Ontogeny of 2-[^{125}I]iodomelatonin binding sites in the chicken (*Gallus domesticus*) kidney and spleen

Y. Song, A.M.S. Poon, G.M. Brown, and S.F. Pang

Abstract: To understand the possible role of melatonin receptors in the development of renal and immune functions, age-related variations of 2-[^{125}I]iodomelatonin binding sites in the chicken kidney and spleen were investigated by radioreceptor assay. Chickens at embryonic day 20, as well as 2 days, 9 days, 2 weeks, 6 weeks, 12 weeks, and 16 weeks after hatching, were kept under a 12 h light : 12 h dark photoperiod and killed at the middle of the light period. Binding sites for 2-[^{125}I]iodomelatonin in membrane preparations of the chicken kidney and spleen were present on embryonic day 20. The maximum binding densities ($B_{\max}$) in the kidney increased to a peak between 9 days and 2 weeks of age, then progressively decreased. $B_{\max}$ values of 2-[^{125}I]iodomelatonin binding sites in the chicken spleen were lower than in the kidney. The peak density in the chicken spleen was recorded at day 2 after hatching and decreased significantly after 6 weeks of age. There were no significant differences in binding affinities (K_d) in kidney and spleen of chicken in the different age groups studied. The unity of Hill coefficients of 2-[^{125}I]iodomelatonin binding sites of the chicken kidney and spleen in all age groups tested suggested that only a single class of binding sites was present in these tissues during development. It is proposed that the developmental changes in 2-[^{125}I]iodomelatonin binding sites in the chicken kidney and spleen may be pertinent to the development of diurnal rhythms of kidney functions and the post-pubertal decline in immune functions of the chicken.

Key words: melatonin receptor, renal system, immune system, pineal gland, development, age-related change.

Résumé : On a examiné les variations selon l'âge des sites de fixations de la 2-[^{125}I]iodomélatonine dans la rate et le rein de poulets, en utilisant une méthode faisant appel à des radiorécepteurs. On a maintenu des embryons de poulets de 20 jours, ainsi que des poulets âgés de 2 et 9 jours, et de 2, 6, 12 et 16 semaines dans un cycle photopériodique de 12 h clarté : 12 h obscurité, et on les a sacrifiés au milieu de la période de clarté. On a relevé des sites de fixation pour la 2-[^{125}I]iodomélatonine dans les préparations de membranes de la rate et du foie de l'embryon de 20 jours. Dans le rein, les densités de fixation maximale ($B_{\max}$) ont atteint un sommet entre l'âge de 9 jours et 2 semaines, puis ont diminué progressivement. Les $B_{\max}$ des sites de fixation de la 2-[^{125}I]iodomélatonine ont été plus faibles dans la rate qu dans le rein. Dans la rate, la densité de crête a été enregistrée à l'âge de 2 jours et a diminué significativement après 6 semaines. Chez tous les groupes examinés, aucune différence significative entre les affinités (K_d) de fixation du rein et de la rate n'a été observée; de plus, l'unité des coefficients de Hill de tous les sites de fixation de la 2-[^{125}I]iodomélatonine du rein et de la rate a indiqué qu'il n'y avait qu'une seule classe de sites de fixation dans ces tissus durant le développement. On suggère que les variations développementales des sites de fixation de la 2-[^{125}I]iodomélatonine dans le rein et la rate de poulets pourraient favoriser le développement des rythmes diurnes des fonctions rénales et le déclin post-pubertaire des fonctions immunes du poulet.

Mots clés : récepteur de mélatonine, système rénal, système immunitaire, glande pinéale, variation liée à l'âge du développement.
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Introduction

In the past few decades, physiological functions of melatonin have been studied extensively. There is considerable evidence that melatonin may be involved in the regulation of kidney functions (Reiter 1991). In patients with essential hypertension, melatonin administration (2 mg/day) was reported to decrease blood pressure (Birau et al. 1981). This finding is supported by reports in rats that pinealectomy elevates blood pressure (Zanoboni and Zanoboni-Muciaccia 1967) and increases renin secretion (Karppanen and Vapaatalo 1971). Furthermore, in rats, melatonin had an antidiuretic action, which was much less potent than that of serotonin (Arutyunyan et al. 1963), and pinealectomy produced an increase in urine excretion (Karppanen and Vapaatalo 1971). In contrast to these studies in the rat, in hamsters melatonin injections (25 μg/day) increased urine output (Richardson et al. 1992), indicating that there may be significant species differences in this relationship (Richardson et al. 1992).

A relationship between melatonin and cation metabolism in the rat was proposed on the basis of a change in sodium levels and elevation of circulating calcium, magnesium, and zinc levels produced by suppression of melatonin secretion by constant light (Morton 1990). Csaba and Bokay (1977) found a decrease in circulating levels of magnesium and calcium following the administration of melatonin (10 $\mu g \cdot 100\ g^{-1}$ body weight · day^{-1}). Furthermore, a reduction in sodium and potassium excretion was reported after pinealectomy (Karppanen and Vapaatalo 1971). The demonstration of 2-[^{125}I]iodomelatonin binding sites in kidneys of several mammalian (Song et al. 1993*a*, 1993*b*) and avian species (Song et al. 1993*a*) is consistent with the hypothesis of a direct action of melatonin on the renal system regulating renin secretion and urine and cation excretion.

Melatonin has been shown to stimulate the mammalian immune system (Maestroni 1993). In birds, melatonin regulates the diurnal rhythm of immune parameters (Skwarlo-Sonta et al. 1991; Rosolowska-Huszca et al. 1991). Pinealectomy modified the circadian rhythms of granulocyte number and serum lysozyme concentration in the chicken, which were restored to normal by daily melatonin injections (10–20 ng/day) (Rosolowska-Huszca et al. 1991). In addition, exogenous melatonin (10–20 ng/day) affects diurnal rhythms of white cell counts and serum agglutinin levels in chickens (Skwarlo-Sonta et al. 1991). However, Skwarlo-Sonta et al. (1992) could not demonstrate any immunostimulatory effects of melatonin (1–100 $\mu g \cdot kg^{-1}$ body weight · day^{-1}) in chickens and concluded that the role and (or) mechanism of action of melatonin may differ between avian and mammalian immune systems. The identification of high affinity 2-[^{125}I]iodomelatonin binding sites in the bursa of Fabricius (Liu and Pang 1993), thymus (Liu and Pang 1992), and spleen (Yu et al. 1991; Pang and Pang 1992; Poon et al. 1993) of various avian species led to the suggestion of a direct action of melatonin on the avian immune system (Poon et al. 1994).

Pineal and plasma melatonin levels are elevated during the nocturnal period (Arendt 1985) and seasonal changes are associated with the natural light–dark cycle (Pang et al. 1993*b*). Age-related changes in pineal and serum melatonin levels have been documented in human (Brown et al. 1979; Pang et al. 1985; Waldhauser et al. 1988), rat (Reiter et al. 1981; Tang and Pang 1988), hamster (Reiter et al. 1980), and chicken (Pang et al. 1993*a*). It is also well known that the risk of developing hypertension increases with age. Renal circadian rhythms are immature in infants and develop fully after months or even years (Koopman et al. 1989). The circadian rhythm of renal functions, which may be related to the diurnal secretion of melatonin, diminishes with age (Koopman et al. 1989). Therefore, a developmental study of 2-[^{125}I]iodomelatonin binding sites in the kidney may provide information relevant to understanding renal melatonin receptor functions.

A decline in immunity with age is also well documented. It has been suggested that the decrease in circulating melatonin levels may contribute to the impaired immunity in aged animals (Pierpaoli and Maestroni 1987; Armstrong and Redman 1991; Grad and Rozencwaig 1993). If melatonin acts directly on the lymphoid tissue (Poon et al. 1994) and is related to the age-related decline in immunity, developmental changes in lymphoid melatonin receptors may also contribute to the decline in immunity with age. In fact, an age-associated decline in the density of the 2-[^{125}I]iodomelatonin binding sites has been reported in rat pituitary (Vanecek 1988; Laitinen et al. 1992), hypothalamus and hippocampus (Zisapel et al. 1989), and caudal and anterior cerebral arteries (Laitinen et al. 1992), and has been related to changes in various neuroendocrine functions with age. In lymphoid tissues, a decrease in 2-[^{125}I]iodomelatonin binding in the chicken bursa of Fabricius at about 12 weeks of age has been reported (Liu and Pang 1993). Whether the age-related decline is due to a change in the binding site density or affinity is unknown. Thus, a survey of the developmental changes in lymphoid 2-[^{125}I]iodomelatonin binding sites is warranted. The chicken was chosen for these studies because of the higher density of 2-[^{125}I]iodomelatonin binding sites in the kidney (Song et al. 1993*a*) and spleen (Poon et al. 1994) in this species. Moreover, in chicken lymphoid tissues, the spleen may contain a subtype of melatonin receptors similar to that of the kidney (Poon and Pang 1994), whereas the bursa of Fabricius may contain another subtype. Therefore, it is of interest to determine whether the chicken kidney and spleen have similar age-related changes in their expression of 2-[^{125}I]iodomelatonin binding sites.

Materials and methods

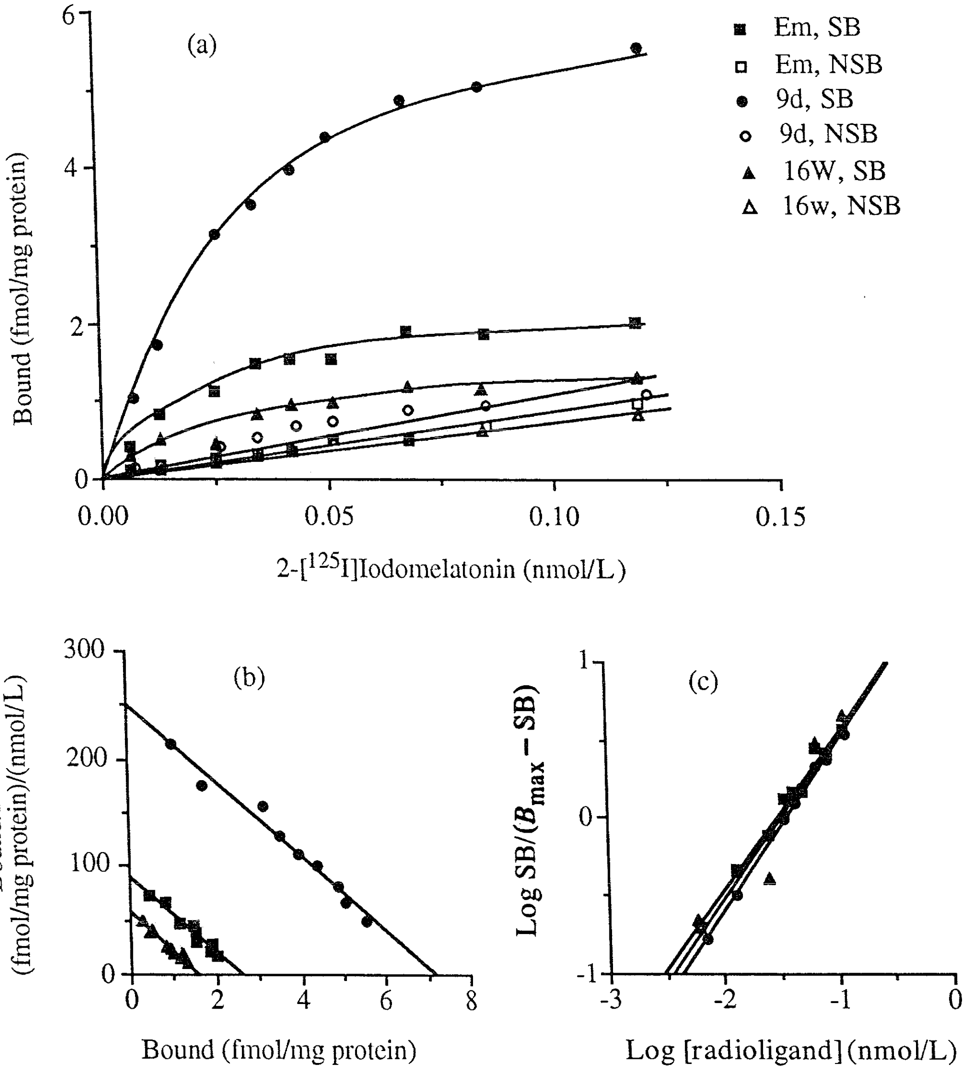
Chemicals

2-[^{125}I]Iodomelatonin (specific activity, 2200 Ci/mmol (1 Ci = 37 GBq)) was obtained from NEN Research Products, Dupont Co., Boston, Mass. Other chemicals were purchased from Sigma Chemical Co., St. Louis, Mo.

Animals, housing and sample collection

Male and female chickens (*Gallus domesticus*) were obtained from the Laboratory Animal Unit, University of Hong Kong, and used in these experiments following the principles and guidelines of the Canadian Council on Animal Care. The animals were housed under a 12 h light : 12 h dark cycle (lights on 03:00–15:00). Light was provided by ceiling-mounted fluorescent tubes at an intensity of approximately 200 lx at the top of cages. Room temperature and humidity

Fig. 1. Saturation study of 2-[^{125}I]iodomelatonin binding sites in the kidney of Em, 9d, and 16w chickens. (*a*) Representative saturation studies of 2-[^{125}I]iodomelatonin in the chicken kidney. SB is defined as TB (data not shown) minus NSB. (*b*) Scatchard transformations of data from Fig. 1*a*, with K_d of 28.6, 28.7, and 27.4 pmol/L and B_{max} of 2.59, 7.16, and 1.59 fmol/mg protein in kidneys of Em, 9d, and 16w chickens, respectively. (*c*) Hill plots with coefficients of 0.981, 1.075, and 1.045 in kidneys of Em, 9d, and 16w chickens, respectively.



were maintained at 20–22°C and 60–70%, respectively. Food and water were available ad libitum. Chickens were sacrificed at embryonic day 20 (Em), and at 2 days (2d), 9 days (9d), 2 weeks (2w), 6 weeks (6w), 12 weeks (12w), and 16 weeks (16w) after hatching. For the kidney studies, samples from Em were pooled from kidneys of 3 chickens, and for the spleen assays, Em, 2d, 9d, and 2w samples were pooled from 12–16, 10–12, 7 or 8, and 3 or 4 chicken spleens, respectively. All chickens were decapitated at the midpoint of the light period in the Minor Operation Room of the Laboratory Animal Unit, University of Hong Kong. Kidney and spleen tissues were removed immediately, frozen in liquid nitrogen, and stored at −70°C. Preliminary data showed that there was no significant change in the binding of 2-[^{125}I]iodomelatonin in the chicken kidney and spleen between those stored at −70°C for 1 week and 3 months (Y. Song, A.M.S. Poon, and S.F. Pang, unpublished data).

Membrane preparations

Frozen tissues were thawed and homogenized in 10 volumes (w/v) of ice-cold 0.05 mol/L Tris-HCl buffer (pH 7.4 at 4°C). Homogenates were centrifuged at 40 000 × *g* for 25 min. Pellets were washed and resuspended in Tris-HCl buffer to a concentration of about 6 g protein/L. The protein concentration of the samples was determined by the method of Lowry et al. (1951), using bovine serum albumen as standard.

Binding assays

The receptor binding assay was carried out by the method previously described (Song and Pang 1992; Pang and Pang 1992). The assay was performed in duplicate. All samples were incubated with 0.005–0.12 nmol/L 2-[^{125}I]iodomelatonin at 37°C for 1 h. Nonspecific binding (NSB) was determined in the presence of 1 μmol/L unlabelled melatonin.

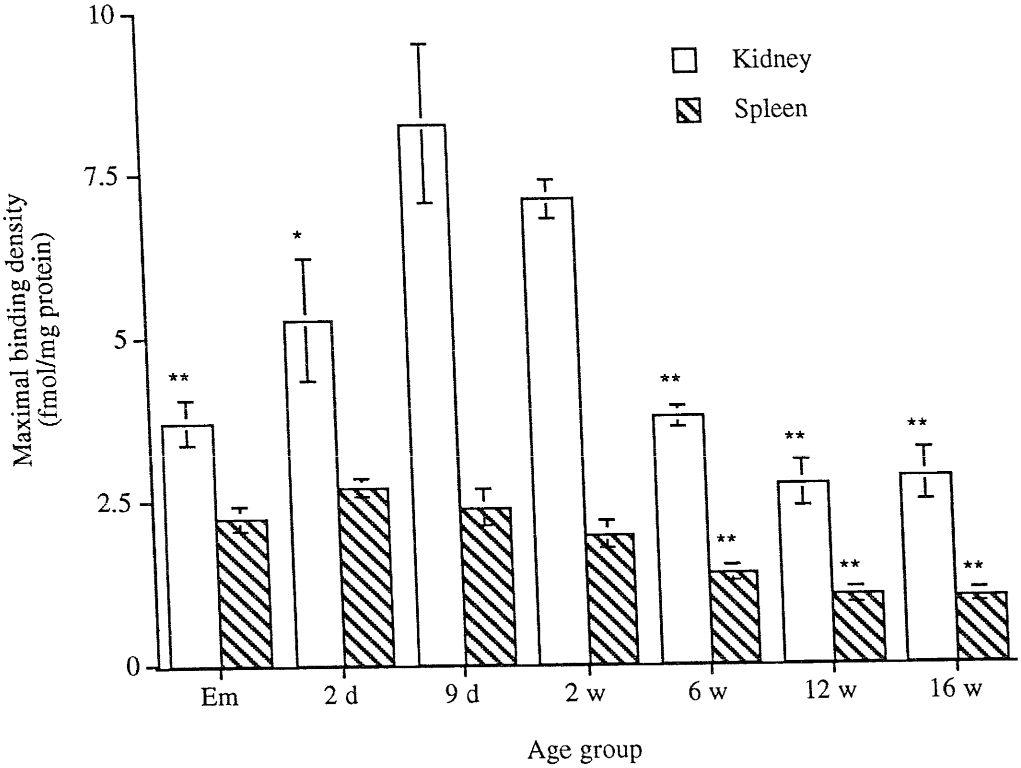


Fig. 2. $B_{\max}$ of 2-[^{125}I]iodomelatonin binding sites in the kidneys and spleens of Em, 2d, 9d, 2w, 6w, 12w, and 16w chickens. Data are means ± SEM of 5–10 tests. Statistical analysis showed significant differences in both kidney ($p < 0.01$, ANOVA, $F_{(6,37)} = 10.6$) and spleen ($p < 0.01$, ANOVA, $F_{(6,47)} = 12.3$). *$p < 0.05$, **$p < 0.01$, vs. corresponding highest $B_{\max}$ group.

Specific binding (SB) was calculated by subtracting NSB from total binding (TB). Radioactivity was determined by a gamma counter (Beckman Instruments Inc., Fullerton, Calif.) with 70% efficiency. The maximal binding density ($B_{\max}$) and equilibrium dissociation constant (K_d) were obtained from Scatchard analysis (Scatchard 1949).

Statistical analysis

Data are expressed as means ± SEM. Group differences were analyzed by one-way analysis of variance (ANOVA) with Systat 5.2.1. followed by Fisher's least significant difference tests of pairwise comparison (Systat, Inc., Evanston, Ill.), taking $p < 0.05$ as the criterion of significance. Preliminary studies in our laboratory showed that binding of 2-[^{125}I]iodomelatonin in the chicken kidney and spleen has no sex difference (Y. Song, A.M.S. Poon, and S.F. Pang, unpublished data). Hence, results of male and female chickens were grouped together for analysis.

Results

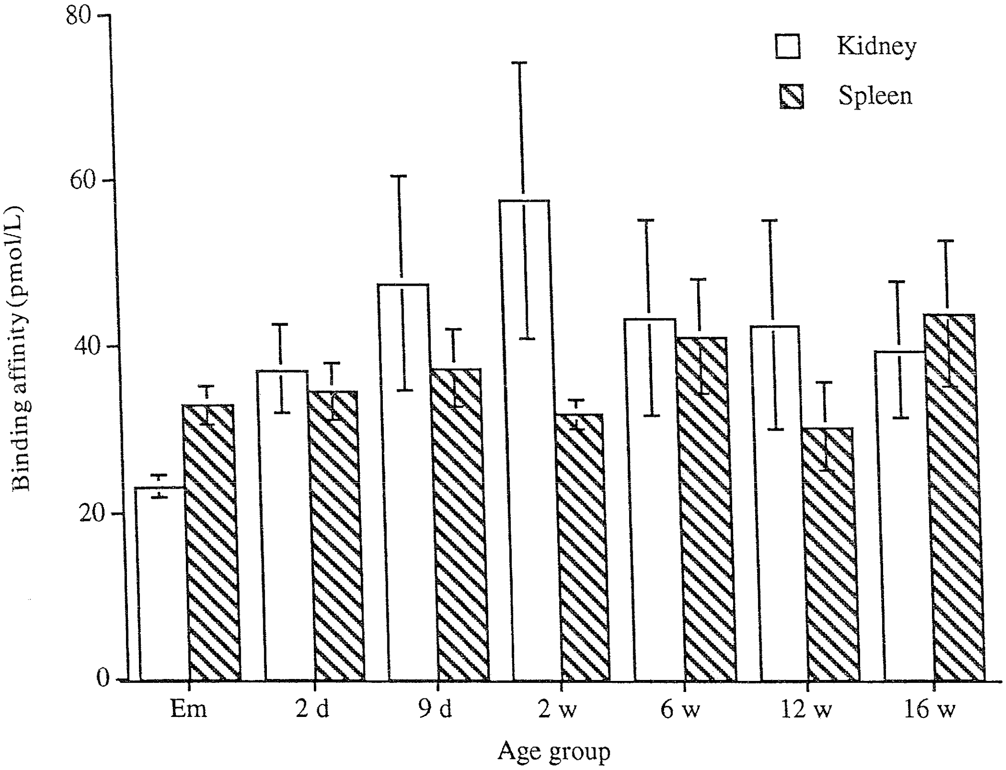
Chicken kidney

Binding of 2-[^{125}I]iodomelatonin was present in chicken kidneys of all age groups tested. TB and NSB increased over the concentration range (0.005–0.12 nmol/L) of 2-[^{125}I]iodomelatonin in all cases tested. SB reached saturation at about 0.07–0.08 nmol/L 2-[^{125}I]iodomelatonin (Fig. 1*a*), which is consistent with data previously reported (Song and Pang 1992; Song et al. 1993*a*). Scatchard plots showed linear regression, as indicated in Fig. 1*b*, which shows representative saturation studies of Em, 2w, and 16w old chickens. Hill coefficients approached unity in all the cases tested (Fig. 1*c*). $B_{\max}$ of 2-[^{125}I]iodomelatonin binding sites in the chicken kidneys differed significantly between various age groups ($p < 0.01$, ANOVA, $F_{(6,37)} = 10.6$) (Fig. 2). $B_{\max}$ gradually increased from Em (3.74 ± 0.35 fmol/mg protein) to 9d (8.28 ± 1.20 fmol/mg protein) and reached its peak level at about 9d–2w. After 2w, $B_{\max}$ of 2-[^{125}I]iodomelatonin binding sites in the chicken kidney progressively decreased, with $B_{\max} = 2.88 \pm 0.40$ fmol/mg protein at 16w. The concentration of 2-[^{125}I]iodomelatonin binding sites in the kidney of 16w chicken was about 35% of the 9d group. The K_d ranged from 23.2 to 57.4 pmol/L and showed no significant difference among the various age groups tested ($p > 0.05$, ANOVA, $F_{(6,37)} = 1.04$) (Fig. 3).

Chicken spleen

Binding of 2-[^{125}I]iodomelatonin in the chicken spleen was present in all age groups tested. Similar to the data reported by Pang and Pang (1992), TB and NSB of 2-[^{125}I]iodomelatonin increased over the range of 0.005–0.12 nmol/L 2-[^{125}I]iodomelatonin tested. SB approached saturation at 0.08–0.10 nmol/L 2-[^{125}I]iodomelatonin (Fig. 4*a*). Scatchard plots of different age groups were linear (Fig. 4*b*), and Hill coefficients approached 1.0 in all the cases examined (Fig. 4*c*). There were significant differences in the $B_{\max}$ of 2-[^{125}I]iodomelatonin binding sites in the chicken spleen at different stages

Fig. 3. Binding affinity of 2-[^{125}I]iodomelatonin binding sites in the kidneys and spleens of Em, 2d, 9d, 2w, 6w, 12w, and 16w chickens. Data are expressed as mean ± SEM of 5−10 examinations. ANOVA shows no significant differences in either kidney ($p > 0.05$, ANOVA, $F_{(6,37)} = 1.04$) or spleen ($p > 0.05$, ANOVA, $F_{(6,47)} = 1.0$).



of development ($p < 0.01$, ANOVA, $F_{(6,47)} = 12.3$) (Fig. 2). B_{max} was 2.26 ± 0.19 fmol/mg protein at Em, 2.73 ± 0.15 fmol/mg protein at 2d, and 2.41 ± 0.27 fmol/mg protein at 9d. The values decreased significantly after 6w, with $B_{max} = 1.140 \pm 0.12$ fmol/mg protein at 6w ($p < 0.01$) and 1.03 ± 0.08 fmol/mg protein at 16w ($p < 0.01$). The K_d values (range 30.4−43.9 pmol/L) did not differ significantly during development ($p > 0.05$, ANOVA, $F_{(6,47)} = 1.0$) (Fig. 3).

Discussion

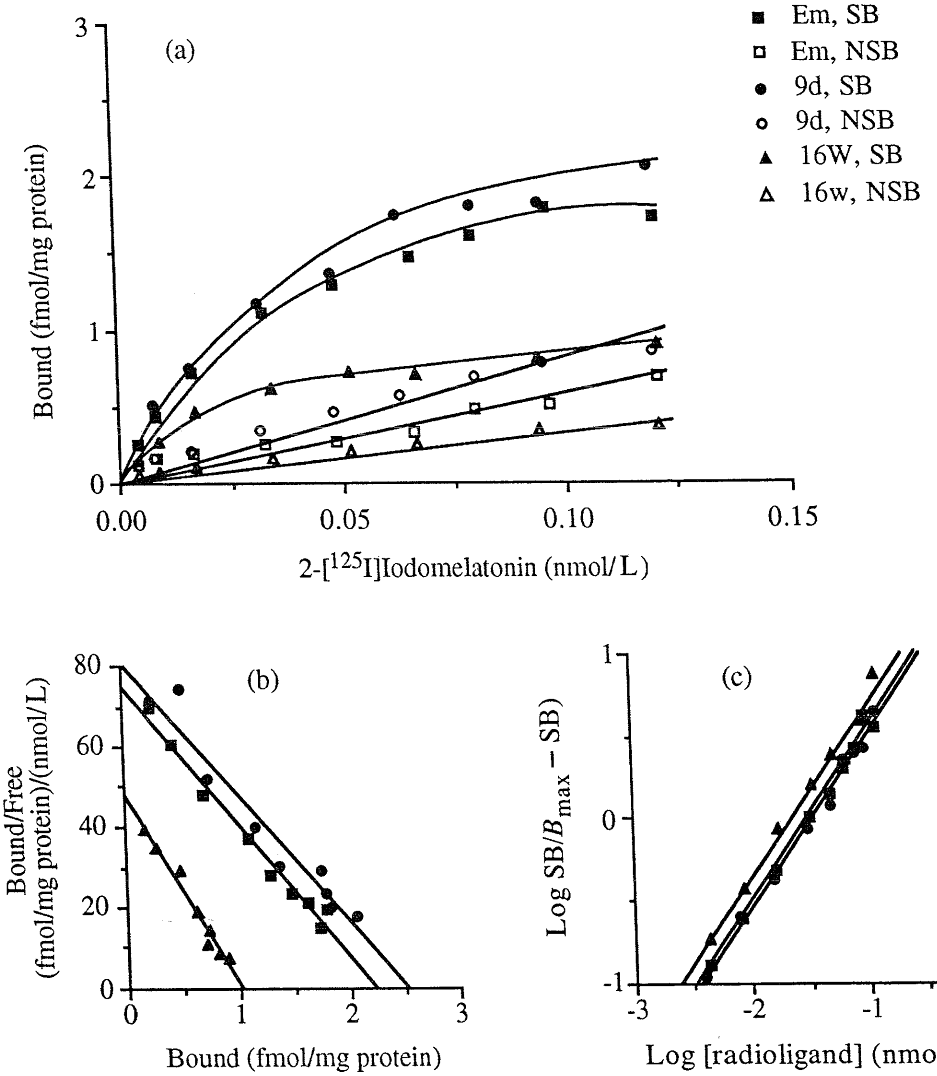
Previous reports of 2-[^{125}I]iodomelatonin binding sites in the chicken kidney (Song and Pang 1992; Song et al. 1993*a*) and spleen (Pang and Pang 1992; Poon et al. 1994) indicated that these binding sites were reversible, saturable, specific, and of high affinity. Reported values of K_d and B_{max} of 2-[^{125}I]iodomelatonin binding sites in the kidney of 800- to 1100-g chickens (about 2−3 months old, $K_d = 30.3 \pm 5.1$ pmol/L, $B_{max} = 2.89 \pm 0.24$ fmol/mg protein; Song and Pang 1992) are comparable with animals with similar ages in the present study. Comparing our data with those reported earlier in the chicken spleen (Pang and Pang 1992), the binding affinities are in the same range. However, we found a higher B_{max} in 2-week-old chicks (1.99 ± 0.20 fmol/mg protein) than 12-week-old chickens (1.05 ± 0.12 fmol/mg protein), whereas in that report (Pang and Pang 1992), a lower B_{max} was recorded in 3-week-old chicks (1.09 ± 0.11 fmol/mg protein) compared with 11-week-old chickens (1.5 ± 0.16 fmol/mg protein). This discrepancy may be due to interassay variations in the previous study. In the present experiment, spleens from all age groups were assayed together within a single assay so that differences among age groups could not be due to interassay variations.

In an earlier report by Yu et al. (1991), receptor binding assays were conducted in duck spleen membranes at 4°C for 5 h, whereas the later, detailed characterization of 2-[^{125}I]-iodomelatonin binding in the chicken spleen (Pang and Pang 1992) was performed at 37°C for 1 h. We have found that, in the chicken spleen, the K_d and B_{max} obtained under these two conditions are not significantly different (A.M.S. Poon and S.F. Pang, unpublished data).

The presence of high affinity 2-[^{125}I]iodomelatonin binding sites in the chicken kidney and spleen suggests that melatonin may play a role in regulating the renal and immune systems via melatonin receptors during growth and development. Over the growth period (embryonic age 20 days to 165 weeks after hatching), there was an age-related difference in B_{max} of 2-[^{125}I]iodomelatonin binding sites in both the chicken kidney and spleen. However, there was no significant change in K_d. The unity of the Hill coefficient and linearity of Scatchard plots for all age groups tested suggest that a single class of binding sites exists in the chicken kidney and spleen during development.

2-[^{125}I]Iodomelatonin binding sites in the kidney and spleen of chickens have similar binding affinities. These binding sites may belong to the ML-1 type of melatonin receptors (Song and Pang 1992; Pang and Pang 1992). It has been demonstrated that these binding sites in the kidney (Pang et al. 1993*a*) and spleen (Poon and Pang 1994) are

Fig. 4. Saturation study of 2-[^{125}I]iodomelatonin binding sites in the spleen of Em, 9d, and 16w chickens. (*a*) Representative saturation studies of 2-[^{125}I]iodomelatonin in the chicken spleen. SB is defined as TB (data not shown) minus NSB. (*b*) Scatchard transformations of data from Fig. 4*a*, with K_d of 29.5, 31.1 and 21.4 pmol/L and B_{max} of 2.22, 2.52 and 1.02 fmol/mg protein in Em, 9d, and 16w chickens, respectively. (*c*) Hill plots with coefficients of 1.041, 1.038, and 1.027 in spleens of Em, 9d, and 16w chickens, respectively.



linked to a G-protein; GTPγS decreased both B_{max} and K_d in the kidney (Pang et al. 1993*a*), as well as spleen (Poon and Pang 1994). These binding sites in both kidney and spleen may be classified as the same subtype of ML-1γ according to Pang et al. (1993*a*). This may explain the similar changes of 2-[^{125}I]iodomelatonin binding sites in the kidney and spleen during growth.

It has been reported that 2-[^{125}I]iodomelatonin binding sites were detected in the brain from embryonic day 8 chickens (Chong and Sugden 1992). In the rat, 2-[^{125}I]iodomelatonin binding sites in the pituitary were found as early as gestation day 15 (Williams et al. 1991) or 20 (Vanecek 1988). 2-[^{125}I]Iodomelatonin binding sites in the 10-day-old hamster fetus could be characterized in the primitive oral pharynx (Rivkees and Reppert 1991). Carlson et al. (1991) reported that 2-[^{125}I]iodomelatonin binding sites appeared in the pituitary, pineal gland, olfactory epithelium, and brain of hamster fetus. These findings are consistent with our identification of 2-[^{125}I]iodomelatonin binding sites in the kidney and spleen of embryonic chickens. *N*-Acetyltransferase, a key enzyme of melatonin biosynthesis in the chicken pineal, could be detected at embryonic days 16–19 (Binkley and Geller 1975). Hydroxyindole-*O*-methyltransferase was also present in primate pineals of near-term fetuses (Reppert et al. 1979, cited in Binkley 1988). The presence of 2-[^{125}I]iodomelatonin binding sites in the kidney and spleen of chicken embryo suggests that melatonin plays a role in regulating the renal and immune systems as early as the late embryonic stage.

Previous reports showed that the expression of melatonin binding sites during development varied with both tissues and species. In the rat pituitary, B_{max} of 2-[^{125}I]iodomelatonin binding sites was highest on embryonic day 20 and then decreased progressively during development, with no significant change of K_d. However, both B_{max} and K_d of 2-[^{125}I]-iodomelatonin binding sites in the median eminence showed no significant differences with age (Vanecek 1988). In the area postrema and suprachiasmatic nuclei of rats, melatonin receptors also did not change during development. The densities of melatonin receptors in the caudal artery, area postrema, and suprachiasmatic nuclei were highest in young rats (9 days old). There were minor changes in K_d, with a lower K_d being recorded in 9-day-old rats than in 96- and 306-day-old animals (Laitinen et al. 1992). Chong and Sugden (1992) reported that 2-[^{125}I]iodomelatonin binding sites in the chicken brain increased during embryonic development and remained stable during the first 30 days after hatching. The highest B_{max} was detected in the 3-month-old chicken. No significant differences of K_d during development were detected. In the chicken bursa, binding for 2-[^{125}I]iodomelatonin was highest in 2-day-old chickens, with an age-associated decrease in older chickens (Liu and Pang 1992). The above findings of minimal changes in K_d of 2-[^{125}I]-iodomelatonin binding sites with age is consistent with our present results of similar K_d during development in the chicken kidney and spleen. Our finding that the B_{max} of 2-[^{125}I]iodomelatonin binding sites in the kidney and spleen was higher in the younger chickens was similar to that reported in the rat pituitary (Vanecek 1988), arteries and anterior pituitary gland (Laitinen et al. 1992), and chicken bursa (Liu and Pang 1992), which differ from the rat median eminence (Vanecek 1988) and area postrema and suprachiasmatic nuclei (Laitinen et al. 1992) and the chicken brain (Chong and Sugden 1992). Tissue and species differences may be responsible for this discrepancy.

Our present studies describe patterns of 2-[^{125}I]iodomelatonin binding sites in the chicken kidney and spleen during development. Diurnal rhythms of blood pressure (Lemmer 1989), renin secretion (Gordon et al. 1966), glomerular filtration rate, urine flow, and sodium secretion (Koopman et al. 1989) have been documented, with higher levels during the day time. These rhythms may be related to postural changes, dietary factors, and daily activities (Lemmer 1989; Koopman et al. 1989). However, regulation by melatonin cannot be ruled out, as melatonin is a diurnally secreted hormone with peak levels at night (Reiter 1991). In addition, as noted earlier in the Introduction, melatonin showed effects on blood pressure (Zanoboni and Zanoboni-Muciaccia 1967; Karppanen and Vapaatalo 1971; Birau et al. 1981), urine output (Arutyunyan et al. 1963; Karppanen and Vapaatalo 1971; Richardson et al. 1992), and cation metabolism (Karppanen and Vapaatalo 1971; Csaba and Bokay 1977; Morton 1990), and developmental changes of diurnal renal functions such as renin secretion, glomerular filtration rate, and urine flow were reported (Koopman et al. 1989). The age-related change in the density of 2-[^{125}I]iodomelatonin binding sites in the chicken kidney may play a role in the development of diurnal rhythms of kidney function in the chicken, as well as in the increasing risk of hypertension in elders. Similarly, the developmental change in the density of 2-[^{125}I]iodomelatonin binding sites in the chicken spleen may be physiologically relevant. The immune system matures as the animal develops, reaching maximum function near the time of sexual maturity and declining after puberty (Kay 1978). The decrease in binding site density after 6 weeks coincides with the decline of immunity after puberty. Since melatonin modulates the immune function, it is possible that the pubertal decrease in melatonin receptor density may contribute to the decline of immune function after puberty. However, the full relevance of developmental changes of 2-[^{125}I]iodomelatonin binding sites in the chicken kidney and spleen for the physiological functions of the immune and renal systems needs further investigation.

Acknowledgments

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