BIBLID : ISSN 0253-9756 中国药理学报 *Acta Pharmacologica Sinica* 1993 Jul; 14 (4) : 292—295

# Specific binding of [$^{125}$I]iodomelatonin in pigeon and quail spleen membrane preparations and effect with hydrocortisone-treatment

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**ABSTRACT** [$^{125}$I]Iodomelatonin binding sites were studied in the spleens of pigeon and quail. Scatchard analyses of the membrane preparations collected at midday (12 : 00) revealed an equilibrium dissociation constant ($K_d$) of 250 ± 74 pmol·L$^{-1}$ and a maximal number of binding sites ($B_{max}$) of 13.4±1.8 fmol/mg protein in the pigeon spleen and $K_d$ = 390 ± 132 pmol·L$^{-1}$, $B_{max}$ = 21.3 ± 3.7 fmol/mg protein in the quail spleen. Circadian studies indicated that spleens collected at midnight (24 : 00) had 53% and 70.9% less [$^{125}$I]iodomelatonin binding sites than the samples collected at midday in the pigeon and quail, respectively. Specificity studies showed only melatonin and 6-chloromelatonin had significant inhibition to [$^{125}$I]iodomelatonin binding. Hydrocortisone 15 mg·kg$^{-1}$·d$^{-1}$ im for 5 d selectively increased [$^{125}$I] iodomelatonin binding sites in pigeon spleen. These results suggest that spleen is a site of melatonin action outside the brain.

**KEY WORDS** melatonin; iodine radioisotopes; binding sites; pigeons; quail; hydrocortisone; spleen

Melatonin (Mel) is a major hormone of the pineal gland. It participates in the regulation of seasonal and circadian functions of endocrine secretions and central neuronal activities. Mel plays its potent biological effects through specific binding sites. The $^{125}$I-melatonin has provided a necessary radioligand for Mel binding studies[1]. [$^{125}$I]Iodomelatonin binding sites from the central nervous system and peripheral organs such as retina, spleen, and gastrointestinal tract have been identified[2-4]. While the central action of Mel is well documented, its immunomodulatory function has also aroused a great deal of interest. It was suggested that Mel has a "up regulation" effect in immune function, and there was a possible complex network connecting the pineal and/or Mel and neuroendocrine and immune system[5,6]. Whether there are more direct relations between them is what we want to know. The present investigation was to establish the specific binding sites of Mel in peripheral immune organ, the spleen in Aves, and the changes after immune depression by hydrocortisone.

Received 1992-03-19 Accepted 1993-03-24

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## MATERIALS AND METHODS

**Reagents and animals** Melatonin, *N*-acetylserotonin, 5-hydroxytryptamine, tryptamine, 5-hydroxyindole-3-acetic acid, norepinephrine, and acetylcholine were purchased from Sigma; 6-chloromelatonin was purchased from Eli-Lilly; hydrocortisone ampule (cortisol) was purchased from Third Pharmacutical Factory of Beijing. [$^{125}$I]Iodomelatonin (81.4 PBq·mol$^{-1}$) was purchased from Du Pont or synthesized in our laboratory[7] (20.4 PBq·mol$^{-1}$). Adult house pigeon (*Columba livia* var *domestica*) and Japanese quail (*Coturnix coturnix japonica*) obtained from the Animal Center, University of Hong Kong were adapted under 12 h light/dark cycle (6 : 00 light on, 18 : 00 light off) for at least 7 d. Water and food *ad lib* were available.

**Membrane preparation**[7] The animals were decapitated at midday (12 : 00) or midnight (24 : 00), spleens collected were either used immediately or frozen at −70℃. The spleen was homogenized in 10 volumes (wt/vol) of ice-cold Tris-HCl buffer 50 mmol·L$^{-1}$ (pH 7.4) and centrifuged at 44 000×*g* for 25 min at 4℃. The pellet was washed once by Tris-HCl buffer and recentrifuged. The crude membrane pellet was suspended in a concentration of approximately 2 — 6 mg protein·ml$^{-1}$ in Tris-HCl buffer. Protein content was determined with bovine serum

albumin as standard.

**Binding assays** For Scatchard analysis, different concentrations of [$^{125}$I]iodomelatonin, aliquots of the membrane preparations, and Tris-HCl buffer were added in the test tubes, the final volume being 350 μl. The non-specific binding tubes had a Mel of 10 μmol·L$^{-1}$. All the tubes were incubated at 4℃ for 5 h. The solution was filtered through Whatman GF/B glass fiber filter. The filters were washed with 3 ml× 2 buffer at 4℃. The filters containing bound [$^{125}$I] iodomelatonin were determined for radioactivity (LKB 1270 Rackgamma Gamma Counter at 70% efficiency). Specific binding was calculated by subtracting the non-specific binding from the total binding.

**Specificity studies** We carried out the binding assays by adding different concentrations of unlabeled indole analogues or neurotransmitters to the test tubes.

**Drug treatment** Twenty aldult ♂ and ♀ pigeons weighing 336±*s* 22 g, after 7 days' adaptation, were randomly assigned to 2 groups. Hydrocortisone 15 mg·kg$^{-1}$·d$^{-1}$ and vehicle (0.5% ethanol/saline) im in a volume of 3 ml·kg$^{-1}$ at 9:00 for 5 d to the experimental and control groups, respectively. One hour after the last injection, the pigeons were decapitated and spleens and brains were frozen for assay.

**Statistics** Each experiment was repeated 2 — 3 times. Data were expressed as $\bar{x}\pm s$. Statistical analyses were carried out using *t* tests.

## RESULTS

**Saturation studies** Crude membrane preparations from spleens were incubated in [$^{125}$I]iodomelatonin at 4℃ for 5 h. Total and specific binding increased with escalating concentrations of [$^{125}$I]iodomelatonin over the range of 27 — 350 pmol·L$^{-1}$ and approached saturation at higher radioligand level (Fig 1A, 1B). Scatchard analyses suggested that a unique class of binding sites existed in pigeon and quail spleens (Fig 1C, 1D).

**Specificity studies** Only melatonin and 6-chloromelatonin were potent inhibitors of [$^{125}$I]iodomelatonin binding in pigeon spleen membranes (Fig 2), the $IC_{50}$ values being 0.81 and 2.3 nmol·L$^{-1}$, respectively.

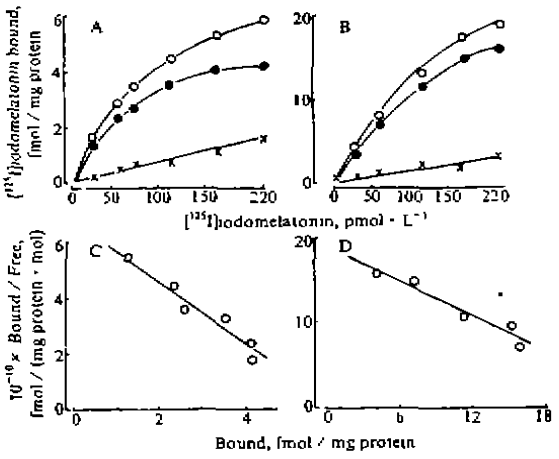


**Fig 1. Binding of [$^{125}$I]iodomelatonin to pigeon # 2 in middark (A) and quail # 1 in midlight (B) spleen membrane preparations.** (○) **total binding,** (●) **specific binding,** (×) **nonspecific binding. Scatchard plots of the specific binding to pigeon # 2 (C) and quail # 1 (D) spleens gave a unique class of binding sites.**

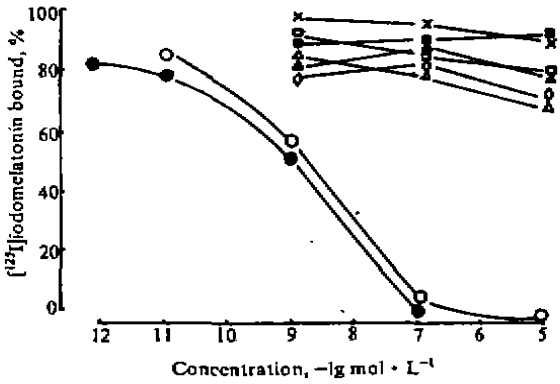


**Fig 2. Competition of indoles and neurotransmitters for [$^{125}$I]iodomelatonin binding sites in pigeon spleen membranes.** (○) **6-chloromelatonin;** (●) **melatonin;** (×) **5-hydroxyindole-3-acetic acid;** (□) ***N*-acetylserotonin;** (■) **5-hydroxytryptamine;** (△) **norepinephrine;** (▲) **tryptamine;** (◇) **acetylcholine.**

**Circadian variation** [$^{125}$I]Iodomelatonin binding capacities at the dark were significantly lower than those at the light by 53.0%

and 70.9% in pigeon and quail spleens, respectively. The $K_d$ values at the dark were smaller too in both of the birds (Tab 1).

**Tab 1.** [$^{125}$]**Iodomelatonin binding in pigeon and quail spleen membrane preparations at midday and midnight.** $\bar{x}\pm s$. $^{*}P>0.05$, $^{***}P<0.01$ **vs midday.**

| Spleen | Time | $n$ | $B_{max}$ fmol/mg protein | $K_d$ pmol·L$^{-1}$ |
|---|---|---|---|---|
| Pigeon | midday | 3 | 13.4±1.8 | 250±74 |
| | midnight | 3 | 6.3±0.9*** | 131±35* |
| Quail | midday | 4 | 21.3±3.7 | 390±132 |
| | midnight | 4 | 6.2±1.4*** | 199±95* |

**Hydrocortisone treatment** There was a significant increase in the number of [$^{125}$I] iodomelatonin binding sites (B) in hydrocortisone-treated pigeons' spleen membrane preparations compared to the control, B = 1.04 ± 0.28 and 0.75 ± 0.26 fmol/mg protein, respectively ($n=10$, $P<0.05$). The binding sites in their brains were 8.6±1.7 and 7.2 ± 1.9 fmol/mg protein, respectively ($P>0.05$).

## DISCUSSION

As spleen is an important immune organ, the presence of [$^{125}$I] iodomelatonin binding sites with high affinity and specificity in it confirmed the hypothesis that the spleen is a site of Mel action.

When the immune function in pigeon was depressed by using pharmacological dose of hydrocortison, the [$^{125}$I]iodomelatonin binding sites were significantly increased, but no statistically variance in their brain Mel receptors. This suggested that the "up regulating" of Mel binding sites in spleen was not mediated through brain or CNS, the spleen was a chief target organ of Mel outside the brain. Although for wanting in the dose-response relations, the direct relevance between immune function and spleen Mel binding sites can not be established yet.

In addition, we found when the concentrations of corticosteroids were higher, for example in hydrocortisone-treated pigeons and normal pigeons during the day, the Mel binding sites in spleen were more. Whether there was interrelations or not between them remains to be elucidated.

The variance in binding affinity have not been confirmed because of insufficient data. Our present results just have provided some clues for exploring the mechanisms of Mel action in immunomodulation.

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292—295 (2)

## [$^{125}$I]碘化褪黑激素与鸽和鹌鹑脾脏膜组份的特异性结合及氢化可的松对其的影响

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**摘要** [$^{125}$I]褪黑激素与鸽及鹌鹑脾膜组份结合的 $B_{max}$ 在日间分别为13.4±1.8和21.3±3.7 fmol/mg

protein，在夜间分别少53.0%和70.9%。褪黑激素和6-氯褪黑激素能竞争性抑制其结合。鸽 im 氢化可的松15 mg·kg$^{-1}$·d$^{-1}$×5 d 能明显增加[$^{125}$I]褪黑激素在脾的结合位点，结果提示褪黑激素对免疫的调节可能有直接作用。

关键词　褪黑激素；碘放射性核素；结合位点；鸽；鹌鹑；氢化可的松；脾

BIBLID：ISSN 0253-9756　中国药理学报　*Acta Pharmacologica Sinica*　1993 Jul；14 (4)：295—297

# Pharmacokinetics and relative bioavailability of nimodipine capsules and tablets in 8 Chinese healthy men

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**ABSTRACT**　A single oral dose of tablets or capsules of 239 μmol nimodipine was given to 8 healthy volunteers of Han nationality in a randomized crossover study. Plasma levels were determined with HPLC method. The plasma concentration-time curve fitted to a first order absorption，1-compartment open model，and the $T_{\frac{1}{2}K}$ was around 2 h. Although the capsules could reach peak level faster，the bioavailability was not significantly different from that of the tablets.

**KEY WORDS**　nimodipine；capsules；tablets；high pressure liquid chromatography；pharmacokinetics；biological availability

Nimodipine belongs to the second generation of 1，4-dihydropyridine group of $Ca^{2+}$ channel antagonists，and is mainly used for the treatment of cerebrovascular diseases[1]. In relation to this compound，there have been studies abroad on the procedures for drug analysis[2]，the characteristics of pharmacokinetics[3]，and the profiles of biotransformation[4]. Domestically，the focus of research has been on drug stability[5,6] and formulation assessment[6-8]，yet no report has been found on investigation in the human body.

Using a high pressure liquid chromatographic (HPLC) method[2,6]，we studied the pharmacokinetics and relative bioavailability of nimodipine capsules and tablets in 8 Chinese healthy men.

Received 1992-09-01　Accepted 1993-02-24

## MATERIALS AND METHODS

**Drug manufacturers**　Nimodipine standard and tablets (20 mg/tablet)，Tianjin Central Pharmaceutical Factory (tablet lot № 911020). Nimodipine capsules (20 mg/capsule)，Suzhou № 3 Pharmaceutical Factory (lot № 911118). Methanol，Shanghai Zhenxing № 1 Chemical Plant (AR，lot № 9105017). Diethyl ether，Shanghai Malu Pharmaceutical Factory (AR，lot № 90031332)

**Instruments**　The HPLC system (Shimadzu Corp，Kyoto，Japan) consisted of 2 LC-6A solvent delivery units，a Rheodyne model 7125 injector，a FCV-2AH high-pressure flow channel selection valve，a SPD-6AV uv-vis spectrophotometric detector，a SCL-6B system controller，and a C-R6A data processing unit. Both the 45 mm×4.6 mm precolumn and 250 mm×4.6 mm analytical column (Dalian Institute of Chemical Physics，Dalian，China) were packed with Spherisorb $C_{18}$ 5 μm.

**Subjects**　Having been informed about the effects of the drug and passed the physical examinations，8 healthy male volunteers of Han nationality were accepted into the study. They were aged 27±*s* 8 a，weighing 64±*s* 6 kg，and all the test results of their blood，urine，liver，kidney，and electrocardiogram were within normal ranges. At least 2 wk before the