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Characterization of a melatonin binding site in Siberian hamster brown adipose tissue

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Abstract

Melatonin has been shown, in various rodent species, to mediate photoperiodic effects on body weight and, consequently, fat mass. Pharmacological investigations indicated that the brown adipose tissue of Siberian hamsters possesses a melatonin binding site with a dissociation constant of 570 ± 300 pM and a density of 3.2 ± 1.8 fmol/mg protein. This binding site can also be detected on mature brown adipocyte membranes. The rank order of potency of a variety of drugs to displace 2-[^{125}I]iodomelatonin from binding sites on Siberian hamster brown adipose tissue was as follows: 2-iodomelatonin > melatonin = prazosin > GR135531 (5-methoxycarbonylamino-*N*-acetyltryptamine) > *N*-acetylserotonin > 6-chloromelatonin > S20304 (*N*-(2-(1-naphthyl)ethyl)cyclobutanecarboxamide) ≫ methoxamine, phenylephrine, serotonin. Mel_{1a} mRNA was not detected by RT-PCR (reverse transcription-polymerase chain reaction) in brown adipose tissue. Melatonin had no effect on either basal or stimulated lipolysis. Moreover, melatonin did not modify intracellular cAMP accumulation or inositol phosphate content. Together, these results suggest that the melatonin binding site characterized in brown adipose tissue is clearly different from the Mel_1 cloned subtype and has some features different from those of the Mel_2 subtype. © 1997 Published by Elsevier Science B.V.

Keywords: Melatonin; Brown adipocyte; Melatonin receptor; (Siberian hamster)

1. Introduction

The photoperiod regulates a variety of physiological processes in mammals including the reproductive state, coat growth, appetite and body weight changes (Goldman and Nelson, 1993). These last changes are always correlated with variations in fat mass (Bartness et al., 1989) and are mainly mediated by the nocturnal synthesis and release of the pineal hormone melatonin. This has been demonstrated by infusing melatonin in pinealectomised hamsters (Bartness and Wade, 1985), or by injecting once daily a melatonin agonist or antagonist into the Garden dormouse (Le Gouic et al., 1996).

In Siberian hamsters, a decrease in fat mass was observed after a few weeks of exposure to a short photoperiod (Wade and Bartness, 1984; Bartness et al., 1989). This decrease was associated with thermogenic activation of brown adipose tissue (Wade and Bartness, 1984; McElroy et al., 1986; Wiesinger et al., 1989; Mercer et al., 1994), which is the main effector of non-shivering thermogenesis in rodents (Himms-Hagen, 1990). This activation of brown adipose tissue is mimicked by melatonin implants (Heldmaier and Steinlechner, 1981) and can be partly explained by an enhancement of sympathetic activity (McElroy et al., 1986). However, a direct effect of melatonin on brown adipose tissue can not be excluded.

Melatonin has been demonstrated to act through specific receptors (Morgan et al., 1994). To date, two melatonin binding sites have been distinguished according to pharmacological and kinetic parameters. A very high-affinity site (K_d in the pM range) sensitive to guanine nucleotides is coupled to inhibition of adenylyl cyclase via a pertussis toxin-sensitive G_i protein (Morgan et al., 1994). Melatonin binding sites exhibiting these features corre-

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spond to the recently cloned melatonin receptor, Mel_1 (Ebisawa et al., 1994; Reppert et al., 1994, 1995). A second binding site with a lower affinity (nanomolar range) and showing a different pharmacological profile has been described in a few studies. This binding site is neither sensitive to guanine nucleotides nor coupled to adenylyl cyclase, and has not yet been cloned (Dubocovich, 1995). Both types of melatonin binding sites have been clearly identified within individual brain nuclei and in a few non-neural sites within the central nervous system (Morgan et al., 1994). Furthermore, the presence of 2-[^{125}I]iodomelatonin specific binding sites in various peripheral tissues, in rodents as well as in humans, has been reported in recent studies (Viswanathan et al., 1990; Pontoire et al., 1993; Song et al., 1995), suggesting that melatonin has peripheral effects. The presence of such peripheral melatonin receptors in the brown adipose tissue of Siberian hamsters suggests that melatonin has a direct effect on this thermogenic tissue. The aim of the present study was to investigate the presence of melatonin binding sites in brown adipose tissue.

2. Materials and methods

2.1. Materials

2-[^{125}I] iodomelatonin (SA 2200 Ci/mmol) was obtained from NEN (Dupont, Boston, MA); melatonin, 2-iodomelatonin, methoxamine and prazosin were from RBI Biochemicals (Natick, MA), and 6-chloromelatonin and GR 135531 (5-methoxycarbonylamino-*N*-acetyltryptamine) were from Tocris Cookson (St. Louis, MO). S20304 (*N*-(2-(1-naphthyl)ethyl)cyclobutanecarboxamide) was provided by Servier (Paris, France). DNase 1 was from Boehringer Mannheim (Mannheim, Germany), Superscript II reverse transcriptase and dithiothreitol were from Life Technologies (Gaithersburg, MD), deoxynucleotides, hexaprimers and placenta ribonuclease inhibitor were from Pharmacia (Uppsala), *Thermus aquaticus* DNA polymerase was from Appligene (Illkirch) and [^{3}H]myo-inositol was from Amersham International (Buckinghamshire). cAMP was measured with a RIA kit from NEN (Dupont, Boston, MA). All other drugs were purchased from Sigma Chemicals (St. Louis, MO).

2.2. Animals

Adult male and female Siberian hamsters (*Phodopus sungorus sungorus*) were obtained from our breeding colony. The animals were kept at 24°C, under a long photoperiod with a 16:8 h light–dark cycle, with food and water ad libitum. They were killed by decapitation. Interscapular and sternal brown adipose tissues were carefully dissected and used for membrane or isolated adipocyte preparations.

2.3. Membrane preparation

Brown adipose tissue was homogenized in ice-cold sucrose buffer (0.32 M sucrose, 50 mM Tris–HCl, pH 7.7, with 0.9 mM phenylmethylsulfonylfluoride, a protease inhibitor). Membranes were prepared by centrifugation at 4°C as described by Sillence and Matthews (1994) with slight modifications. The homogenate was centrifuged at 15,000 g for 15 min in order to eliminate the nuclear and mitochondrial fractions. Supernatants were centrifuged at 100,000 g for 90 min. Pellets were resuspended in the same buffer. Membranes (around 20 μg/μl) were stored at −80°C until used for binding experiments.

2.4. Radioligand binding studies

Binding assays were performed in 50 mM Tris–HCl buffer (pH 7.7) with 0.5 mM $MgCl_2$. For saturation studies, the reaction was initiated by addition of 100 μl membranes (2–3 μg protein/μl) to tubes containing increasing concentrations of 2-[^{125}I]iodomelatonin (from 0.03 to 2 nM). Non-specific binding was estimated at each concentration of radioligand with 1 μM melatonin. The levels of total, non-specific and specific binding to crude membrane preparations were correlated with total protein concentration (from 200 to 500 μg). The maximum variation in values obtained for a given binding condition did not exceed 10% and the values were highly reproducible. For competition studies, 500 pM 2-[^{125}I]iodomelatonin was incubated with drugs at various concentrations (from 10^{-13} to 10^{-4} M). In all cases, the binding of 2-[^{125}I]iodomelatonin was measured in triplicate after incubation at 4°C for 3 h in the dark. Reactions were terminated by addition of 4 ml of ice-cold Tris–HCl buffer, and the contents were immediately filtered through glass-fiber filters (Whatman GF/C) soaked in 0.5% polyethylenimine solution. Each filter was washed briefly (less than 10 s) three times with 4 ml of the cold buffer. The radioactivity was determined in a gamma counter. Protein concentration was measured by the method of Bradford (1976).

Data for saturation and competition experiments were analyzed by use of the EBDA/LIGAND program (McPherson, 1983).

2.5. Reverse transcription polymerase chain reaction experiment

Total RNA was isolated from frozen tissues of Siberian hamsters according to the single step method of Chomczynski and Sacchi (1987). The RNA pellet was dried and dissolved in distilled water. RNA were subjected to digestion with DNase 1 for 20 min at 37°C. Digested RNA was extracted with phenol/chloroform and precipitated with ethanol. DNA-free RNA (1 μg) was treated with 200 U reverse transcriptase (RT) in 20 μl RT buffer containing deoxynucleotides (4 mM), hexaprimers (5 μM), dithio-

threitol (10 nM), and 30 U placenta ribonuclease inhibitor. The reaction was carried out at 25°C for 10 min, then at 42°C for 50 min and the tubes were heated to 95°C for 5 min and chilled on ice.

PCR amplification was performed with 10 μl of each cDNA preparation with 0.5 μM of each primer and 1.25 U *Thermus aquaticus* DNA polymerase in a final volume of 50 μl. Samples were denatured for 3 min at 95°C and then PCR was performed for 35 cycles (30 s at 94°C, 20 s at 58°C, 30 s at 72°C). The primers used were selected in the coding region of the Mel_{1a} sequence, to amplify a band with an expected size of 336 bp. The sense primer (5′-TCCTGATATGGGTGTTGACG-3′) matched at a position corresponding to the beginning of the fourth transmembrane segment. The antisense primer (5′-CCCGATGAAATTGAGTGGCG-3′) matched at a position corresponding to the sixth transmembrane segment (as described by Reppert et al., 1994). The PCR products were analyzed on 2% agarose gels stained with ethidium bromide.

2.6. Isolated adipocyte preparation

Dissected brown adipose tissue was quickly placed in Krebs–Ringer bicarbonate buffer (KRB) equilibrated with 95% O_2–5% CO_2, pH 7.4, and containing 20 mM HEPES (*N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid]). Brown adipocytes were isolated as previously described by D'Allaire et al. (1995).

2.7. Lipolysis

Adipocytes were incubated for 90 min in 1 ml KRB containing 4% fatty acid-free bovine serum albumin (KRBA) at 37°C with gentle shaking (40 cycles/min) as previously described by Simard et al. (1994). For measurement of the sensitivity of the adipocytes to isoproterenol, the dose–response curves plotted for 10^{-9}–10^{-6} M were fitted by a computerized program (Sigma plot–Jandel Scientific). The EC_{50} is defined as the concentration of agonist inducing the half-maximal response.

2.8. Measurement of cAMP accumulation

Adipocytes were incubated at 37°C for 10 min in KRBA 4% buffer with 0.1 μM isobutylmethylxanthine (IBMX) (an inhibitor of phosphodiesterase). The reaction was stopped by addition of 2 ml of methanol/formic acid (95:5, v:v). After centrifugation at 3000 *g* for 15 minutes at 4°C, 100 μl of supernatant were evaporated under a flow of nitrogen at 60°C. One hundred microliters of phosphate buffer (pH 7.4) was added and the cAMP content was measured with a RIA kit from NEN.

2.9. Measurement of phosphoinositide metabolism

Isolated adipocytes from the sternal brown adipose depot were incubated in a KREBS buffer, 3.5% bovine serum albumin, supplemented with myo-[^{3}H]inositol (20 μCi/ml) and lithium chloride (10 mM) for 3 h at 37°C. The cells were washed three times in an isotope-free buffer containing 10 mM lithium chloride. Then 2.5 ml of packed cells was diluted to 20 ml with an isotope-free buffer containing 10 mM lithium chloride and 2 mM of cold myo-inositol. After 1 h, the incubation was stopped by addition of 1 ml of chloroform/methanol/HCl (2:1:0.08), and the two phases were separated by centrifugation at 2000 *g* for 10 min. Radioactive total inositol phosphates were chromatographed on a column packed with Dowex-1-X8 (formate form), and eluted with a solution of 1 M ammonium formate and 100 mM formic acid, according to Berridge et al. (1983).

2.10. Statistical analysis

Results are expressed as means ± S.E.M. Statistical analyses were performed by using Student's *t*-test for unpaired or paired data.

3. Results

3.1. Characterization of 2-[^{125}I]iodomelatonin binding on brown adipose tissue membranes

3.1.1. Pharmacological characterization

Melatonin binding sites were identified in crude preparations of brown adipose tissue membranes. Kinetic studies with 2-[^{125}I]iodomelatonin showed that binding to brown adipose tissue membranes was saturable and reversible. 2-[^{125}I]iodomelatonin binding at 4°C reached equilibrium within 60 min and was stable for at least 5 h of incubation (Fig. 1A). After 3 h of incubation, addition of melatonin (1 μM) led to a rapid and complete dissociation of 2-[^{125}I]iodomelatonin binding within 2 min (Fig. 1B). The k_{+1} value was calculated from the k_{obs} and the k_{-1}. The apparent dissociation constant ($K_d = 0.13$ nM) was calculated from k_{+1} and k_{-1} values (6.86×10^9 M^{-1} min^{-1} and 0.89 min^{-1}, respectively).

Saturation studies were performed with increasing concentrations of radioligand (from 0.03 to 2 nM) (Fig. 2). At 4°C, the binding was saturable at about 2 nM ligand. Scatchard analysis of the specific binding data was linear and showed a dissociation constant in the nanomolar range ($K_d = 0.57 \pm 0.3$ nM; $B_{max} = 3.2 \pm 1.8$ fmol/mg proteins; $r = 0.72$; $n = 5$) (Fig. 2A). K_d and B_{max} values obtained when the incubation temperature was 27°C ($K_d = 1.47 \pm 0.8$ nM; B_{max} 3.9 ± 1.5 fmol/mg proteins; $r = 0.82$) were not significantly different from the values obtained at 4°C (Fig. 2B). Nevertheless, at 27°C the non-specific binding was higher than at 4°C (60–70% at 27°C versus 30% at 4°C).

Melatonin binding sites were also detected on crude membranes prepared from isolated brown adipocytes (K_d

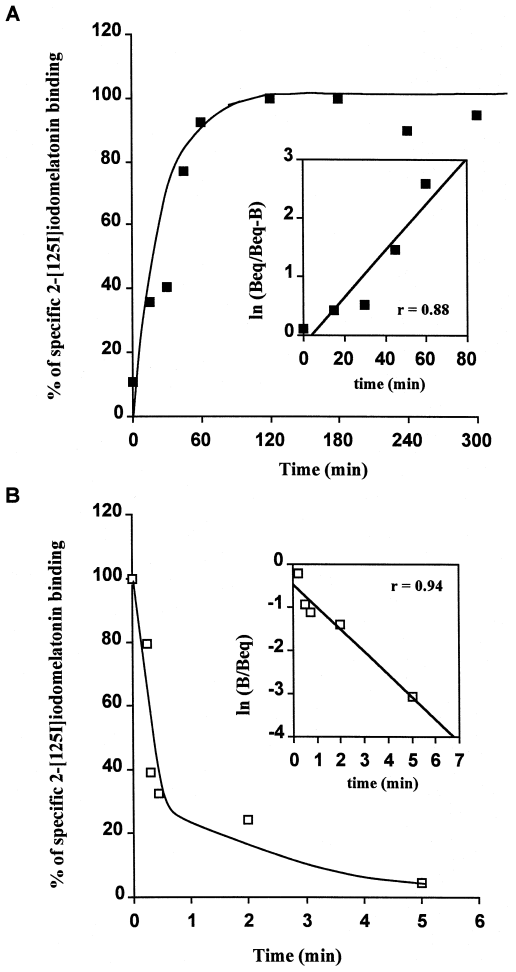

Fig. 1. Time course of association and dissociation of 2-[^{125}I]iodomelatonin to brown adipose tissue membranes from Siberian hamsters. (A) membranes were incubated with 250 pM of 2-[^{125}I]iodomelatonin at 4°C for the indicated times. Only specific binding is presented. Data linearization (insert) allowed calculation of the observed association rate constant (k_{obs}). (B) after binding equilibrium was reached, unlabelled melatonin (1 μM) was added to initiate dissociation of 2-[^{125}I]iodomelatonin binding. Data linearization (insert) allowed calculation of the dissociation rate constant (k_{-1}). Data are means of triplicate determinations. r is the correlation coefficient.

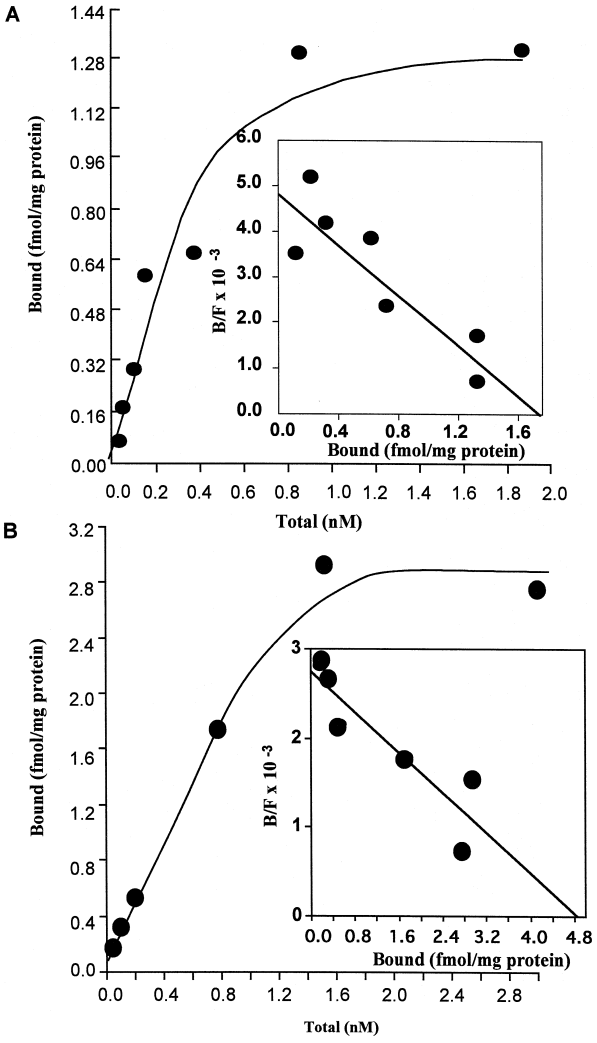

Fig. 2. Saturation and Scatchard analysis of 2-[^{125}I]iodomelatonin binding to brown adipose tissue membranes from Siberian hamsters. (A) membranes were incubated 3 h at 4°C with increasing amounts of radioligand (from 0.03 to 2 nM) in a total volume of 200 μl. Non-specific binding was measured in the presence of 1 μM melatonin. (B) membranes were incubated 30 min at 27°C. The data shown are from a representative experiment, and each point is the mean of triplicate determinations.

$= 0.36 \pm 1.1$ nM; $B_{max} = 1.9 \pm 0.2$ fmol/mg proteins).

Competition studies with 2-[^{125}I]iodomelatonin (500 pM) were carried out with drugs that have a chemical structure similar to that of melatonin, to define the pharmacological profile of the high-affinity 2-[^{125}I]iodomelatonin binding site in brown adipose tissue membranes. The K_i values are presented in Table 1. The most potent competitors of 2-[^{125}I]iodomelatonin binding to the membranes of brown adipose tissue from Siberian hamsters were melatonin, prazosin (α_1-adrenoceptor antagonist) and GR135531 (melatonin receptor agonist). The melatonin analog, S20304, displaced 2-[^{125}I]iodomelatonin binding to brown adipose tissue membranes with a K_i value of around 10^{-7} M. Serotonin, phenylephrine (α_1-adrenoceptor agonist) and methoxamine (α_{1A}-adrenoceptor agonist)

Table 1
Pharmacological profile of the 2-[^{125}I]iodomelatonin binding site in brown adipose tissue membranes prepared from Siberian hamsters: affinities of various drugs

Drugs	K_i (nM)
Melatonin	15 ± 7.2
Prazosin	14 ± 2.5
GR135531	19 ± 2.5
N-Acetylserotonin	25 ± 5
6-Chloromelatonin	40 ± 5
S20304	200 ± 59
Serotonin	1350 ± 340
Methoxamine	3000 ± 650
Phenylephrine	$> 100{,}000$

K_i values represent the mean of 3 independent determinations. Inhibition of specific binding of 2-[^{125}I]iodomelatonin (500 pM) to brown adipose tissue membranes was determined with competing drugs at concentrations between 10^{-13} and 10^{-4} M.

Table 2
Effect of melatonin on basal and forskolin or isoproterenol stimulated lipolysis in Siberian hamster brown adipocytes

	Glycerol (μmol/90 min/100 mg lipid)	
	control	melatonin (10^{-6} M)
Basal	0.11 ± 0.05 ($n = 5$)	0.13 ± 0.04 ($n = 5$)
Forskolin (10^{-5} M)	2.19 ± 0.23 ($n = 5$)	2.41 ± 0.45 ($n = 5$)
Isoproterenol (10^{-6} M)	3.96 ± 0.02 ($n = 4$)	4.08 ± 0.12 ($n = 4$)

Data are means $\pm$ S.E.M., n = number of independent experiments.

Table 3
Effect of melatonin on cAMP accumulation in Siberian hamster brown adipocytes

	cAMP (pmol/100 mg lipid)	
	control	melatonin (10^{-6} M)
Basal	0.25 ± 0.07 ($n = 4$)	0.31 ± 0.10 ($n = 4$)
Forskolin (10^{-5} M)	1.90 ± 0.19 ($n = 3$)	1.76 ± 0.08 ($n = 3$)

Data present means $\pm$ S.E.M., n = number of independent experiments.

were weak competitors of 2-[^{125}I]iodomelatonin binding on brown adipose tissue membranes. Norepinephrine and epinephrine did not displace 2-[^{125}I]iodomelatonin binding (data not shown).

3.1.2. Molecular investigation

RT-PCR studies (Fig. 3A) with specific primers for the Siberian hamster Mel_{1a} sequence were performed on RNA from brown adipose tissue. RNAs from Siberian hamster total brain were used as a positive control (Reppert et al., 1994). RT-PCR of β-actin mRNA was used as a control of RNA integrity (Fig. 3B). Results shown in Fig. 3 are representative of 3 independent experiments. As expected, in the presence of reverse transcriptase, a 336-bp band was revealed in brain (lane 3). In the absence of reverse transcriptase (lane 4), no signal was detected, thus excluding the presence of contaminant genomic DNA in the samples. No amplification product corresponding to Mel_{1a} was identified in brown adipose tissue RNAs (Fig. 3, lane 1 and lane 2). Under the same conditions, actin mRNA, used as a positive control in the experiment, was detected.

3.2. Effect of melatonin on lipolysis and intracellular cAMP

Melatonin (10^{-6} M and 10^{-8} M) did not alter basal or forskolin or isoproterenol stimulated glycerol release (Table 2). The maximum response or the sensitivity of the lipolytic response to isoproterenol, as assessed by the EC_{50} values of the dose–response curve, was not modified irrespective of the melatonin concentration used (EC_{50} = 46 ± 11.1 nM versus 40 ± 6.3 nM and 38 ± 7.4 nM with melatonin 10^{-6} M and 10^{-8} M respectively). Treatment of isolated brown adipocytes with forskolin (10^{-5} M) significantly increased basal lipolysis. Moreover, melatonin (10^{-6} M) did not alter either basal or forskolin-stimulated cAMP accumulation (Table 3).

3.3. Effect of melatonin on intracellular inositol phosphate accumulation

Phenylephrine (10^{-5} M) (a non specific α_1-adrenoceptor agonist) stimulated total inositol phosphate accumulation 8 times above basal values (82209 ± 10839 versus

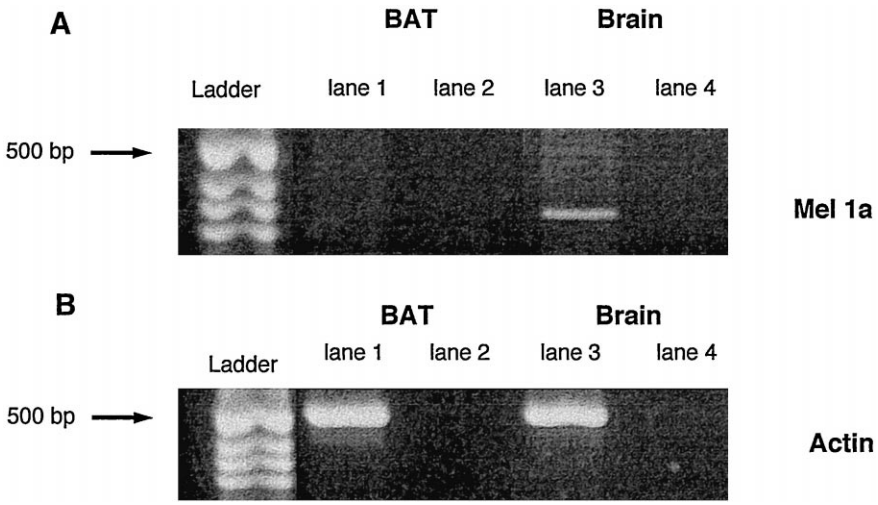

Fig. 3. RT-PCR analysis of Mel_{1a} receptor (A) and β-actin (B) gene expression in total brain and brown adipose tissue from Siberian hamsters. Lanes 1 and 3: RT-PCR experiments were performed in the presence of reverse transcriptase, lanes 2 and 4: RT-PCR experiments were performed in the absence of reverse transcriptase.

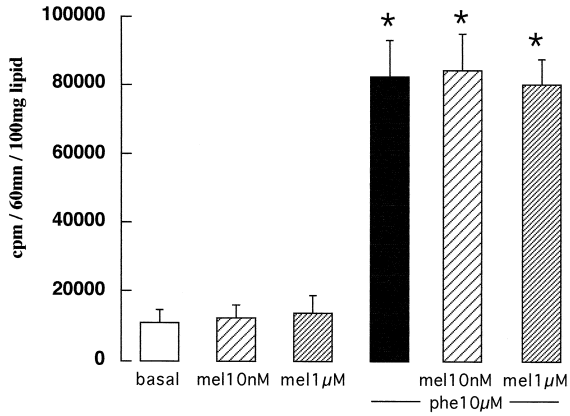

Fig. 4. Effects of melatonin (mel) on basal and phenylephrine (phe)-induced stimulation of inositol phosphate production by sternal brown adipocytes. Adipocytes were isolated from the sternal depot as described in Section 2 and were incubated in the presence of 10 nM or 1 μM of melatonin as indicated. Values are means ± s.e.m. of 5 independent experiments performed in triplicate. * Values statistically different from the basal values, $P < 0.05$ (control with vehicle DMSO).

10861 ± 3982 cpm/100 mg lipid) (Fig. 4). However, melatonin (10^{-8} or 10^{-6} M) did not significantly change the basal inositol phosphate content after 10 min of incubation, or the intracellular accumulation of inositol phosphates after phenylephrine stimulation.

4. Discussion

The present study demonstrated the presence of a specific binding site for 2-[^{125}I]iodomelatonin in brown adipose tissue and brown adipocytes of the Siberian hamster. Specific binding was reversible, saturable, of high affinity (around 10^{-10} M) and detectable at 4°C as well as at 27°C. Nevertheless, at 27°C the elevated non-specific binding values impaired to analysis of the competition studies. An incubation temperature of 4°C is usually used to investigate the presence of peripheral melatonin binding sites (Dubocovich, 1995).

In this study, the dissociation kinetics were very fast, which is surprising considering the incubation temperature and filtration technique used. Nevertheless, similar values were previously described with the same technique for glycine and adenosine binding sites (Popik et al., 1995; Zocchi et al., 1996). Moreover, at a low incubation temperature (15°C), the dissociation kinetics of 2-[^{125}I]iodomelatonin for human blood lymphocytes membranes are similar (Lopez-Gonzalez et al., 1992).

The brown adipose tissue binding site does not correspond to known 5-HT receptors because of the weak ability of 5-HT to displace 2-[^{125}I]iodomelatonin binding. The high ability of prazosin, which is generally classified as a specific α_1-adrenoceptor antagonist, to displace 2-[^{125}I]iodomelatonin binding could suggest the presence of an alpha-1 adrenergic binding site. However, the weak abilities of phenylephrine (a non-selective α_1-adrenoceptor agonist) and methoxamine (a specific α_{1A}-adrenoceptor agonist) to displace 2-[^{125}I]iodomelatonin binding in brown adipose tissue membranes clearly rejects the possibility that the binding site described in the current study corresponds to known α_1-adrenoceptors. The pharmacological profile for the iodomelatonin binding site described on brown adipose tissue was: melatonin = prazosin > GR135531 > *N*-acetyl serotonin > 6-chloromelatonin > S20304. This pharmacological profile has some similarity with the one described for Hamster brain and for RPMI 1846 melanoma cells (Dubocovich, 1995). Indeed, prazosin and GR135531 have a strong ability to displace iodomelatonin binding in brown adipose tissue and in Hamster brain. Until now, two receptors (Mel_{1a} and Mel_{1b}) of the melatonin receptor family have been cloned (Reppert et al., 1994, 1995). RT-PCR studies clearly demonstrate the lack of Mel_{1A} expression in Siberian hamster brown adipose tissue. Weaver et al. (1996) have shown that, in Siberian hamster, the Mel_{1b} receptor is a natural knockout because of the presence of two stop codons (Weaver et al., 1996). These molecular studies definitively rule out that the melatonin binding site found on Hamster brown adipose tissue belongs to the Mel_1 family.

Of the previously described non-Mel_1 binding sites, the brown adipose tissue melatonin binding site is quite different from the one reported by Pickering and Niles (1992). Indeed, the K_i values for melatonin as well as *N*-acetyl serotonin are 10-fold higher for the site present in Syrian hamster RPMI 1846 melanoma cells than for the site in brown fat. However, even if the brown adipose tissue site is more related to the one identified by Duncan et al. (1988) in hamster brain, the relative order of potency is somewhat different (melatonin = prazosin > GR135531 > *N*-acetyl serotonin > 6-chloromelatonin > S20304 in brown adipose tissue compared to 6-chloromelatonin > prazosin > *N*-acetyl serotonin > GR135531 ≥ melatonin, in hamster brain) (Dubocovich, 1995). These differences could indicate that there is another melatonin binding site subtype. It is noteworthy that in brown adipose tissue, melatonin was more potent in displacing iodomelatonin than its precursor, *N*-acetyl serotonin. The melatonin K_i value is compatible with the circulating melatonin concentration, which is the nanomolar range in Siberian hamsters (Darrow and Goldman, 1985).

The presence of a melatonin binding site in brown adipose tissue and particularly on brown adipocytes opens the question as to its associated transduction pathway, and its associated physiological effect. The main characteristic of brown adipocytes is their thermogenic capacity, due to the presence of a specific mitochondrial protein, the uncoupling protein (Himms-Hagen, 1990). Heldmaier and Steinlechner (1981) have demonstrated, in vivo, that a melatonin implant elicits a thermotropic response in brown adipose tissue from Siberian hamsters. The presence of a melatonin binding site on brown adipocytes logically sug-

gests that melatonin directly modulates this function at a cellular level. It is known that the rate of lipolysis, which is controlled by the intracellular cAMP content, is a good index of the state of brown adipocyte activation (Bukowiecky et al., 1981). The present data demonstrate that acute treatment with melatonin does not modulate lipolysis stimulated by isoproterenol (non-selective β-adrenoceptor agonist) or by forskolin, a direct activator of adenylyl cyclase. This indicates that an acute effect of melatonin on brown adipose tissue thermogenesis through peripheral melatonin receptors can be rejected. The absence of a melatonin effect on lipolysis is to be related to the lack of effect on cAMP accumulation. This result is consistent with our previous conclusion that the brown adipose tissue melatonin binding site is different from the Mel_1 subtype. It is noteworthy that the coupling of the melatonin receptor to signal transduction pathways other than the cAMP pathway has been reported in different systems (Dubocovich, 1995; Fischer et al., 1996). In RPMI 1846 melanoma cells, the melatonin binding site, which has been characterized as a Mel_2 receptor, is coupled to phosphoinositide metabolism (Eison and Mullins, 1993). This pathway appears not to be coupled to the melatonin binding sites on the brown adipose tissue of Siberian hamsters because melatonin did not affect total inositol phosphate accumulation. Together, these data confirm our previous hypothesis that the melatonin binding site characterized in brown adipose tissue is different from the Mel_2 subtype described by Dubocovich (1995).

In conclusion, this study, which was performed with a photoperiodic-sensitive species, is the first one to describe a melatonin binding site on brown adipocytes. Our results indicate that this binding site is clearly different from the Mel_1 and Mel_2 sites. Several points remain to be clarified, such as the nature of the receptor transduction pathway, and the characterization of its biological effects on brown adipose tissue. The information obtained will lead to a better understanding of the physiological effects of melatonin on this thermogenic tissue and on the regulation of energy metabolism.

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