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REVIEW

Melatonin receptors, heterodimerization, signal transduction and binding sites: what's new?

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Melatonin is a neurohormone that has been claimed to be involved in a wide range of physiological functions. Nevertheless, for most of its effects, the mechanism of action is not really known. In mammals, two melatonin receptors, MT_1 and MT_2, have been cloned. They belong to the G-protein-coupled receptor (GPCR) superfamily. They share some specific short amino-acid sequences, which suggest that they represent a specific subfamily. Another receptor from the same subfamily, the melatonin-related receptor has been cloned in different species including humans. This orphan receptor also named GPR50 does not bind melatonin and its endogenous ligand is still unknown. Nevertheless, this receptor has been shown to behave as an antagonist of the MT_1 receptor, which opens new pharmacological perspectives for GPR50 despite the lack of endogenous or synthetic ligands. Moreover, MT_1 and MT_2 interact together through the formation of heterodimers at least in cells transfected with the cDNA of these two receptors. Lastly, signalling complexes associated with MT_1 and MT_2 receptors are starting to be deciphered. A third melatonin-binding site has been purified and characterized as the enzyme quinone reductase 2 (QR2). Inhibition of QR2 by melatonin may explain melatonin's protective effect that has been reported in different animal models and that is generally associated with its well-documented antioxidant properties.
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Introduction

Melatonin (5-methoxy-*N*-acetyltryptamine) is a neurohormone synthesized during the night by the pineal gland irrespective of the species considered (Arendt, 1998). In humans and all other diurnal species, plasma levels of melatonin are high during the sleep period, whereas in nocturnal species (that is, the majority of laboratory animals) melatonin concentrations are high during the active period. Its secretion is regulated by circadian and seasonal variations in daylight length. The circadian rhythm of pineal melatonin synthesis and release is driven by the circadian 'clock' located in the suprachiasmatic nuclei (SCN) of the hypothalamus that project to the pineal gland via a multi-synaptic pathway (Reppert and Weaver, 2002). The clock rhythm is entrained to a 24-h period by environmental light (the photoperiod) that is directly sensed by the retina and conveyed to the SCN via the retino-hypothalamic tract. Melatonin is very lipophilic and is released without storage directly into the blood and the CSF. All the structures that present melatonin-binding sites will receive this information on photoperiod through the melatonin signal. Melatonin also acts directly on melatonin receptors expressed in the SCN to modulate the clock itself.

The discovery of a radioligand allowing binding as well as autoradiography studies was a major breakthrough in the field of melatonin research (Vakkuri *et al*., 1984). This radioligand, 2-[^{125}I]iodomelatonin (^{125}I-MLT), has a high affinity (in the picomolar range) for melatonin receptors and is still the only available radioligand. This high affinity was the key point for the characterization of the distribution of melatonin-binding sites. Indeed, melatonin-binding sites have the unusual feature of being expressed in very low density even in tissues most sensitive to melatonin. This low density is probably related to the high affinity of melatonin for its endogenous receptors. A few pharmacological studies were also performed with [^{3}H]melatonin but the low specific activity of this radioligand prevented widespread use in tissues with low-density melatonin-binding sites (Kennaway *et al*., 1994; Browning *et al*., 2000).

The purpose of this review is to report on the recent developments in the field of melatonin receptor subtypes and binding sites including the recently characterized MT_3-binding site, and the orphan GPR50, which modulates melatonin receptor function. All the other topics regarding the physiological role of melatonin, the regulation of its

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synthesis, the therapeutic perspectives of melatonin ligands and the chemistry of melatonin ligands have been discussed in recent reviews (Masana and Dubocovich, 2001; von Gall *et al*., 2002b; Simonneaux and Ribelayga, 2003; Witt-Enderby *et al*., 2003; Arendt, 2005; Boutin *et al*., 2005; Zlotos, 2005; Maronde and Stehle, 2007).

Melatonin receptors: distribution

In 1994, the first melatonin receptor was cloned from *Xenopus laevis* immortalized melanophore, but this receptor subtype, Mel_{1C}, was found to be only expressed in non-mammalian species (that is, birds, chicken, fishes) (Ebisawa *et al*., 1994). Subsequently, two other melatonin receptors, MT_1 (Mel_{1A}) and MT_2 (Mel_{1B}), were cloned from humans (Reppert *et al*., 1994, 1995). The official international nomenclature for these receptors is MT_1 and MT_2 (Dubocovich *et al*., 2001; Alexander *et al*., 2007). They both belong to the G-protein-coupled receptor (GPCR) superfamily of membrane receptors and show high homology at the amino-acid level (about 55% overall and 70% within transmembrane domains). Moreover, they share specific short amino-acid sequences, which suggest that they represent a specific subfamily.

In human brain, the MT_1 mRNA was detected by RT-PCR or *in situ* hybridization in SCN, cortex, hippocampus, thalamus and cerebellum, whereas the MT_2 mRNA was detected in the retina and in whole brain and the hippocampus, although in lower amounts (Reppert *et al*., 1994, 1995; Mazzucchelli *et al*., 1996; Weaver and Reppert, 1996; Al-Ghoul *et al*., 1998; Uz *et al*., 2005). The MT_2 receptor subtype has not yet been reported in the SCN in humans, most likely due to the limited number of receptors in this small brain structure. Nevertheless, MT_2 receptors have been detected in the SCN of other species such as mouse and rat (Liu *et al*., 1997; Rivera-Bermudez *et al*., 2004). The localization of the human MT_1 receptor protein was recently confirmed in the SCN, hippocampus, nucleus accumbens, amygdala, substantia nigra, hypothalamus and cerebellum by immunohistochemistry or western immunoblotting studies using specific antibodies (Song *et al*., 1997; Savaskan *et al*., 2001, 2002a; Dillon *et al*., 2002; Uz *et al*., 2005; Wu *et al*., 2007). Expression of the human MT_2 receptor protein was demonstrated in hippocampus and cortex by immunohistochemical studies (Savaskan *et al*., 2005; Brunner *et al*., 2006).

So far, no reliable antibody directed against MT_1 or MT_2 receptors has been reported for species other than humans. Consequently, the receptor expression pattern in other species was only determined at the mRNA level using the reverse transcriptase-PCR (RT-PCR) or *in situ* hybridization. For instance, the MT_1 mRNA was expressed in the majority of the central and peripheral tissues studied in rats (Poirel *et al*., 2003b).

Expression of the MT_1 receptor is also regulated in a circadian manner as demonstrated in the SCN and the *pars tuberalis* (PT) in rodents. These rhythms are regulated in the SCN by the light–dark cycle even in pinealectomized rats (Masson-Pevet *et al*., 1996) and by melatonin itself in the PT (Guerrero *et al*., 2000). The level of MT_1 and MT_2 receptor expression is altered in ageing and in Alzheimer's disease (Savaskan *et al*., 2002a, 2005; Wu *et al*., 2007). Melatonin levels decrease with ageing, which may explain the flattening of circadian rhythms (van Coevorden *et al*., 1991). This decrease in the robustness of the circadian rhythm can be restored by a chronic treatment of rats with a melatonin agonist (Koster-van Hoffen *et al*., 1993).

Melatonin receptors: signal transduction

Signal-transduction pathways triggered by MT_1 and MT_2 receptors were characterized in various primary cell cultures and tissues (Table 1) and in different mammalian cell lines expressing the recombinant receptors (Table 2) (see also Morgan *et al*., 1994; Masana and Dubocovich, 2001; von Gall *et al*., 2002b; Witt-Enderby *et al*., 2003 for more details). These signalling pathways involve the activation of both pertussis toxin-sensitive and -insensitive G proteins. Indeed, both receptors preferentially couple to G_i proteins. MT_1, and most likely MT_2, couple also to $G_{q/11}$ proteins, although to a lesser extent (Brydon *et al*., 1999a,b; Jarzynka *et al*., 2006).

G-protein activation of the MT_1 receptor modulates several signal-transduction pathways. Melatonin typically inhibits forskolin-stimulated cAMP formation (Morgan *et al*., 1989; Brydon *et al*., 1999a,b), protein kinase A activity (Morgan *et al*., 1994; Witt-Enderby *et al*., 1998) and phosphorylation of the cAMP-responsive element binding (Witt-Enderby *et al*., 1998; McNulty *et al*., 1994). Activation of the MT_1 receptor also increases the phosphorylation of the mitogen-activated protein kinase kinases 1 and 2 (MEK1 and MEK2), the extracellular signal-regulated kinases 1 and 2 (ERK1 and ERK2) (Witt-Enderby *et al*., 2000) and c-Jun N-terminal kinase (JNK) via pertussis toxin-sensitive and -insensitive G proteins (Chan *et al*., 2002). Potentiation of ATP and prostaglandin F2α-induced phosphoinositide turnover through the activation of $\beta\gamma$ subunits of pertussis toxin-sensitive G proteins has also been reported (Godson and Reppert, 1997; Roka *et al*., 1999).

Similarly, activation of the MT_2 receptor inhibits forskolin-stimulated cAMP production (Reppert *et al*., 1995; MacKenzie *et al*., 2002) and stimulates JNK (Chan *et al*., 2002) and phosphoinositide turnover (MacKenzie *et al*., 2002).

Collectively, the repertoire of G-protein-dependent signalling pathways activated by MT_1 and MT_2 receptors is very similar. Subtype-specific differences have only been reported in some cases. For instance, the MT_2 receptor inhibits cGMP formation through the soluble guanylyl cyclase pathway, but MT_1 does not in HEK293 cells (Brydon *et al*., 1999a,b; Petit *et al*., 1999). Furthermore, activation of PKC in the SCN occurs only through MT_2 receptors and not MT_1 despite the expression of both subtypes (Hunt *et al*., 2001).

Proteins interacting with melatonin receptors

Recent evidence indicates that GPCRs not only couple to heterotrimeric G proteins but also physically associate with

Table 1 Melatonin-regulated signalling responses in cells or tissues expressing endogenous receptors

Signalling response	*Cells/tissues*	*Functional effect*	*Receptor subtype predominantly involved*	*References*
cAMP formation	Ovine pars tuberalis primary cells	↓	MT_1 and/or MT_2	Carlson *et al.* (1989); McNulty *et al.* (1994)
	Rabbit cortex explants	↓	MT_1 and/or MT_2	Stankov *et al.* (1992)
	Rat neonatal pituitary primary cells	↓	MT_1 and/or MT_2	Vanecek and Vollrath (1989); Slanar *et al.* (2000)
	SCN2.2 cells	↓	MT_1 and/or MT_2	Rivera-Bermudez *et al.* (2004)
cGMP formation	Rat neonatal pituitary primary cells	↓	MT_1 and/or MT_2	Vanecek and Vollrath (1989)
pCREB	Mouse SCN slices	↓	MT_1	von Gall *et al.* (2000)
	Ovine pars tuberalis primary cells	↓	MT_1 and/or MT_2	McNulty *et al.* (1994); McNulty *et al.* (1996)
pJNK, pERK1/2	MCF-7 cells	↑	MT_1	Chan *et al.* (2002)
DAG/PKC	Rat neonatal pituitary primary cells	↓	MT_1 and/or MT_2	Vanecek and Vollrath (1990)
	Human monocytes	↑	MT_1 and/or MT_2	Morrey *et al.* (1994)
	Rat SCN slices	↑	MT_1 and/or MT_2	Mc Arthur *et al.* (1997)
	Rat SCN slices	↑	MT_2	Hunt *et al.* (2001)
	SCN2.2 cells	↑	MT_2	Gerdin *et al.* (2004); Rivera-Bermudez *et al.* (2004)
c-Fos	Rat neonatal pituitary	↓	MT_1 and/or MT_2	Sumova and Vanecek (1997)
Arachidonic acid	Rat neonatal pituitary primary cells	↑	MT1 and/or MT2	Vanecek and Vollrath (1990)
Ca^{2+}-dependent, large conductance K^+ channels (BKCa)	Rat cerebral arteries	↓	MT_1 and/or MT_2	Geary *et al.* (1997)
	Rat tail arteries	↓	MT_1 and/or MT_2	Geary *et al.* (1997)
K^+ conductance	Mouse SCN slices	↑	MT_1 and/or MT_2	Jiang *et al.* (1995)
Inward-rectifying cation current (I_k)	Mouse SCN slices	↓	MT_1 and/or MT_2	Jiang *et al.* (1995)
$GABA_A$-mediated current	Rat SCN slices	↑	MT_1	Wan *et al.* (1999)
	Rat hippocampus slices	↓	MT_2	Wan *et al.* (1999)
Ca^{2+} influx	Rat neonatal pituitary primary cells	↓	MT_1 and/or MT_2	Slanar *et al.* (2000)
Intracellular Ca^{2+}	Rat neonatal pituitary primary cells	↓	MT_1 and/or MT_2	Vanecek and Klein (1992)
		↑	MT_1	Brydon *et al.* (1999a)

↓, decrease, inhibition; ↑, increase, activation.

Table 2 Melatonin-regulated signalling responses in cell lines expressing the recombinant MT_1, or MT_2 receptors

Signalling response	*Cell line*	*Functional effect*		*References*
		hMT_1	*hMT_2*	
cAMP formation	NIH3T3	↓	↓	Reppert *et al.* (1995); Godson and Repper (1997)
	CHO	↓	↓	Witt-Enderby and Dubocovich (1996); MacKenzie *et al.* (2002)
	HEK293	↓	↓	Brydon *et al.* (1999a, b); Roka *et al.* (1999)
	AtT-20	↓	ND	Nelson *et al.* (2001)
cGMP formation	HEK293	=	↓	Petit *et al.* (1999)
pCREB	CHO	↓	ND	Witt-Enderby *et al.* (1998)
pMEK1/2	CHO	↑	=	Witt-Enderby *et al.* (2000)
pERK1/2	CHO	↑	=	Witt-Enderby *et al.* (2000)
	HEK293	↑	↑	Daulat *et al.* (2007)
pJNK	COS-7	↑	↑	Chan *et al.* (2002)
PKA activity	CHO	↓	nd	Witt-Enderby *et al.* (1998)
K^+ currents	AT.-T20	↑	nd	Nelson *et al.* (2001)
Intracellular Ca^{2+}	HEK293	↑	nd	Brydon *et al.* (1999a, b)
Ca^{2+} influx	AT.-T20	↓	nd	Nelson *et al.* (2001)
PI turnover	NIH3T3	↑	nd	Godson and Reppert (1997)
	HEK293	↑	nd	Roka *et al.* (1999)
	CHO	↑	↑	MacKenzie *et al.* (2002)

Abbreviations: CHO, Chinese hamster ovary; ERK, extracellular signal-regulated kinase; ND, not determined; JNK, c-Jun N-terminal kinase; MEK, mitogen-activated protein kinase kinase; PI, phosphotnositide.
↓, decrease; ↑, increase; =, no effect.

other intracellular proteins (Bockaert *et al.*, 2004; Pluder *et al.*, 2006). Several approaches have been developed to identify protein complexes associated with the intracellular domains (loops and C-tail) of GPCRs. Indeed, the nature of the interacting proteins that bind to the intracellular parts of GPCRs can determine its targeting to a specific cellular compartment, its association with other signalling or structural proteins and the fine-tuning of its signal

transduction such as desensitization and resensitization. Therefore, the identification of the protein complexes associated with GPCRs constitutes an important step towards the development of new drugs that could be used to disrupt or strengthen specific interactions between GPCRs and their associated proteins.

An original proteomic approach was recently described by Daulat *et al*. (2007) and gave an overview of the protein complexes able to interact with the MT_1 and MT_2 receptors in HEK293 cells. This approach is based on the tandem affinity purification (TAP) of MT_1 and MT_2 receptors that are expressed in HEK293 cells as C-terminal TAP-tag fusion proteins. The TAP method is based on two successive affinity chromatography steps to purify the protein of interest and the associated complexes. The protein of interest is expressed as fusion protein of the TAP tag, which is composed of two IgG-binding domains, a TEV (Tobacco Etch Virus) protease cleavage site and a calmodulin-binding domain. The complex is first immobilized on an IgG column, then specifically liberated by the addition of the TEV protease and finally bound to a calmodulin column in the presence of calcium. The final eluate is typically separated by SDS-polyacrylamide gel electrophoresis and the recovered proteins were identified by mass spectrometry. The TAP-tag method presents several advantages compared to other techniques as full-length protein of interest, including membrane proteins, can be expressed in mammalian cells where subcellular localization and post-translational modifications are conserved. Despite the fact that this two-step purification protocol generates only few false positives, control conditions in the absence of the TAP-tagged protein should always be run in parallel to identify these nonspecific proteins. Using this method, we confirmed the coupling of both receptors to G_i proteins and allowed the purification of protein complexes associated with GPCRs under native conditions. Several new and potentially functionally relevant MT_1- and MT_2-associated proteins were identified; some of them were common to both receptors, and others were specific for each subtype.

Filamin A and insulin receptor substrate 4 (IRS4) were identified as common members of MT_1- and MT_2-associated complexes. The actin-binding protein filamin A has been already shown to interact with several other members of the GPCR family, including dopamine D_2/D_3 (Li *et al*., 2000; Lin *et al*., 2001) and calcium-sensing receptors (Awata *et al*., 2001; Hjalm *et al*., 2001). Interestingly, filamin A was reported to form a signalling complex with D_3 receptors and β-arrestins, which can be destabilized by D_3 receptor or GRK2/3 activation (Kim *et al*., 2005). Filamin A interacts also with μ-opioid receptors and regulates receptor trafficking (Onoprishvili *et al*., 2003). Finally, a role for filamin in endocytic sorting and recycling of internalized calcitonin receptor has been reported (Seck *et al*., 2003). The role of IRS4 is less well documented. Involvement of IRS4 in fibroblast growth factor receptor signalling (Hinsby *et al*., 2004) and interaction with the protein phosphatase 4 has been described (Mihindukulasuriya *et al*., 2004).

The MT_1 melatonin receptor was shown to specifically interact with Rac1, Rap-1A, the 2′,3′-cyclic-nucleotide 3′-phosphodiesterase and the protein elongation factor 1-γ (eEF-1Bγ) (Daulat *et al*., 2007). The small GTPases Rac1 and Rap-1A have been shown to function downstream of 5-HT_4 receptors and the cAMP guanine nucleotide exchange factor Epac1 (Maillet *et al*., 2003). Both Rap and Rac1 have been reported to be activated upon stimulation of several other GPCRs (Pelletier *et al*., 2003; Weissman *et al*., 2004). The 2′,3′-cyclic-nucleotide 3′-phosphodiesterase, belonging to the PDE3A family, is involved in the degradation of second messengers such as cAMP and cGMP (Lugnier, 2006). eEF-1Bγ and other elongation factors have been reported to modulate GPCR function by direct interaction with the receptor (McClatchy *et al*., 2002, 2006; Cho *et al*., 2003).

Specific interaction partners of the MT_2 receptor were also identified by the TAP approach, including catenin δ1 (p120 catenin) and the protein phosphatase 2Cγ (PP2Cγ) (Daulat *et al*., 2007). Catenin δ1 is a regulator of cadherin stability and an important modulator of Rho GTPase activities (Reynolds, 2007). This protein was shown to interact with mGluR1 receptors and dissociate upon activation of the receptor by L-glutamate (Jones *et al*., 2002). However, its specific role in GPCR signalling is currently unknown. Several serine/threonine phosphatases participate in the dephosphorylation of activated GPCRs (Shih *et al*., 1999). Phosphatases of the PP2A and PP2B subfamilies have been reported to target GPCRs, whereas PP2C subfamily members have been shown to dephosphorylate the metabotropic glutamate receptor 3 (Flajolet *et al*., 2003). Specific dephosphorylation of the MT_2 receptor by the PP2Cγ remains to be demonstrated. Taken together, the TAP method defined the protein complexes associated with transfected TAP-tagged MT_1 and MT_2 receptors in intact HEK293 cells. It will be interesting to expand this study to neuronal and endocrine cells known to express endogenous melatonin receptors, and to study the components of these complexes in native tissues by co-immunoprecipitation experiments.

One interesting feature of the MT_1 receptor is the presence of a class III recognition motif for PSD-95/Disc-large/Zona Occludens-1 (PDZ) domains at its C-terminal extremity (D-S-V). PDZ domains are modular protein interaction domains that are specialized for binding to C-terminal peptide motifs of proteins. PDZ domain-based scaffolds typically assemble large molecular complexes at specific subcellular sites, like synapses. Using protein microarrays and quantitative fluorescence polarization, Stiffler *et al*. (2007) have characterized the binding selectivity of 157 mouse PDZ domains with respect to 217 genome-encoded peptides encompassing the 10 C-terminal residues of mouse proteins, including the MT_1 receptor. By this approach, the C-terminal extremity of the MT_1 receptor was shown to interact with the channel-interacting PDZ domain protein Cipp, the serine protease HtrA1, and MUPP1. The interaction with MUPP1 has been recently confirmed in the PT and has been shown to promote coupling of MT_1 to the cAMP pathway (Guillaume *et al*., 2008). Another PDZ domain-containing protein has been already described as potential interacting candidate of the MT_1 receptor, the neuronal NO synthase (Stricker *et al*., 1997). Using a C-terminal peptide display strategy, Stricker *et al*. screened 13 billion distinct C-terminal peptides to select sequences specific to the PDZ domain of neuronal NO synthase and found that the positive peptides had a D-X-V C-terminal consensus sequence. Searching the non-redundant

protein database NCBInr revealed 484 matches, including the glutamate receptor 6 and the MT_1 receptor. The interaction between nNOS and MT_1 has been recently confirmed (Maurice *et al*., 2008), however the functional consequences on melatonin-mediated cell signalling remain to be demonstrated. The MT_2 receptor also contains a class III PDZ domain-binding motif at its C-terminal extremity (-A-D-A-L). However, no information has been reported in the literature regarding this motif, and a functional role of this motif remains to be demonstrated.

Melatonin receptors: MT_1/MT_2 heterodimerization

Oligomerization (or dimerization) has emerged as a general trademark of GPCRs that is supported by multiple lines of evidence. Recently, the IUPHAR proposed general guidelines for recognition and acceptance of such oligomeric receptors (Pin *et al*., 2007). Receptor dimers may be composed of the same (homodimers) or different (heterodimers) GPCRs. As GPCR dimerization may have a profound impact on receptor pharmacology, signalling and regulation, the issue is of primary importance for basic science and drug development.

Investigation of GPCR dimerization has been limited for a long time by the use of biochemical techniques that were based on detergent extraction of receptors from their natural environment, the plasma membrane. New non-invasive energy transfer-based approaches were introduced in the field of GPCR dimerization late in the 1990s. BRET (bioluminescence resonance energy transfer) is one of these techniques that allows real-time measurement of protein–protein interactions in intact cells (Pfleger and Eidne, 2006). This approach is based on the energy transfer between two receptor fusion proteins. One receptor is fused to the energy donor *Renilla* luciferase (Rluc) and the second to the energy acceptor, the yellow variant of the green fluorescence protein (YFP). Significant BRET can be measured if the donor and acceptor are in close proximity (10–100 Å) and if their respective orientation is appropriate. MT_1 and MT_2 receptors have been among the first GPCRs, whose homo- and heterodimerization have been demonstrated by BRET in transfected HEK293 cells (Ayoub *et al*., 2002). Interestingly, the propensity of MT_2 homodimer formation is 3- to 4-fold lower than that of MT_1/MT_2 heterodimer and MT_1 homodimer formation (Ayoub *et al*., 2004), suggesting that MT_2 may be preferentially engaged into heterodimers in cells co-expressing equimolar quantities of both receptors. It is important to note that melatonin receptors may potentially heterodimerize with other GPCRs. Some other GPCRs have been tested for their ability to heterodimerize with melatonin receptors. The CCR5 chemokine receptor, the β_2-adrenoceptor (Ayoub *et al*., 2002, 2004) and the serotonin 5-HT_4 receptor (Berthouze *et al*., 2005) do not heterodimerize with melatonin receptors, whereas the orphan GPR50 does (Levoye *et al*., 2006a) as determined by BRET. Further experiments will be necessary to determine the complete heterodimerization profile of melatonin receptors.

The BRET assay has also been successfully applied to the determination of the pharmacological profile of MT_1/MT_2 heterodimers. Indeed, a general problem for the determination of the functional properties of heterodimers is the concomitant presence of the respective homodimers. This also applies to MT_1 and MT_2 receptors, which bind ^{125}I-MLT and most melatonin receptor-specific ligands with similar affinities. The choice of the appropriate energy transfer couple in the BRET assay avoids the 'contamination' of the heterodimer-specific profile by that of the two corresponding homodimers (Figure 1). For instance, if MT_1-Rluc and MT_2-YFP fusion proteins are co-expressed, energy transfer can only occur between MT_1-Rluc and the MT_2-YFP fusion proteins but not between two MT_1-Rluc or two MT_2-YFP fusion proteins. Accordingly, ligand binding to heterodimers can be monitored in such an assay if the ligand-promoted conformational change within the heterodimer modifies the relative distance and orientation of Rluc and YFP.

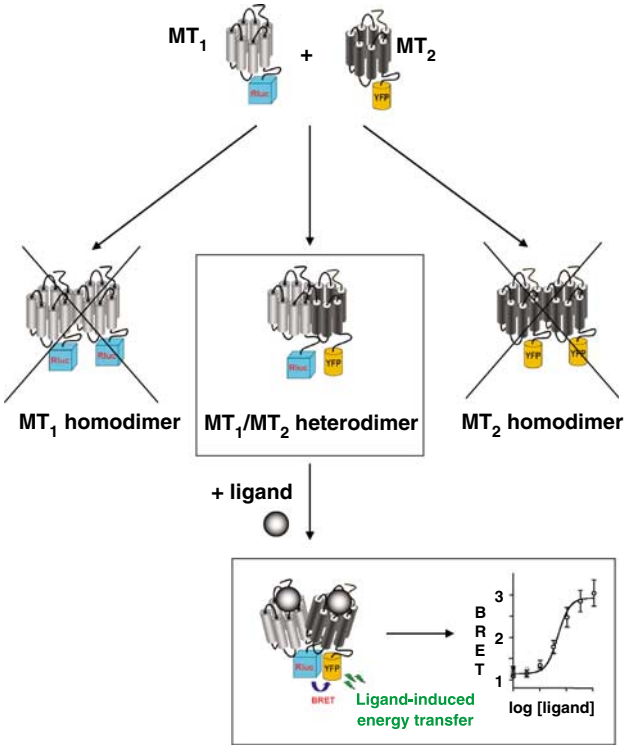

Figure 1 Determination of the pharmacological profile of MT_1/MT_2 heterodimers by BRET (bioluminescence resonance energy transfer). Co-expression of MT_1-Rluc (*Renilla* luciferase) and MT_2-YFP (yellow variant of the green fluorescence protein) fusion proteins results in the formation of at least three different oligomeric receptor species, MT_1-Rluc homodimers, MT_2-YFP homodimers and MT_1-Rluc/MT_2-YFP heterodimers. Whereas all three species are detected in classical ^{125}I-MLT-binding assays, BRET occurs only between MT_1-Rluc and MT_2-YFP. The observed ligand-promoted BRET change of melatonin receptor ligands is therefore exclusively generated by the MT_1/MT_2 heterodimer and not 'contaminated' by the other dimeric receptor species.

Using this approach, a specific pharmacological profile has been established for the MT_1/MT_2 heterodimer and several heterodimer-selective ligands have been identified (Ayoub *et al*., 2004). Luzindole (*N*-acetyl-2-benzyltryptamine) and 4P-PDOT (4-phenyl-2-propionamidotetralin) are the two most widely used pharmacological tools to discriminate between MT_1 and MT_2 in native tissues and animal studies (Dubocovich *et al*., 1998). Our BRET data indicate that these

MT_2-selective compounds have similar or even higher affinity for MT_1/MT_2 heterodimers. This high affinity for MT_1/MT_2 heterodimers has to be considered for the correct interpretation of data obtained in cellular systems co-expressing MT_1 and MT_2. Functional consequences of MT_1/MT_2 heterodimerization, that is, on receptor signalling and trafficking, are currently unknown.

Several candidate tissues for MT_1/MT_2 heterodimer formation may already be identified. Functional melatonin receptors were first characterized in the retina (Dubocovich, 1983), a tissue that predominantly expresses the MT_2 subtype (Dubocovich and Takahashi, 1987). Immunohistochemical experiments in human retina show that both receptors are expressed in virtually every ganglion and photoreceptor cell, strongly suggesting heterodimer formation in these cells (Meyer *et al*., 2002; Savaskan *et al*., 2002b, 2007).

Co-expression of MT_1 and MT_2 in pyramidal neurons of the hippocampus, as shown by RT-PCR (Musshoff *et al*., 2002), is consistent with the possible existence of MT_1/MT_2 heterodimers in these cells. An immunohistochemical analysis showed that MT_1 and MT_2 expression patterns overlap with MT_1 predominantly expressed in the CA1 hippocampal subfield and MT_2 in the CA3/CA4 subfields (Savaskan *et al*., 2002a, 2005).

Expression of MT_1 and MT_2 in SCN neurons is widely accepted (von Gall *et al*., 2002b): the SCN contains ^{125}I-MLT-binding sites (Reppert *et al*., 1988), both receptor mRNAs have been detected by *in situ* hybridization (Reppert *et al*., 1994; Dubocovich *et al*., 1998) and MT_1 expression in human SCN has been recently confirmed by immunohistochemistry (Wu *et al*., 2007). Although a detailed analysis has not been performed, the extensive labelling throughout the SCN strongly indicates co-expression of both receptors, at least in sub-populations of SCN neurons. Arteries (Krause *et al*., 1995) and adipose tissue (Brydon *et al*., 2001) are two further tissues co-expressing MT_1 and MT_2.

Modification of the MT_1/MT_2 ratio is likely to influence the dimerization pattern of these receptors. Such modifications have been observed in the brain and retina of Alzheimer's disease patients (see Levoye *et al*., 2006c; Wu *et al*., 2007 for review) and may modify the functional melatonin response. Future efforts have to focus on the identification of MT_1/MT_2 heterodimers in native tissues and primary cell cultures to firmly establish their physiological relevance. Faster progress in this field is still hampered by the absence of adequate high-affinity melatonin receptor-specific antibodies for co-immunoprecipitation experiments.

Melatonin receptors: *in vivo* functions

In this review, we will discuss only melatonin receptor functions that have been confirmed *in vivo*. Consequently, all the results obtained *in vitro* on tissues or in cell lines either transfected with the receptor subtypes or naturally expressing these receptors will not be discussed here. These data are described in detail in the reviews mentioned above. There are at least two different ways to study the *in vivo* function of a receptor: the use of receptor-specific pharmacological tools (antagonist and agonist) and knockout mice. Only a few selective ligands have been described for MT_1 or MT_2 receptors. Most of these ligands are antagonists or partial agonists and most of them have a weak selectivity, which limits their use *in vivo* (Dubocovich, Delagrange, Olcese, IUPHAR Receptor database, 2007, http://www.iuphar-db.org/GPCR/ChapterMenuForward?chapterID = 1291). Today, the 4P-PDOT compound is the only specific molecule available for *in vivo* studies. 4P-PDOT is an MT_2 antagonist with a selectivity of 100–1000 times (Dubocovich *et al*., 1997; Audinot *et al*., 2003). Because of this lack of pharmacological tools, only few *in vivo* physiological functions have been clearly attributed to each melatonin receptor type.

At the level of the SCN, MT_1 and MT_2 receptors are involved in different functions, which have been mainly studied on tissue slices. Whereas MT_1 receptors are necessary for the acute inhibitory action of melatonin or melatonin receptor agonists on neuronal activity in the SCN, MT_2 receptors mediate the phase shift of circadian rhythm of neuronal activity in the SCN (Liu *et al*., 1997; Hunt *et al*., 2001). These effects have been confirmed for MT_1 receptors on SCN slices by both pharmacological experiments and by studies performed on MT_1 receptor knockout mice (Liu *et al*., 1997; Dubocovich *et al*., 2005). For MT_2, these effects have been only confirmed by *in vivo* treatment with the MT_2-selective antagonist 4P-PDOT (Hudson *et al*., 2005). The interpretation of *in vivo* results obtained with MT_2 knockout mice is more difficult and suggests a functional interaction between these two receptors (Dubocovich *et al*., 2005). Nevertheless, the resynchronizing effects of melatonin observed for both slices and *in vivo* treatments, are always reported in the evening corresponding in rats or mice to the light–dark transition (Redman *et al*., 1983). Phase-response curves obtained in humans confirmed this window of sensitivity for both melatonin and melatonin receptor agonists (Wirz-Justice *et al*., 2002; Arendt, 2005). This window of sensitivity may be explained by the desensitization of the MT_2 receptor at the beginning of the night, which internalizes upon exposure to its endogenous agonist, melatonin, as most GPCRs. *In vitro* studies have recently validated this hypothesis (Gerdin *et al*., 2004). This rapid desensitization may turn out to be tissue specific. Indeed, melatonin receptors play an important role in the transmission of the photoperiodic message, which is given by the duration of melatonin synthesis (Bartness and Goldman, 1989). Consequently, to sense this message, melatonin receptors have to stay at the plasma membrane for at least 12 h, as observed in the ovine PT. Prolonged exposure of primary PT cell cultures with melatonin (8–16 h) induced a sensitization of the cells, which increased with the duration of melatonin incubation (Hazlerigg *et al*., 1993). A decrease of melatonin receptor density was only observed after 24 h of melatonin incubation.

The SCN is the master pacemaker of the organism. The generation of circadian rhythms involves, at the cellular level, clock genes and associated proteins, which are organized in a transcriptional–translational autoregulatory loop that generates molecular oscillations of these clock genes (Ko and Takahashi, 2006). Melatonin receptors are expressed in the SCN and melatonin is able to advance or to

resynchronize the clock. However, the molecular mechanisms underlying this chronobiotic effect are poorly understood. Melatonin treatments have no effect on the expression of clock genes in the SCN (Poirel *et al*., 2003a). The only effect reported recently for melatonin in the SCN is the phase advance in melatonin-treated rats of rev-erbα mRNA expression (Agez *et al*., 2007). This nuclear orphan receptor is involved in the functional link between the regulatory loops of the molecular clock. In the PT, melatonin regulates clock gene expression. Melatonin infusion in sheep induced cryptochrome1 expression and suppressed the expression of other clock genes (Johnston *et al*., 2006b). These effects of melatonin probably involve the MT_1 receptor. Indeed, the expression of most clock genes is reduced in the PT of MT_1 receptor knockout mice but not in MT_2 knockout mice (von Gall *et al*., 2005).

The phenotype of knockout mice either for MT_1 or MT_2 or both receptor subtypes is not very different from wild-type mice (Jin *et al*., 2003). Indeed, the changes associated with targeted disruption of either the MT_1 or MT_2 receptors are subtle and have been reported mainly at the molecular levels, like the regulation of clock genes, transcription factors (CREB) or neuronal activity (Liu *et al*., 1997; von Gall *et al*., 2000, 2002a, 2005; Wang *et al*., 2005). These deletions induce no major behavioural changes under normal conditions according to the limited number of publication related to this field. The lack of phase-shifting effect of melatonin in MT_1 knockout mice, as mentioned above, and a deficit in memory, based only on the elevated plus-maze test in MT_2 knockout mice were until recently the only behavioural effect reported *in vivo* (Dubocovich *et al*., 2005; Larson *et al*., 2006). Another publication reported that knockout mice for the MT_1 receptor exhibited a depressive behaviour in an animal model of depression, the forced swimming test (Weil *et al*., 2006). These results do not demonstrate that melatonin receptors are directly involved in mood disorders, but suggest that their dysregulation might participate indirectly in the installation of depression. In depressed patients, circadian rhythms are generally also dampened or even disrupted (Turek, 2007). The efficacy of agomelatine as an antidepressant may result in part from its activity as an MT_1/MT_2 agonist, although melatonin receptor activation alone is not sufficient for effectiveness in treating depression (Dalton *et al*., 2000; Bertaina-Anglade *et al*., 2006).

The orphan GPR50: progress on the understanding of its function

GPR50 is one of the more than 100 orphan GPCRs that resists the de-orphanization efforts of academic and industrial research. GPR50, also called melatonin-related receptor (MRR), was cloned in 1996 and classified as a member of the melatonin receptor subfamily due to its high homology (45%) with MT_1 and MT_2 at the amino-acid level and due to the presence of characteristic signatures of this subfamily (Reppert *et al*., 1996). More than 10 years later, the function of GPR50 is still poorly understood. Recent genetic studies provided first hints on potential functions. Three polymorphisms located in exon 2 of the human GPR50 gene have been documented (Figure 2a). All three polymorphisms induce amino-acid changes in the C-tail of the human GPR50 (Δ502–505, $Thr^{532}Ala$ and $Val^{606}Ile$). The Δ502–505 variant corresponds to the deletion of four amino acids (Thr-Thr-Gly-His) and has been associated in a Scottish population with increased risk of developing either bipolar affective and major depressive disorder in women (Thomson *et al*., 2005). However, this association was not replicated in a Swedish population (Alaerts *et al*., 2006). A further genetic study confirmed the existence of these three variants in two independent obese cohorts and described an additional variant upstream of the GPR50 coding region (C-16X2GPR50T) (Bhattacharyya *et al*., 2006). No association was observed with body mass index, but carriers of all three variants (Δ502–505, $Thr^{532}Ala$ and $Val^{606}Ile$) had significantly higher fasting circulating triglyceride levels and

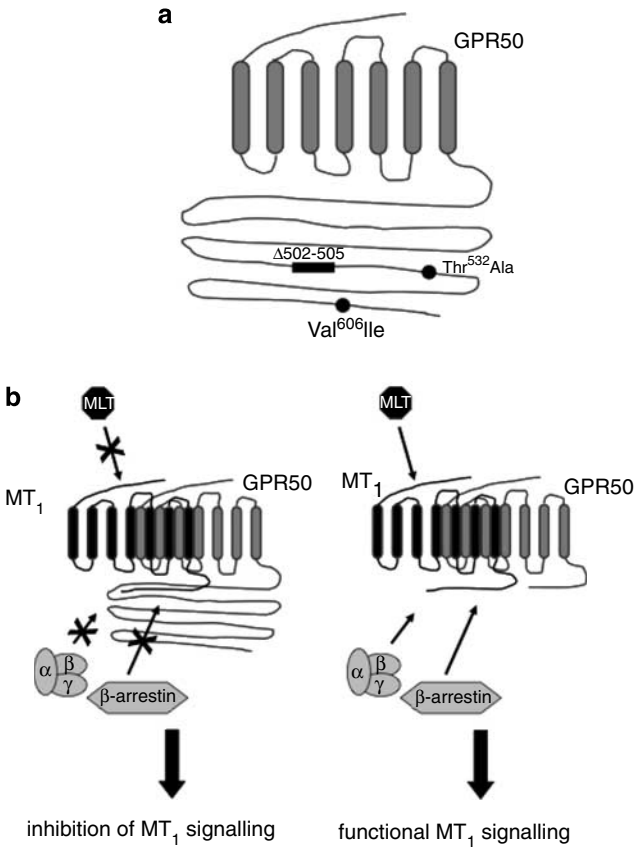

Figure 2 GPR50 polymorphisms and functional properties. (**a**) Localization of the three polymorphisms reported in the literature for GPR50 (Thomson *et al*., 2005; Alaerts *et al*., 2006; Bhattacharyya *et al*., 2006; Feng *et al*., 2007). 1: Δ502–505 corresponds to the insertion/deletion of the four amino acids Thr-Thr-Gly-His (TTGH) at position 502 (Δ502–505 variant); 2: $Thr^{532}Ala$ corresponds to the substitution of Thr (T) to Ala (A) at position 532; 3: $Val^{606}Ile$ corresponds to the substitution of Val (V) to Ile (I) at position 606. The reader is referred to the publication of Bhattacharyya *et al*. (2006) for the haplotype frequencies of these three polymorphisms in GPR50. (**b**) Regulation of MT_1 signalling by heterodimerization with GPR50. Engagement of GPR50 (wild type) into heterodimers with MT_1 receptors prevents high-affinity agonist binding, heterotrimeric G-protein coupling and β-arrestin binding to the MT_1 receptor. When engaged into heterodimers with a C-terminally truncated GPR50 mutant, the MT_1 receptor functions properly.

carriers of the C-16X2GPR50T variant had lower circulating high-density lipoprotein cholesterol levels. The functional differences of these variants and their implication in mental disorders and lipid metabolism remain to be shown. The phenotype of mice with targeted disruption of the GPR50 gene has been described recently (Ivanova *et al*., 2007). In agreement with the genetic studies, these mice have an altered metabolism (increased metabolic rate). Phenotypic alterations associated with mental disorders have not been studied in these animals.

An unexpected function of GPR50 was revealed in co-expression experiments with MT_1 and MT_2 receptors (Levoye *et al*., 2006a). GPR50 was shown to constitutively heterodimerize with MT_1 and MT_2 but not with β_2-adrenoceptors and CCR5 chemokine receptors. Whereas engagement of GPR50 into heterodimers with MT_1 completely inhibited the high-affinity agonist binding of the latter, engagement of GPR50 with MT_2 does not modify the agonist-binding properties of MT_2. Binding of GPR50 to MT_1 had other profound consequences on MT_1 function such as the inhibition of heterotrimeric G-protein coupling and β-arrestin binding (Figure 2b). A more detailed analysis showed that the long C-tail of GPR50 prevents recruitment of intracellular interaction partners such as G proteins and β-arrestins to the MT_1 receptor in the heterodimer.

The potential significance of the inhibitory effect of GPR50 on MT_1 receptor function was shown in immortalized human endothelial cerebral hCMEC/D3 cells that express both proteins endogenously (Levoye *et al*., 2006a). Whereas MT_1 activity was undetectable in cells expressing GPR50, MT_1 receptors became fully functional upon GPR50 silencing, demonstrating that endogenous GPR50 expression levels can indeed regulate MT_1 activity. Two major tasks remain to fully understand the physiological significance of MT_1/GPR50 heterodimerization: the identification of tissues co-expressing MT_1 receptors and GPR50 and the regulation of GPR50 expression. GPR50 mRNA is expressed in the hypothalamus, pituitary, retina, testis, kidney and several other central and peripheral sites known to express MT_1 receptors (Reppert *et al*., 1996; Drew *et al*., 1998, 2001; Vassilatis *et al*., 2003). As a first step to study GPR50 expression at the protein level, we recently developed specific antibodies against GPR50 (Ould-Hamouda *et al*., 2007). Immunohistochemical analysis detected the presence of GPR50 in rat pituitary and in human hippocampus, two structures also expressing MT_1 receptors that are thus promising candidate tissues for further analysis.

Formation of MT_1/GPR50 heterodimers is constitutive and is expected to depend on the relative expression levels of both proteins. MT_1 receptor expression is known to be regulated during development and the circadian cycle (Poirel *et al*., 2002; Johnston *et al*., 2003, 2006a). Little is known about the regulation of GPR50 expression. Recently, GPR50 mRNA levels were shown to be downregulated in short-day photoperiods specifically in tanycytes of the ependymal layer of the hypothalamus (Barrett *et al*., 2006). Hypothalamic expression of GPR50 is also highly responsive to energy status with decreased expression upon fasting and feeding with high-energy diet (Ivanova *et al*., 2007). The function of MT_1 in the heterodimer might also be regulated post-translationally by the proteolysis of the GPR50 C-tail as heterologous expression studies showed that the MT_1 receptor is fully functional when engaged in heterodimers with a C-terminally truncated GPR50 mutant (Levoye *et al*., 2006a) (Figure 2b). This regulatory mechanism may warrant further attention as recent data suggest that GPR50 is indeed sensitive to proteolytic degradation (Ould-Hamouda *et al*., 2007).

GPR50 may have further functions that remain to be discovered. In particular, the question of an endogenous ligand and the possibility of ligand-dependent signalling is difficult to exclude. GPR50 heterodimerizes with MT_2. However, the consequences of GPR50 on the MT_2 function are currently unknown but warrant further attention. Heterodimerization between an orphan and non-orphan GPCR as exemplified by the MT_1/GPR50 couple, may be a more general strategy of cells to regulate GPCR function and may in addition open new perspectives for drug design (Levoye *et al*., 2006b; Levoye and Jockers, 2007).

Melatonin-binding site MT_3 is QR2: facts, remaining questions and hypotheses

MT_3, previously called ML-2, was initially described by Duncan *et al*. (1988). By 1999, only a dozen articles describing this binding were published. This binding site presented a 'low' affinity for melatonin (5–50 nM in contrast to the less than 1 nM for MT_1 and MT_2 receptors, see Molinari *et al*., 1996 for complete discussion), very fast association and dissociation kinetics and a pharmacological profile slightly different from MT_1 and MT_2 receptors. A specific ligand for MT_3 was identified in 1996, 5-methoxycarbonylamino-*N*-acetyltryptamine (MCA-NAT), which presents nanomolar affinity for MT_3 and only micromolar affinity for MT_1 and MT_2 (Molinari *et al*., 1996). Further studies extended the pharmacological characterization of MT_3 (Paul *et al*., 1999). By using a mild extraction procedure and affinity chromatography onto an immobilized analogue of MCA-NAT, a single protein was isolated (Nosjean *et al*., 2000) that, once sequenced, turned out to be a long forgotten quinone reductase, QR2 (Zhao *et al*., 1997).

Further studies on this enzyme firmly established that MT_3 and QR2 are the same protein. Indeed, transfection of QR2 in Chinese hamster ovary cells revealed an MT_3-type binding site (Nosjean *et al*., 2000). Similarly, tissues from various species showed a correlation between QR2 activity and MT_3 binding (Nosjean *et al*., 2001). Furthermore, and most importantly, $QR2^{-/-}$ mice did not have any measurable MT_3-binding sites (Mailliet *et al*., 2004). Very recently, the co-crystallization of QR2 with melatonin and 2-iodo-melatonin, and the docking of MCA-NAT onto this structure confirmed that QR2 corresponds to MT_3 (Calamini *et al*., 2008).

Nevertheless, different properties of melatonin reported to involve MT_3 cannot be explained so far according to what is known today about QR2. For example, MT_3 was originally described as a membrane-bound binding site in hamster brains (Duncan *et al*., 1988), while QR2 is a cytosolic enzyme, which has so far never been reported to be membrane-associated. Specific MT_3 binding at the membrane level

could be detected in wild-type mice but not in QR2$^{-/-}$ mice. Moreover, MT_3-binding sites have a pharmacological profile different from MT_1 and MT_2 receptors and were reported to be functionally coupled to phosphoinositide hydrolysis in Syrian hamster RPMI 1846 melanoma cells (Pickering and Niles, 1992; Eison and Mullins, 1993). This observation is the key to the nature of MT3. These results may imply that the MT_3-binding site present on QR2 induces new functions for this enzyme. Finally, a set of molecules with strong affinity for the MT_3 membrane-binding sites does not have the same capacity to inhibit QR2 catalytic activity (Mailliet *et al*., 2005), suggesting that the characteristics of the MT_3-binding site at the membrane level were different from that of the cytosolic site involved in the control of QR2 catalytic activity. However, biophysical experiments did not confirm this hypothesis so far.

To rule out any possible bias, these points should be addressed *de novo* at the bench level. At least two theories should be explored. These pharmacological differences might be explained by the presence of QR2 monomers at the membrane level. As a reminder, QR2 is a homodimer. The monomer would not have any catalytic activity—in line with the fact that both catalytic sites of the QR2 homodimer are formed of amino acids belonging to each monomer—but would have a large portion of its sequence, of hydrophobic nature and normally implicated in the monomer–monomer interface, available for association with membranes. The simplest explanation, though, would be that a portion of the QR2 homodimers is associated with the plasma membrane. One hypothesis is that QR2 possesses a cryptic myristoylation site. Myristoylation is known to play a role in membrane addressing (Boutin, 1997). This site could become available for *N*-myristoyltransferase—which catalyses the transfer of myristate moiety onto the N-terminal glycine of target proteins—after cleavage by caspase, as demonstrated for another protein, bid, a member of the bcl2 antiapoptotic protein family (Degli Esposti *et al*., 2003). These issues should be explored using confocal microscopy, specific antibodies, subcellular fractionations (Antoine *et al*., 1993) and directed mutagenesis.

In contrast to a common belief (see Testa and Kramer, 2007 for review), QR2 might be an activating enzyme. Indeed, in three different models, the toxicity of menadione is enhanced by QR2: in K562 cells treated with QR2 siRNA (Buryanovskyy *et al*., 2004), in HT22 cells treated with QR2 shRNA (Chomarat *et al*., 2007) and in QR2$^{-/-}$ mice (Long *et al*., 2002). In all these cases, menadione was more toxic for biological systems in the presence of QR2 than in its absence. These are important features of QR2 and its MT_3-binding site. Indeed, their role in redox is now clearly stated and strongly suggests that its inhibition is beneficial for living organisms.

Resveratrol is a potent QR2 inhibitor (Buryanovskyy *et al*., 2004; Calamini *et al*., 2008). Resveratrol and melatonin have been reported in many studies to have antioxidant activities and protective effects, which might be due to their potencies to inhibit QR2 activity. The fact that inhibition of QR2 expression induces an upregulation of different enzymes with antioxidant properties supports this hypothesis (Buryanovskyy *et al*., 2004). The 'antioxidant' effect of melatonin is generally observed at high concentrations ($>1\,\mu M$) (Reiter *et al*., 1995) far from the physiological levels. Nevertheless, it should be kept in mind that melatonin can also be ingested with food (Tan *et al*., 2002; Reiter *et al*., 2007). Obviously, the local concentration of melatonin in subcellular compartments or in organs has not been reported so far, even after massive melatonin ingestion or treatment.

Although a review should be meant to generalize the observations and to try to make sense out of them, it seems that some other features of QR2 (see also Vella *et al*., 2005, for discussion) should be listed to complete the overall portrait of MT_3/QR2. For instance, Brouillette and Quirion (2007) reported that ageing rats (24 months and beyond) could be separated into two groups: one in which animals can retain a 'normal' level of learning, and another one with animals that cannot. Microarray experiments with rat brains of both groups showed that QR2 was among the genes that were the most strongly induced in the learning deficiency group. This suggests that QR2 has some deleterious effects in relation to the memory processes.

Finally, it is worth stressing that QR2 does not recognize the classical NAD(P)H co-substrates as a hydride donors, but rather some of their putative breakdown products, such as *N*-methyl- and *N*-ribosyl-nicotinamide (Zhao *et al*., 1997). As described recently by Belenky *et al*. (2007) in a review on NAD metabolism, these breakdown products are still of unknown origin(s). This is also a quite interesting fact, as without these NAD(P)H breakdown products QR2 is not capable of having any kind of catalytic activity, which strongly suggests an yet new level of QR2/MT_3 catalytic activity regulation.

An association between Parkinson's disease or schizophrenia and polymorphisms of QR2 has been reported (Harada *et al*., 2001, 2003). This polymorphism consists in an insertion of 29-bp nucleotides in the promoter region of the QR2 gene. Human cells expressing a promoter containing this insertion polymorphism demonstrate significantly higher QR2 gene expression (Wang and Jaiswal, 2006) and the catalytic activity thereof. Consequently, some neurological disorders including schizophrenia and Parkinson's disease might be associated with a higher expression of QR2, which would result in a higher susceptibility to external factors.

Conclusions/perspectives

After more than 10 years since the cloning of the melatonin receptor subtypes, their pharmacology is still not very well known. This may be due in part to the reasons mentioned above and to the lack of appropriate pharmacological tools. All the melatonin ligands reported until now are more or less close in chemical structure to that of melatonin or melatonin bio-isosteres. New ligands, mainly full and stable antagonists, not structurally related to melatonin should lead to new pharmacological tools. Nevertheless, the weak acute effect of melatonin in different animal models, with the exception of the clear but subtle effect of melatonin on the circadian rhythm, is also a possible reason. Indeed, melatonin or specific melatonin receptor agonists generally

have modulator effects against other molecules such as neurotransmitters and their pharmacology might involve other partners.

The finding that MT_3 and QR2 are the same protein, initially taken with much caution by the scientific community, might indeed shed new light on the role of melatonin at high concentrations (that is, $>1\,\mu M$), in oxidative stress and, somehow, one might now hope to better understand at least some of the antioxidant properties of melatonin through a molecular mechanism of the neurohormone at the QR2 level.

Furthermore, the existence of other not yet identified melatonin receptor types is very plausible. Although two melatonin receptor subtypes have been cloned from mammalian species, the MT_2 receptor is not present as a functional receptor in all mammals. For instance, in Siberian hamster the gene encoding the MT_2 receptor contains a premature stop codon, which means that the protein is not expressed. Nevertheless, these animals are highly seasonal and melatonin treatments mimic short-day exposure and induce all the characteristic behavioural and physiological modifications induced by short photoperiod (body weight decrease, pelage colour change, gonadal regression, hormonal status, etc.) (Bartness and Goldman, 1988). It is difficult to understand how a single melatonin receptor subtype may be involved in all these changes on top of the circadian effects.

Further complexity may be generated by the formation of heterodimers between MT_1 and MT_2, at least in cells expressing the two melatonin receptor subtypes. The orphan receptor GPR50 does not bind melatonin and its endogenous ligand is still unknown. Nevertheless, this receptor has been shown to behave as an antagonist of the MT_1 receptor, which opens new pharmacological perspectives for GPR50 despite the lack of identified endogenous or synthetic ligands and emphasize the complexity of the melatonin receptor field.

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Conflict of interest

JA Boutin and P Delagrange are employees of the Institut de Recherches Servier.

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