity of RZRα-mediated gene activity. As in the previous experiment, we transfected the CAT reporter construct containing the optimized RZR response element together with the expression vector for human RZRα into SL-3 cells and stimulated them with graded concentrations of either melatonin or CGP 52608 in the absence of FCS (Fig. 3). We obtained typical dose–response curves providing EC_{50} values of 1.1 nM for melatonin and 3.2 nM for CGP 52608. For comparison, in an identical experimental setup, rat RZRβ provided a EC_{50} value of about 3 nM for melatonin (14).

We next asked whether RZRα binds both melatonin and CGP 52608 directly. We obtained specific binding of 2-[^{125}I]-iodomelatonin (Fig. 4) and [^{3}H]-CGP 52608 (Fig. 4) to nuclear extracts of SL-3 cells that had been transfected with RZRα. Scatchard plot analysis yielded K_d values of 1.71 and 4.84 nM, respectively. As a control, nuclear extracts of mock-transfected SL-3 cells did not bind 2-[^{125}I]-iodomelatonin or [^{3}H]-CGP 52608 specifically. A competition experiment (Fig. 4) for displacement of 2-[^{125}I]-iodomelatonin from nuclear extracts of RZRα-transfected SL-3 cells showed IC_{50} values of 1.8 nM for melatonin and 5.2 nM for CGP 52608. These two IC_{50} values fit very well with the respective K_d-values of the radiolabelled ligands. This indicates that both ligands have binding properties nearly identical to those of their radiolabelled derivatives. Furthermore, these results suggest that melatonin and CGP 52608 compete for a very close, if not identical binding site in the RZRα LBD. This establishes CGP 52608 as a functional analogue of melatonin. Consequently we were interested to know whether CGP 52608 also binds to the high affinity membrane receptor for melatonin. Displacement studies with 2-[^{125}I]-iodomelatonin bound to chicken brain

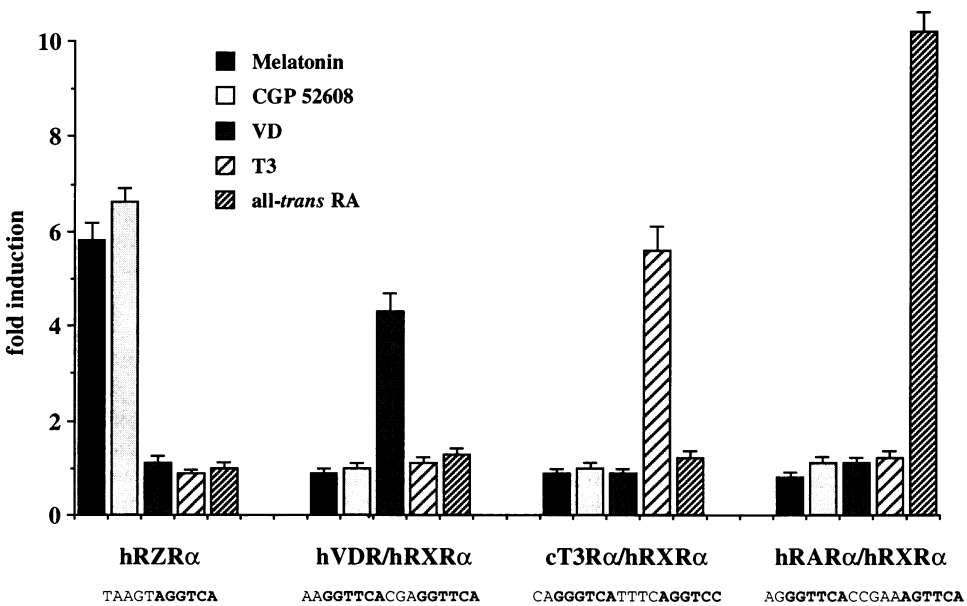

Figure 2. Specificity of RZRα induced gene activation for melatonin and CGP 52608. *Drosophila* SL-3 cells were transfected with the expression vectors for human RZRα, human VDR, chicken T_3Rα or human RARα (the last three in combination with an expression vector for human RXRα) and the CAT reporter construct containing their specific response elements, as indicated. The cells were treated with the indicated ligands (100 nM each, no addition of FCS), harvested 16 h after addition of the ligands and CAT-assays were performed as described (31). The CAT activities were normalized to β-galactosidase activity. Columns represent mean values of at least three independent experiments; the bars indicate standard deviations.

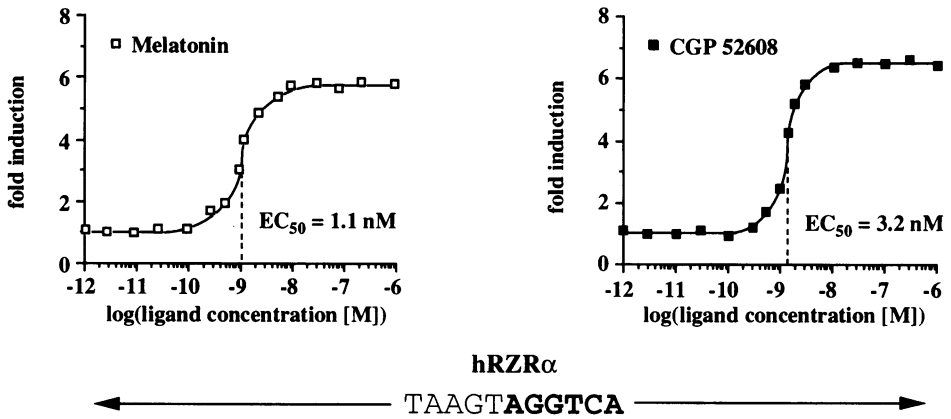

Figure 3. Dose–response curves for melatonin and CGP 52608. *Drosophila* SL-3 cells were transfected with the expression vector for human RZRα and the CAT reporter construct containing the RZR response element TAAGTAGGTCA (26). The cells were treated with increasing concentrations of melatonin or CGP 52608 (no addition of FCS), as indicated. CAT activities were determined 16 h later and stimulation was calculated in comparison to solvent controls. Each point represents the mean of triplicates; the standard deviation was always <10%.

membranes showed that CGP 52608 up to a concentration of 10 μM did not compete for binding (Table 1). This result suggests that CGP 52608 does not bind to the membrane receptor for melatonin.

Table 1. Competition of 2-[^{125}I]-iodomelatonin-binding to chicken brain membranes by melatonin and CGP 52608

Test compound	IC_{50}
2-iodomelatonin	0.1 nM
melatonin	2 nM
6-hydroxymelatonin	20 nM
N-acetyl-5-hydroxytryptamine	2 μM
5-methoxytryptol	15 μM
5-methoxytryptamine	100 μM
5-methoxyindole	200 μM
6-methoxytryptamine	>1 mM
CGPP 52608	inactive at 10 μ

Results are given as IC_{50} values for reference compounds (NovaScreen®). CGP was tested in duplicate at concentrations of 1 nM, 100 nM and 100 μM.

The experiments presented so far establish melatonin and CGP 52608 as directly binding and specifically transactivating ligands of RZRα. Since RZRα is the fourth N-terminal splicing variant of RORα, they share an identical ligand binding domain. We therefore conclude that RORα1–3 are also nuclear melatonin receptors. However, the most prominently expressed representative, RORα1 shows high constitutive transactivational activity (24,25). Therefore, we compared, again in SL-3 cells, RZRα- and RORα1-mediated gene activity on the optimized RZR response element (Fig. 5A). In fact, in the presence of FCS, we observed with both receptors rather high constitutive activity that could be enhanced only by about 30% through the addition of saturating concentrations of melatonin (100 nM). However, in the absence of FCS, the constitutive activity of RORα1 was reduced by a factor of 2.5 and that of RZRα even by a factor of 5. The addition of melatonin, however, reconstituted the activity of both receptors again to the same levels as observed in the presence of FCS.

For several nuclear receptors an increase in DNA binding affinity due to the addition of ligand has been observed. The most prominent example is RXR, for which the DNA binding affinity as a homodimer could be enhanced by 9-*cis* RA (11). Therefore, we tested whether the binding affinity of RZRα and RORα1 to the optimized RZR response element could be enhanced by the addition of melatonin or CGP 52608. Figure 5B shows that the DNA binding affinities of both receptors increased by a factor of about 2 after binding of either melatonin or CGP 52608.

DISCUSSION

The discovery that the pineal gland hormone melatonin is a specific ligand for the RZR/ROR family of nuclear orphan receptors, is rather surprising. From the historical perspective, genomic effects of hormones such as VD, T_3, RA or steroids had been proposed long before their respective nuclear receptors were cloned and the list of true nuclear hormone receptors appeared to be complete. It is a current hypothesis that orphan nuclear receptors are evolutionary precursors of nuclear hormone receptors, which, if ever, are activated by nutrients or similar compounds (42). Peroxisome proliferator activated receptors (PPARs) seem to be a good example; they can be activated by certain fatty acids (e.g. arachidinoic and linoleic acid) (43).

For melatonin binding sites with K_d values in the picomolar range have been described in membrane fractions of different brain tissues and retina (18,19) and a membrane receptor for melatonin with a K_d-value of 0.063 nM has been recently cloned from frog skin (21). We have shown coincidence between membrane binding sites for melatonin in the pineal gland, the superchiasmatic nuclei and certain layers of the retina with high expression of the brain-specific RZR family member RZRβ (14). Future experiments have to show whether nuclear and membrane receptors for melatonin are co-expressed in the same cell type, or

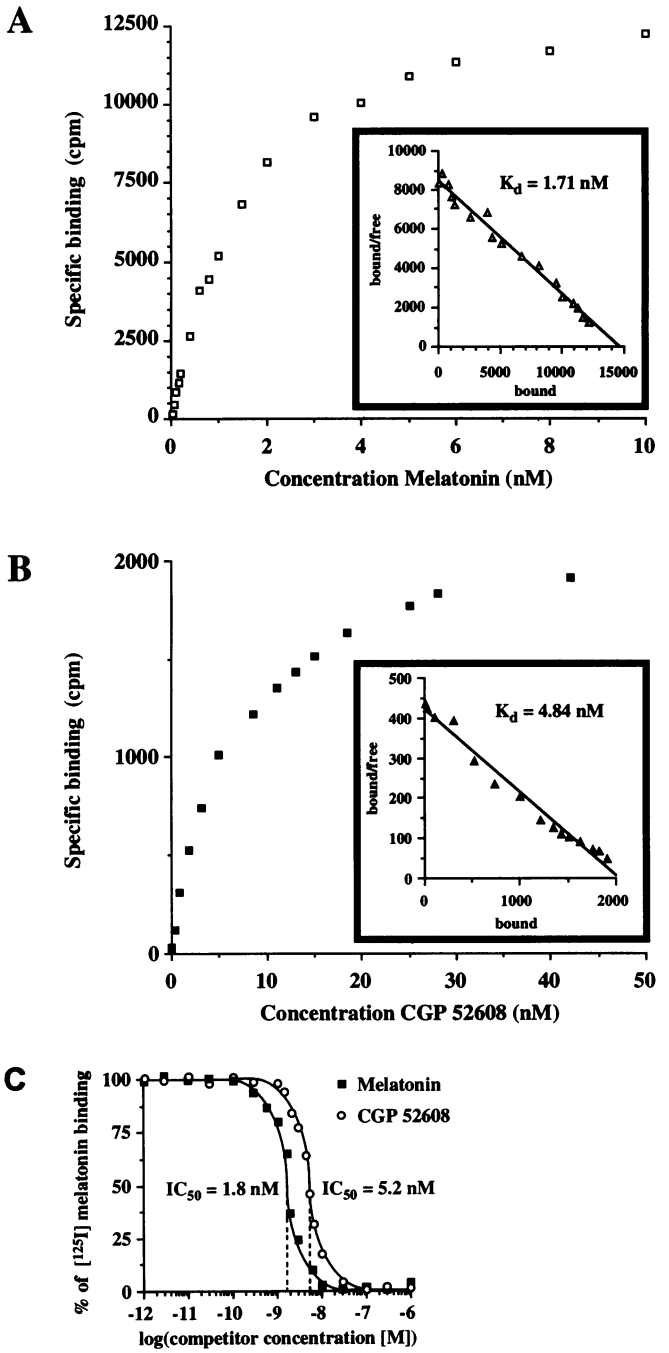

Figure 4. Ligand binding to RZRα. Ligand binding studies were performed with nuclear extracts from RZRα overexpressing *Drosophila* SL-3 cells; as control, nuclear extracts from non-transfected SL-3 cells were used. Increasing concentrations of 2-[^{125}I]-iodomelatonin (**A**) and [^{3}H] CGP 52608 (**B**) were used and specifically bound radioactivity was measured. Scatchard analyses of the data are shown in the insets. Competition experiments (**C**) were performed with nuclear extracts from SL-3 cells using 2-[^{125}I]-iodomelatonin and increasing concentrations of non-labelled melatonin and CGP 52608. Results were expressed as the percentage of specifically bound 2-[^{125}I]-iodomelatonin in the absence of competitor.

whether both signalling processes are independent events in different cell types.

Nuclear signalling by melatonin has been repeatedly proposed by Russel Reiter and co-workers (16,17,44,45). Melatonin exhibits anti-stress, anti-ageing and oncostatic properties and influences various immunological and endocrinological functions. There is growing evidence that melatonin is involved in basic mechanisms controlling cell growth and differentiation and regulatory effects of melatonin on cell cycle kinetics (46–48) have been proposed to play a role in a variety of mammalian tissues (45). These effects could be controlled by gene regulatory events that may be mediated by the nuclear melatonin receptor RZR/RORα. The challenge is now to identify natural responding genes for RZR/RORα and relate their function to cellular actions of melatonin.

The first synthetic RZR-ligand, the thiazolidine dione CGP 52608, may be a useful tool for these investigations. CGP 52608 binds and activates RZRα in a similar fashion as melatonin and is therefore considered to be a functional melatonin agonist. Interestingly, CGP 52608 appears not for be a ligand to the membrane melatonin receptor. Therefore, CGP 52608 will specifically address only the nuclear signalling pathway of melatonin.

Conventional approaches to ligand screening have adapted *cis/trans* transient transfection assays to test for activation by potential ligands. Cell lines used in this assays only survive several hours without FCS. As we demonstrated here for the RZR/ROR family, this may hide the effects of the ligand. We therefore used a more robust cell line, *Drosophila* SL-3 cells. However, the observation of a high constitutive activity of the RZR/ROR family members raises the question whether these receptors actually need a ligand for their proper function. It is generally accepted that the classical nuclear receptors are phosphoproteins. Consequently, it seems reasonable that phosphorylation regulates their function. Phosphorylation by protein kinases activated through signal transduction from membrane bound receptors is probably one mechanism of orphan nuclear receptor activation (49). We hypothesize that ligand binding and phosphorylation are two fundamental activation mechanisms applicable for all members of the nuclear receptor superfamily. However, for each individual member the extent to which the activation is achieved by one or the other mechanism seems to be characteristic. Classical nuclear hormone receptors are most probably activated under physiological conditions by ligand binding, whereas some orphan receptors may not have a true ligand and may be activated predominantly by phosphorylation. We assume that both activation mechanisms are physiologically relevant for the members of the RZR/ROR family. Very recent studies indicate that in certain cellular systems melatonin is able to repress the action of RZRs (MS and CC, unpublished results). In this case the effect of melatonin on transcriptional regulation is reversed to the effect of phosphorylation and may therefore be even more important for fine tuning the system.

In summary, the discovery of the RZR/ROR family of nuclear receptors having melatonin as their common physiological ligand may help in understanding how 'the hormone of darkness' can elicit its diverse biological functions.

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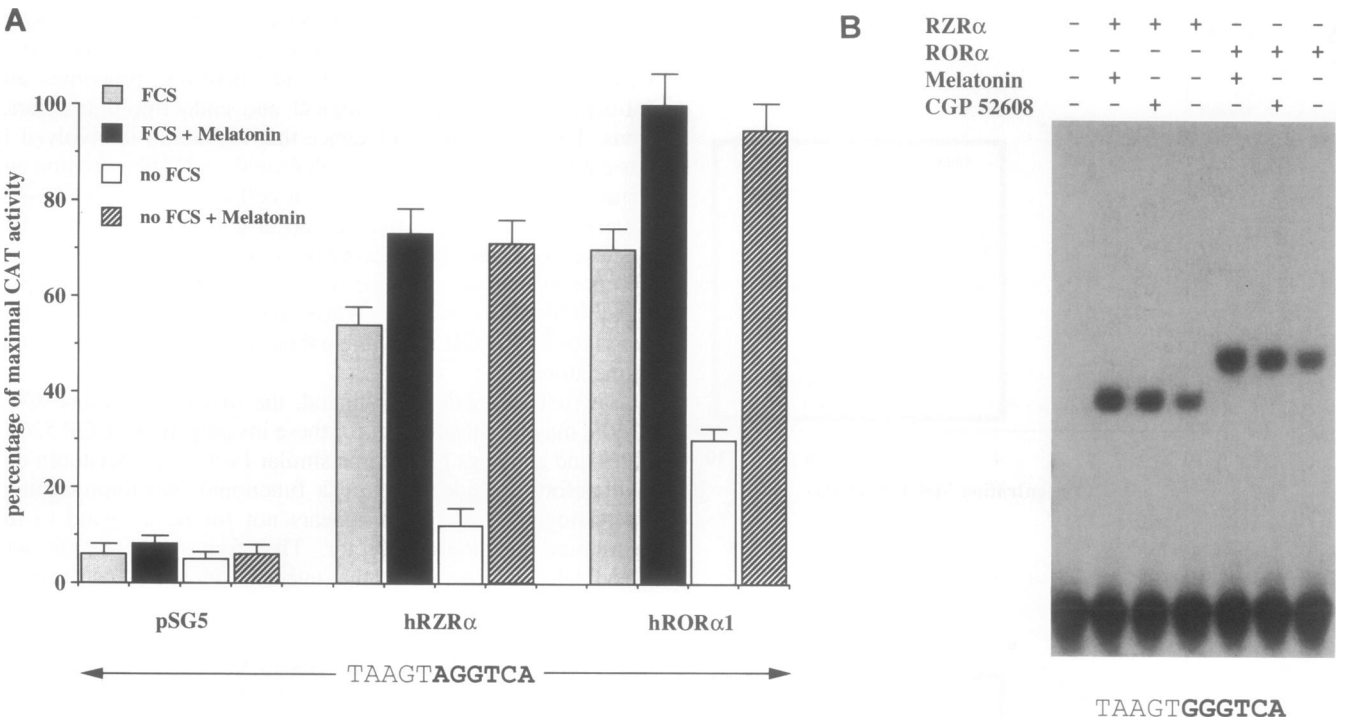

Figure 5. Comparison of RZRα and RORα1. (**A**) *Drosophila* SL-3 cells were transfected with the expression vectors for human RZRα, human RORα1 or, for control, with the parental expression vector pSG5 and the CAT reporter construct containing the RZR response element TAAGTAGGTCA (26). The cells were treated with 100 nM melatonin or solvent in the presence or absence of 10% FCS (as indicated) and CAT activities were determined 16 h later (compare Fig. 2). Columns represent mean values of at least three independent experiments; the bars indicate standard deviations. (**B**) A gel shift analysis was performed using *in vitro* translated RZRα and RORα1 (pre-incubated with 1 μM melatonin, CGP 52608 or solvent, as indicated) and the RZR response element probe. The specificities of the shifted bands were proved by comparison with unprogrammed lysate (first lane).

RARα-expression vector. This work was supported by the Swiss National Foundation (No. 3100-040314.94 to CC).

REFERENCES

1 Evans, R.M. (1988) *Science*, **240**, 889–895.
2 Laudet, V., Hänni, C., Coll, J., Catzeflis, F. and Stehelin, D. (1992) *EMBO J.*, **11**, 1003–1013.
3 Glass, C.K. (1994) *Endocrine Rev.*, **15**, 391–407.
4 Heyman, R.A., Mangelsdorf, D.J., Dyck, J.A., Stein, R.B., Eichele, G., Evans, R.M. and Thaller, C. (1992) *Cell*, **68**, 397–406.
5 Levin, A.A., Sturzenbecker, L.J., Kazmer, S., Bosakowski, T., Huselton, C., Allenby, G., Speck, J., Kratzeisen, C., Rosenberger, M., Lovey, A. and Grippo, J.F. (1992) *Nature*, **355**, 359–361.
6 Mangelsdorf, D.J., Ong, E.S., Dyck, J.A. and Evans, R.M. (1990) *Nature*, **345**, 224–229.
7 Yu, V.C., Delsert, C., Andersen, B., Holloway, J.M., Devary, O.V., Näär, A.M., Kim, S.Y., Boutin, J.-M., Glass, C.K. and Rosenfeld, M.G. (1991) *Cell*, **67**, 1251–1266.
8 Kliewer, S.A., Umesono, K., Mangelsdorf, D.J. and Evans, R.M. (1992) *Nature*, **355**, 446–449.
9 Leid, M., Kastner, P., Lyons, R., Nakshatri, H., Saunders, M., Zacharewski, T., Chen, J.-Y., Staub, A., Garnier, J.-M., Mader, S. and Chambon, P. (1992) *Cell*, **68**, 377–395.
10 Zhang, X.-K., Hoffmann, B., Tran, P.B.-V., Graupner, G. and Pfahl, M. (1992) *Nature*, **355**, 441–446.
11 Zhang, X.-K., Lehmann, J., Hoffmann, B., Dawson, M.I., Cameron, J., Graupner, G., Herrmann, T., Tran, P. and Pfahl, M. (1992) *Nature*, **358**, 587–591.
12 Lehmann, J.M., Zhang, X.-K., Graupner, G., Lee, M.-O., Hermann, T., Hoffmann, B. and Pfahl, M. (1993) *Mol. Cell. Biol.*, **13**, 7698–7707.
13 Schräder, M., Nayeri, S., Kahlen, J.-P., Müller, K.M. and Carlberg, C. (1994) submitted.
14 Becker-André, M., Wiesenberg, I., Schaeren-Wiemers, N., André, E., Missbach, M., Saurat, J.-H. and Carlberg, C. (1994) *J. Biol. Chem.*, **269**, 28531–28534.
15 Reiter, R.J. (1993) *Experimentia*, **49**, 654–664.
16 Acuna-Castroviejo, D., Pablos, M.I., Menendez-Pelaez, A. and Reiter, R.J. (1993) *Res. Commun. Chem. Pathol. Pharmacol.*, **2**, 253–256.
17 Acuna-Castroviejo, D., Reiter, R.J., Menendez-Pelaez, A., Pablos, M.I. and Burgos, A. (1994) *J. Pineal Res.*, **16**, 100–112.
18 Krause, D.N. and Dubocovich, M.L. (1991) *Annu. Rev. Pharmacol. Toxicol.*, **31**, 549–568.
19 Morgan, P.J., Barrett, P., Howell, H.E. and Helliwell, R. (1994) *Neurochem. Int.*, **24**, 101–146.
20 Sugden, D. (1994) *Neurochem. Int.*, **24**, 147–157.
21 Ebisawa, T., Karne, S., Lerner, M.R. and Reppert, S.M. (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 6133–6137.
22 Becker-André, M., André, E. and DeLamarter, J.F. (1993) *Biochem. Biophys. Res. Comm.*, **194**, 1371–1379.
23 Giguère, V., Tini, M., Flock, G., Ong, E., Evans, R.M. and Otulakowski, G. (1994) *Genes Dev.*, **8**, 538–553.
24 Forman, B.M., Chen, J., Blumberg, B., Kliewer, S.A., Henshaw, R., Ong, E.S. and Evans, R.M. (1994) *Mol. Endocrinol.*, **8**, 1253–1261.
25 Retnakaran, R., Flock, G. and Giguère, V. (1994) *Mol. Endocrinol.*, **8**, 1234–1244.
26 Carlberg, C., Hooft van Huijsduijnen, R., Staple, J., DeLamarter, J.F. and Becker-André, M. (1994) *Mol. Endocrinol.*, **8**, 757–770.
27 Luckow, B. and Schütz, G. (1987) *Nucleic Acids Res.*, **15**, 5490.
28 Schräder, M., Müller, K.M., Becker-André, M. and Carlberg, C. (1994) *J. Mol. Endocrinol.*, **12**, 327–339.
29 Schräder, M., Becker-André, M. and Carlberg, C. (1994) *J. Biol. Chem.*, **269**, 6444–6449.
30 Carlberg, C., Saurat, J.-H. and Siegenthaler, G. (1993) *Biochem. J.*, **295**, 343–346.
31 Pothier, F., Ouellet, M., Julien, J.-P. and Guérin, S.L. (1992) *DNA Cell Biol.*, **11**, 83–90.

32 Shapiro, D.J., Sharp, P.A., Wahli, W.W. and Keller, M.J. (1988) *DNA Cell Biol.*, **7**, 47–55.
33 Dubocovich, M.L., Shankar, G. and Mickel, M. (1989) *Eur. J. Pharmacol.*, **162**, 289–299.
34 Noda, M., Vogel, R.L., Craig, A.M., Prahl, J., DeLuca, H.F. and Denhardt, D.T. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 9995–9999.
35 Sap, J., Munoz, A., Schmitt, J., Stunnenberg, H. and Vennström, B. (1989) *Nature*, **340**, 242–244.
36 de Thé, H., Vivanco-Ruiz, M.M., Tiollais, P., Stunnenberg, H. and Dejean, A. (1990) *Nature*, **343**, 177–180.
37 Carlberg, C. (1993) *Biochem. Biophys. Res. Comm.*, **195**, 1345–1353.
38 Carlberg, C., Bendik, I., Wyss, A., Meier, E., Sturzenbecker, L.J., Grippo, J.F. and Hunziker, W. (1993) *Nature*, **361**, 657–660.
39 Schräder, M., Bendik, I., Becker-André, M. and Carlberg, C. (1993) *J. Biol. Chem.*, **268**, 17830–17836.
40 Schräder, M., Wyss, A., Sturzenbecker, L.J., Grippo, J.F., LeMotte, P. and Carlberg, C. (1993) *Nucleic Acids Res.*, **21**, 1231–1237.
41 Schräder, M., Müller, K.M. and Carlberg, C. (1994) *J. Biol. Chem.*, **269**, 5501–5504.
42 O'Malley, B.W. and Conneely, O.M. (1992) *Mol. Endocrinol.*, **6**, 1359–1361.
43 Keller, H. and Wahli, W. (1993) *Trends Endocrinol. Nutr.*, **4**, 291–296.
44 Menendez-Pelaez, A., Poeggeler, B., Reiter, R.J., Barlow-Walden, L., Pablos, M.I. and Tan, D.-X. (1993) *J. Cell. Biochem.*, **53**, 373–382.
45 Menendez-Pelaez, A. and Reiter, R. (1993) *J. Pineal Res.*, **15**, 59–69.
46 Blask, D.E. and Hill, S.M. (1986) *J. Neural. Transm.*, **21**, 433–449.
47 Hill, S.M. and Blask, D.E. (1988) *Cancer Res.*, **48**, 6121–6126.
48 Cos, S., Blask, D.E., Lemos-Wilson, A. and Hill, A.B. (1991) *J. Pineal Res.*, **10**, 36–42.
49 Power, R.F., Conneely, O.M. and O'Malley, B.W. (1992) *Trends Pharmacol. Sci.*, **13**, 318–323.