Melatonin Binding Sites in the Brain of European Sea Bass
(Dicentrarchus labrax)

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ABSTRACT—Characteristics, day-night changes, guanosine 5′-O-(3-thiotriphosphate) (GTPγS) modula-
tion, and localization of melatonin binding sites in the brain of a marine teleost, European sea bass
Dicentrarchus labrax, were studied by radioreceptor assay using 2-[¹²⁵I]iodomelatonin as a radioligand. The
specific binding to the sea bass brain membranes was rapid, stable, saturable and reversible. The radio-
ligand binds to a single class of receptor site with the affinity (Kd) of 9.3±0.6 pM and total binding capacity
(Bmax) of 39.08±0.86 fmol/mg protein (mean±SEM, n=4) at mid-light under light-dark (LD) cycles of 12:12.
Day-night changes were observed neither in the Kd nor in the Bmax under LD 12:12. Treatment with
GTPγS significantly increased the Kd and decreased the Bmax both at mid-light and mid-dark. The binding
sites were highly specific for 2-phenylmelatonin, 2-iodomelatonin, melatonin, and 6-chloromelatonin. Dis-
tribution of melatonin binding sites in the sea bass brain was uneven: The Bmax was determined to be
highest in mesencephalic optic tectum-tegmentum and hypothalamus, intermediate in telencephalon, cer-
ebellum-vestibulolateral lobe and medulla oblongata-spinal cord, and lowest in olfactory bulbs with the Kd
in the low picomolar range. These results indicate that melatonin released from the pineal organ and/or
retina plays neuromodulatory roles in the sea bass brain via G protein-coupled melatonin receptors.

Key words: sea bass Dicentrarchus labrax, melatonin receptor, daily rhythm, brain

INTRODUCTION

Melatonin (N-acetyl-5-methoxytryptamine) is an indoleam-
ide synthesized in the pineal organ and retina of verte-
brates. Generally, melatonin production exhibits a clear daily
rhythm with high levels observed during the dark phase
under light-dark (LD) cycles. The duration of nocturnal
increase in melatonin production is influenced by day length
with longer duration under short photoperiod than under

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long photoperiod. These changes in melatonin regulate sea-
sonal rhythms such as reproduction and moulting (Binkley,
1987; Yu and Reiter, 1991).

In addition to the regulation by light, melatonin produc-
tion is regulated by circadian clock. In the mammalian pineal
gland, the circadian clock that regulates melatonin produc-
tion in the pineal locates in the suprachiasmatic nucleus
(SCN) of the hypothalamus, the site of primary circadian
pacemaker. The photic information is received in the retina
and sent to the SCN, which in turn regulates the pineal via
sympathetic nervous system (Binkley, 1987; Yu and Reiter,
1991). On the contrary, melatonin production in the pineal of
non-mammalian vertebrates and in the retina of vertebrates is regulated by the circadian clocks located in these photo-receptive structures (Iigo et al., 1994a; Cahill et al., 1991; Falcón, 1999). Thus, the pineal organ, retina and melatonin could be important components of the vertebrate circadian system.

The European sea bass (Dicentrarchus labrax) provides an interesting opportunity to analyze the roles of melatonin in the teleostean circadian system. Recent studies demonstrated the presence of daily and circadian feeding rhythms with a dual phasing capacity: some individual fed during the light phase (i.e. diurnal) but the other fed during the dark phase under LD (i.e. nocturnal) (Sánchez-Vázquez et al., 1995a,b, 1998). The information on such feeding habits provides some insights into the development of feeding regimes in aquaculture farms because this species is of great commercial values in Mediterranean regions. In addition, the presence of reversed melatonin profiles in the retina (with high ocular contents during the light phase under LD) has been demonstrated (Sánchez-Vázquez et al., 1997; Iigo et al., 1997d). The melatonin rhythms in the plasma and eyes were also influenced by environmental temperature, day length and light conditions (Iigo et al., 1997d; García-Allegue et al., 2001; Bayarri et al., 2002, 2003). Thus, this species is an interesting model to analyze the circadian system in marine fish.

In this study, in order to make the roles of melatonin in the sea bass circadian system clear, we characterized melatonin binding sites in the brain by radioreceptor assay using 2-[125I]iodomelatonin as the radioligand. Here we report characteristics, day-night changes, guanosine 5′-O-(3-thiotriphosphate) (GTP·S) modulation, and distribution of melatonin binding sites in the sea bass brain.

MATERIALS AND METHODS

Materials

2-[125I]iodomelatonin (81.4 TBq/mmol) were obtained from Daiichi Pure Chemicals (Tokyo, Japan). Unlabeled 2-iodomelatonin was obtained from Research Biochemicals Inc. (Natick, MA). Other indole derivatives, bovine serum albumin (BSA), polyethylenimine, dopamine, noradrenaline and GTP·S (tetra lithium salt) were obtained from Sigma (St. Louis, MO). Acetylcholine chloride was obtained from Nakarai Tesque, Inc. (Kyoto, Japan). Ordinary chemicals were obtained from commercial sources.

Membrane preparations and binding assays

Membrane preparations and binding assays were performed according to the procedure as previously described (Iigo et al., 1994b, 1997c) unless otherwise stated. In the present study, 50 mM Tris-HCl buffer containing 4 mM CaCl2 (pH 7.4) was used as the assay buffer. The binding of 2-[125I]iodomelatonin was routinely measured in duplicate after incubation at 25°C for 2 hr with the exception of kinetic studies where incubation duration varied. Protein contents in the incubation mixture were determined by the method of Bradford (1976) with bovine γ-globulin as a standard. Nonspecific binding was defined as the binding in the presence of 10 μM melatonin. Specific binding was calculated by subtracting nonspecific binding from total binding and expressed as fmol/mg protein. A preliminary study demonstrated the specific binding increased linearly at least up to 0.2 mg protein/tube.

Characterization, day-night changes and GTP·S modulation of melatonin binding sites in the sea bass whole brain

Sea bass (approx. 170 g in body weight) reared in net cages were supplied by a local fish farm (Culmarex, S.A., Aguillas, Murcia, Spain). Fish were reared in 350 l experimental tanks filled with artificial seawater (hw-Marinemix, Wiegandt GmgH, Germany) of 2.3% salinity at 24°C. Illumination (200 lx at the water surface) was supplied by 40 W daylight blue bulbs for the light phase of LD cycles (LD 12:12; light on 0800–2000 hr). During this period, sea bass were fed using demand feeding devices (Sánchez-Vázquez et al., 1997), displaying mainly nocturnal feeding behavior (data not shown).

Fish were anesthetized with 2-phenoxyethanol (0.6 ml/l around mid-light (1400 hr) or mid-dark (0200 hr). Blood samples were taken from caudal vasculature with a heparinized syringe with a 23-G needle and collected in test tubes on ice. Plasma was later separated by centrifugation and melatonin contents were determined by radioimmunoassay as previously described (Iigo et al., 1997e; Sánchez-Vázquez et al., 1997). Fish were then decapitated and whole brains were dissected out, frozen on dry ice and later used for kinetic, saturation and competition studies. Saturation studies with or without GTP·S (10−4 M) were performed using a range of 2-[125I]iodomelatonin concentration from 6.1 to 189.6 pM. Kinetic and competition studies were performed using a 2-[125I]iodomelatonin concentration of 49.9 and 48.0 pM, respectively.

Localization of melatonin binding sites in discrete brain areas

Sea bass (approx. 540 g in body weight) were obtained from the Laboratorio de Cultivos Marinos (CASEM, University of Cádiz, Spain). Fish were reared in 1000 l experimental tanks continuously supplied with running borehole water of 3.9% salinity at a temperature of 19.4°C under natural photoperiod. Fish were fed with commercial extruded pellets (Trouw Aquaculture, Spain).

On June 14, 2001, fish were decapitated during the light phase (around 1700 hr). Brain regions (olfactory bulbs, telencephalon, hypothalamus, mesencephalic optic tectum-tegmentum, cerebellum-vestibulolateral lobe, and medulla oblongata-spinal cord; see Fig. 1) were immediately dissected out, rapidly frozen on dry ice and later used for saturation analysis using 2-[125I]iodomelatonin concentration from 6.0 to 200.0 pM.

Data analysis

Data obtained from the kinetic, saturation and competition experiments were analyzed as previously described to calculate association rate constants (k+1), dissociation rate constants (k−1),

Fig. 1. Schematic representation of the sea bass brain (sagittal view) that depicts dissections used in the present study to localize melatonin binding sites in the sea bass brain: 1: olfactory bulbs; 2: telencephalon; 3: hypothalamus; 4: mesencephalic optic tectum-tegmentum; 5: cerebellum-vestibulolateral lobe; 6: medulla oblongata-spinal cord; 7: pituitary gland.
affinity (Kd), total binding capacity (Bmax), Hill coefficients (nH), and Ki values (Iigo et al., 1994b). Day-night changes in plasma concentrations of melatonin and in the Kd and Bmax of melatonin binding sites in the brain were analyzed by t-test. The Kd and Bmax in the GTP\gammaS-treated group were compared with respective control values by paired t-test. Analysis was performed using the GraphPad Prism program (GraphPad Software, San Diego, CA).

RESULTS

Kinetic Studies

The time course of the association of 2-[\text{\textsuperscript{125}}I]iodomelatonin (48.6 pM) to sea bass brain membranes at 25°C is shown in Fig. 2. The specific binding of 2-[\text{\textsuperscript{125}}I]iodomelatonin was rapid, stable and reversible. Association of 2-[\text{\textsuperscript{125}}I]iodomelatonin to sea bass brain membranes proceeded rapidly and reached a steady state at 2 hr. The k\textsubscript{1} determined from the pseudo-first-order plot was 8.84×10^{-8}±0.57×10^{-8} M^{-1} min^{-1} (n=5). The binding of 2-[\text{\textsuperscript{125}}I]iodomelatonin was stable during 2–4 hr of incubation. After 2 hr of incubation with 2-[\text{\textsuperscript{125}}I]iodomelatonin, dissociation was initiated by the addition of 8 µM melatonin to several of the tubes. The k\textsubscript{−1} calculated from the first-order regression analysis was 2.83×10^{-3}±0.34×10^{-3} min^{-1} (n=5). The kinetic dissociation constant calculated from the ratio k\textsubscript{−1}/k\textsubscript{1} was 3.45±0.60 pM.

Saturation studies

Membranes prepared from the sea bass brain collected both at mid-light and mid-dark were used for saturation studies using a range of radioligand concentrations from 6.1 to 189.6 pM with or without GTP\gammaS. Saturation curves, Scatchard plots, and calculated Kd, Bmax and nH values were shown in Figs. 3 and 4 and Table 1. The specific binding reached a plateau at approximately 100 pM (Fig 3A). A linear Scatchard plot (Fig. 3B) and the nH close to unity (1.05±0.02) revealed that 2-[\text{\textsuperscript{125}}I]iodomelatonin binds to a single class of sites with the Kd of 9.3±0.6 pM and the Bmax of 39.08±0.86 fmol/mg protein (n=4) at mid-light.

Day-night changes in the Kd, Bmax and nH and modulation by GTP\gammaS of melatonin binding sites in the sea bass brain are exhibited in Fig. 4 and Table 1. Although plasma concentrations of melatonin exhibited significant day-night changes with higher levels at mid-dark than at mid-light (P<0.05), neither the Kd nor the Bmax exhibited day-night changes (Table 1). Treatment with GTP\gammaS induced signifi-

![Fig. 2. The time course of association and dissociation of 2-[\text{\textsuperscript{125}}I]iodomelatonin (48.6 pM) to sea bass brain membranes at 25°C. Values shown are the means±SEM (n=5). The SEM is not visible when it is smaller than the symbol. The arrow indicates the addition of melatonin (8 µM) for the initiation of dissociation. The data were analyzed by pseudo-first-order plotting to estimate the association rate constant (k\textsubscript{1}=8.84×10^{-8}±0.57×10^{-8} M^{-1} min^{-1}, and by first-order plotting to calculate the dissociation rate constant (k\textsubscript{−1}=2.83×10^{-3}±0.34×10^{-3} min^{-1}) after the addition of melatonin.](https://bioone.org/journals/Zoological-Science/)

![Fig. 3. Results of a saturation study using whole brain membranes of the sea bass collected at mid-light. (A) A saturation curve of melatonin binding sites in the sea bass brain collected at mid-light. Values shown are the means±SEM (n=5). The SEM is not visible when it is smaller than the symbol. Total binding (TB): open circles; specific binding (SB): closed circles; nonspecific binding (NSB): open triangles. (B) A representative Scatchard plot.](https://bioone.org/journals/Zoological-Science/)
Fig. 4. Day-night changes of melatonin binding sites in the sea bass whole brain and their modulation by GTPγS. Saturation curves of melatonin binding sites in the sea bass brain collected at mid-light (A) and mid-dark (C). Values shown are the means±SEM (n=5). The SEM is not visible when it is smaller than the symbol. Representative Scatchard plots at mid-light (B) and mid-dark (D). The data for the mid-light control is the same as shown in Fig. 3. Open circles, the control group; solid circles, the GTPγS-treated group. The Kd, Bmax and nH values calculated from these data are shown in Table 1.

Table 1. Day-night changes of the Kd and Bmax and nH of melatonin binding sites in the brain and plasma melatonin concentrations of the sea bass under LD 12:12 and modulation of melatonin binding sites by GTPγS.

<table>
<thead>
<tr>
<th>Time</th>
<th>Group</th>
<th>Kd (pM)</th>
<th>Bmax (fmol/mg protein)</th>
<th>nH</th>
<th>Melatonin (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mid-light</td>
<td>Control</td>
<td>9.3±0.6</td>
<td>39.08±0.86</td>
<td>1.05±0.02</td>
<td>18.3±1.8</td>
</tr>
<tr>
<td></td>
<td>GTPγS</td>
<td>26.9±3.5**</td>
<td>18.29±2.44***</td>
<td>1.04±0.04</td>
<td>–</td>
</tr>
<tr>
<td>Mid-dark</td>
<td>Control</td>
<td>10.9±1.0</td>
<td>43.45±2.35</td>
<td>1.04±0.06</td>
<td>167.7±51.5a</td>
</tr>
<tr>
<td></td>
<td>GTPγS</td>
<td>31.8±5.6*</td>
<td>19.22±3.75**</td>
<td>1.05±0.06</td>
<td>–</td>
</tr>
</tbody>
</table>

The Kd, Bmax and nH values were calculated from the data obtained from saturation experiments (shown in Fig. 4) using 2-[125I]iodomelatonin concentrations from 6.1 to 189.6 pM. Melatonin concentrations in the plasma were determined by radioimmunoassay. The data shown are the means±SEM (n=4 for mid-light and n=3 for mid-dark). *P<0.05, **P<0.01, ***P<0.001 vs. respective control values. a P<0.05 vs. the mid-light value.
cant changes both in the Kd and Bmax with no change in the nH both at mid-light and mid-dark (Table 1).

Specificity
Specificity of melatonin binding sites in the sea bass brain is shown in Fig. 5. Competition experiments carried out using 2-[^125]Iiodomelatonin (48.0 pM) with several indole compounds and neurotransmitters demonstrated the following order of potency to inhibit specific binding: 2-phenylmelatonin = 2-iodomelatonin > melatonin > 6-chloromelatonin > 6-hydroxymelatonin > N-acetylserotonin> 5-methoxytryptophol = 5-methoxytryptamine. 5-hydroxytryptamine. Acetylcholine, dopamine and noradrenaline exhibited no inhibition even at a concentration of 10^-4 M (data not shown).

Localization of melatonin binding sites in discrete brain areas
Saturable 2[^125]Iiodomelatonin binding was detected in all brain areas with the Kd values in the low picomolar range (Fig. 6 and Table 2). However, melatonin binding sites in the sea bass brain exhibited uneven distribution: The Bmax values were highest in mesencephalic optic tectum-tegmentum and hypothalamus, intermediate in telencephalon, cerebellum-vestibulolateral lobe and medulla oblongata-spinal cord, and lowest in olfactory bulbs.

DISCUSSION
European sea bass has been used as an experimental model to analyze the circadian system in marine fish (Sánchez-Vázquez et al., 1995a, b, 1997, 1998; Iigo et al., 1997d; Garcia-Allegue et al., 2001; Bayarri et al., 2002, 2003). In the present study, in order to make the sites of melatonin action in the sea bass circadian system clear, we have examined characteristics of melatonin binding sites in the brain of the European sea bass.
1 subtype, MT1 (Mel 1a), MT2 (Mel 1b) and Mel 1c subtypes least three subtypes of melatonin receptors among the ML-
recent molecular studies demonstrated the presence of at
brates (Dubocovich, 1988, 1995; Reppert, 1997). Although
ity melatonin receptors (ML-1 melatonin receptors) in verte-
the sea bass brain was almost identical to that of high-affin-
plots and the nH close to unity. Finally, competition studies
demonstrated that the binding sites are highly specific to
melatonin and its related analogues. The Kd values
obtained from the kinetic and saturation studies are consist-
tent with picomolar affinity. The Kd values in discrete brain
areas of the sea bass (6.6–15.9 pM) are also well within the
physiological range of plasma melatonin concentrations
described above (~100 pM at night) (Iigo et al., 1997d; Sánchez-Vázquez et al., 1997; Garcia-Allegue et al., 2001; Bayarri et al., 2002, 2003).

Melatonin binding sites in the sea bass brain are highly
specific for melatonin and its related compounds that pos-
sess both 5-methoxy- and N-acetyl-substituents. Especially,
2-phenylmelatonin, 2-iodomelatonin, melatonin and 6-chlo-
romelatonin exhibited a high affinity. Melatonin precursors
such as N-acetylserotonin or metabolites such as 6-
hydroxymelatonin were less effective as compared to the
compounds described above. Furthermore, compounds
known to act at serotonergic, dopaminergic, cholinergic and
adrenergic sites were ineffective. These results indicate that
the sites labeled by 2-[125I]iodomelatonin are specific bind-
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potency of melatonin analogs to inhibit specific binding in
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1 subtype, MT1 (Mel1a), MT2 (Mel1b) and Mel1c subtypes
(Reppert, 1997), the subtype(s) expressed in the sea bass
brain remains to be determined. Further molecular biological
and pharmacological studies are required to elucidate this
subject in future.

In the present study, a non-hydrolyzable GTP analog
(GTPγS) was used to examine whether the melatonin bind-
ing sites in the sea bass brain are coupled to G protein phar-
macologically. Treatment of sea bass brain membranes with
GTPγS affected both the Kd and Bmax of melatonin binding
sites both at mid-light and mid-dark, indicating that they are
coupled to G protein as in other vertebrate species (Rep-
pert, 1997; Davies et al., 1994; ligo et al., 1997a, b; Gaildrat
et al., 1998; Amano et al., 2003). Further identification of the
type(s) of G protein and the effector system should be
required to elucidate the signal transduction pathway of
melatonin in this species.

Table 2. Localization of melatonin binding sites in the sea bass brain.

<table>
<thead>
<tr>
<th>Brain area</th>
<th>Kd (pM)</th>
<th>Bmax (fmol/mg protein)</th>
<th>nH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olfactory bulbs</td>
<td>15.9±3.0</td>
<td>2.27±0.23</td>
<td>1.00±0.12</td>
</tr>
<tr>
<td>Telencephalon</td>
<td>6.6±1.3</td>
<td>19.56±0.57</td>
<td>0.97±0.10</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>9.9±1.4</td>
<td>41.12±5.18</td>
<td>1.22±0.04</td>
</tr>
<tr>
<td>Optic tectum- tegmentum</td>
<td>12.4±2.4</td>
<td>57.30±8.95</td>
<td>1.21±0.02</td>
</tr>
<tr>
<td>Cerebellum-vestibulolateral lobe</td>
<td>6.6±0.4</td>
<td>15.77±1.06</td>
<td>0.97±0.11</td>
</tr>
<tr>
<td>Medulla oblongata-spinal cord</td>
<td>6.8±0.4</td>
<td>7.88±0.37</td>
<td>0.98±0.08</td>
</tr>
</tbody>
</table>

The Kd, Bmax and nH values were calculated from the data obtained from saturation experiments (shown in
Fig. 6) using 2-[125I]iodomelatonin concentrations from 6.0 to 200.0 pM in six brain regions as depicted in Fig. 1. Results are represented as the mean±SEM (n=5 except for the olfactory bulbs where n=3).

The specific binding of a radioiodinated melatonin ago-
nist 2-[125I]iodomelatonin to sea bass brain membranes ful-
fills almost all the criteria for binding to a functional receptor
site. First, kinetic studies demonstrated that the specific
binding was rapid, stable and reversible. Second, saturation
studies showed that the binding is of low capacity and high
affinity and that the radioligand appeared to label a single
class of binding sites as is evident from linear Scatchard
plots and the nH close to unity. Finally, competition studies
demonstrated that the binding sites are highly specific to
melatonin and its related analogues. The Kd values
obtained from the kinetic and saturation studies are consist-
tent with picomolar affinity. The Kd values in discrete brain
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type(s) of G protein and the effector system should be
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melatonin in this species.

In several teleost species such as the goldfish, catfish,
gilthead sea bream, pike, and masu salmon, the Bmax and/
or the Kd of melatonin binding sites in the brain exhibit daily
variations under LD (Iigo et al., 1994b, 1995, 1997c, 2003;
Falcón et al., 1996; Gaildrat et al., 1998; Amano et al.,
2003b). However, in the present study, neither the Kd nor
Bmax of melatonin binding sites in the sea bass brain
showed day-night changes, whereas plasma concentrations
of melatonin exhibited significant day-night changes. Similar
results were obtained in other teleost species such as rain-
bow trout, Atlantic salmon and Arctic charr (Davies et al.,
1994; Pang et al., 1994). These results indicate that the reg-
ulatory mechanism of melatonin binding sites differ among
species. Alternatively, melatonin binding sites in the brain do
exhibit daily variation but the use of whole brain homoge-
nates for binding assays and/or the sampling at only two
time points a day may have obscured variations in restricted
brain areas. This possibility should be verified in future.

Several studies demonstrated widespread distribution
of melatonin binding sites in the teleostean brain (Martinoli
et al., 1991; Ekström and Vaneczek, 1992; Davies et al.,
1994; ligo et al., 1994b, 1997c; Vernadakis et al., 1998;
Mazurais et al., 1999). The present study on the sea bass
brain also demonstrated a similar uneven distribution: Local-
ization of melatonin binding sites in discrete brain areas was
determined to be highest in the mesencephalic optic tectum-
tegmentum and hypothalamus, intermediate in the telen-
cephalon, cerebellum-vestibulolateral lobe and medulla
oblongata-spinal cord, and lowest in the olfactory bulbs. The
present results are consistent with the previous reports on
the goldfish, catfish, Atlantic salmon and rainbow trout.
Melatonin Receptors in Sea Bass Brain

The highest density in the optic tectum, a primary retinorecipient area, is a conserved feature among nonmammalian vertebrates, indicating importance of melatonin in the visual signal transduction (Rivkees et al., 1989; Wiechmann and Wirsig-Wiewehmann, 1993, 1994). High levels of melatonin binding in the hypothalamus were also notable among vertebrates. Melatonin binding sites in this area may modulate neuroendocrine functions. Furthermore, the SCN of the hypothalamus, the site of primary circadian pacemaker in mammals, is a target of melatonin in mammals and melatonin is involved in the resetting of the circadian clock (Yu and Reiter, 1992). It is of great interest to examine whether the SCN homologue of the sea bass expresses melatonin receptors. Furthermore, localization of melatonin binding sites in the other brain areas (olfactory bulbs, telencephalon, cerebellum-vestibulolateral lobe and medulla oblongata-spinal cord) indicates a variety of roles of melatonin in the central nervous system in fish. Further studies using in vitro autoradiography should be required to demonstrate precise localization of melatonin binding sites in the sea bass brain.

The present study also demonstrated the heterogeneity in the affinity of melatonin binding sites in discrete brain area of the sea bass, i.e., the Kd values were not uniform among brain areas. Relatively high Kd values (9.9–15.9 pM) were seen in the olfactory bulbs, optic tectum and hypothalamus while in the other brain areas the Kd values were in the range of 6.6–6.8 pM. These suggest site-specific expression of different subtypes of melatonin receptors in the sea bass brain. Recently, subtype selective melatonin receptor ligands such as 4-phenyl-2-propionamidotetralin (4P-PDOT) were developed and used to differentiate melatonin receptor subtypes expressed not only in cells transfected melatonin receptor subtype cDNAs but also in native tissues (Dubocovich et al., 1997, 1998). Further pharmacological studies using these selective ligands will be required to examine the subtype of melatonin receptors expressed in each brain area of the sea bass. In addition, molecular cloning of melatonin receptors and in situ hybridization histochemistry will be required to elucidate this subject in more details.

In several vertebrate species, melatonin binding sites are localized not only in the brain but also in the retina (Dubocovich and Takahashi JS, 1987; Lu et al., 1991; Wiechmann and Wirsig-Wiewehmann, 1994; Iigo et al., 1997b). Since the melatonin profile in the retina of the European sea bass is reversed (with high values during the light phase under LD cycles), it is of great interest to examine whether melatonin binding sites are localized in the sea bass retina. If so, to examine characteristics and daily variations of the melatonin binding sites in the retina may help to elucidate distinct roles of melatonin produced in the pineal organ and retina in the sea bass circadian system that regulates locomotor and feeding rhythms.

In conclusion, the present study has characterized melatonin binding sites in the brain of European sea bass. The results suggest that melatonin produced in the pineal organ act via melatonin binding sites (putative melatonin receptors) characterized in this study. Further studies using the sea bass will help to elucidate the roles of melatonin in the teleostean circadian system.

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These experiments comply with the Principles of Animal Care of the Institute of Health (1985), and also with the laws of the countries in which the experiments were conducted.

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