

## Ontogenesis of Melatonin Receptors in Anterior Pituitary and Pars Tuberalis of Golden Hamsters

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### Summary

The ontogenesis of melatonin receptors in the anterior pituitary and pars tuberalis of the Golden hamster was studied using [<sup>125</sup>I]iodomelatonin as a ligand. The affinity of the binding site to the ligand ( $K_d$ ) was in the range 21 to 54 pM and it did not change significantly during development. The concentration of the [<sup>125</sup>I]iodomelatonin binding sites in the anterior pituitary was highest in one-day-old hamsters ( $B_{max}$  = 14 fmol/mg protein) and thereafter gradually decreased. In adults it reached to about 6 % of the neonatal values. In contrast, the concentration of the binding sites in pars tuberalis did not change significantly during ontogenesis and it was in the range of 3 to 5 fmol/mg protein.

### Key words

Melatonin receptor – Anterior pituitary – Pars tuberalis – Ontogenesis – Golden hamster

Melatonin receptor density in the rat anterior pituitary is high immediately before and after birth, but it gradually decreases in the course of postnatal development and declines below 10 % of the neonatal values within 30 days (Vaněček 1988). In pars tuberalis, the high concentration of melatonin receptors is maintained throughout development and in adulthood it has the highest density of the receptors from all tissues tested (Vaněček *et al.* 1987).

Similarly to rats, the density of the melatonin receptors in adult Golden hamsters is much higher in pars tuberalis than in pars distalis of the anterior pituitary (Vaněček and Janský 1989). Although hamsters are markedly photoperiodic and are often used for studies of the melatonin effects (Reiter *et al.* 1974), ontogenesis of the melatonin receptors in this species has not been described. In this report, the postnatal development of melatonin receptors in the hamster anterior pituitary and pars tuberalis was investigated.

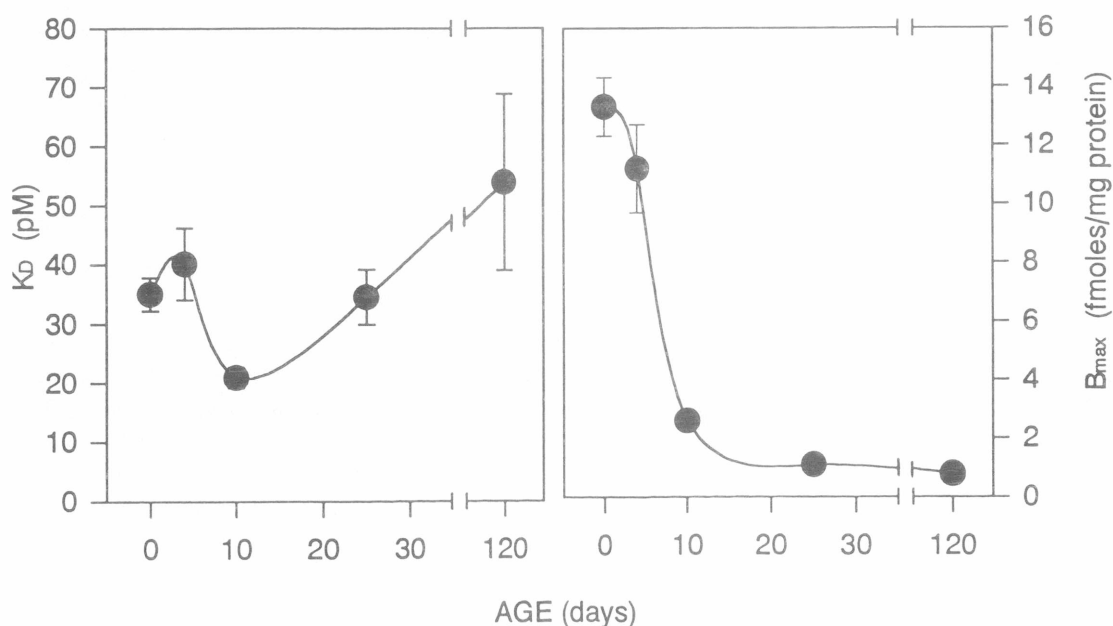
[<sup>125</sup>I]iodomelatonin (specific activity ~1000 Ci/mmol) was prepared by iodination ([<sup>125</sup>I]NaI from Amersham) of melatonin in the presence of iodo-gen (Sigma Chemical Co.) as described by Vakkuri *et al.* (1984). For each binding assay, a group of 40 Golden

hamsters were killed by decapitation between 10.00 and 12.00 h. Because no differences in the melatonin binding between males and females were found in preliminary experiments, the animals of both sexes have been used. After decapitation, the anterior pituitary and pars tuberalis were quickly dissected and frozen on solid CO<sub>2</sub>. The tissues were homogenized in 50 mM Tris-HCl (pH 7.4) with 4 mM CaCl<sub>2</sub> and centrifuged (15 000 × g, 20 min). The pellet was rehomogenized in the buffer and 100 µl aliquots were incubated with various concentrations of [<sup>125</sup>I]iodomelatonin with or without cold melatonin (1 µM) as described previously (Vaněček *et al.* 1987). The bound and free ligand were separated by vacuum filtration using GF/C Whatman glass fibre filters. The filters were washed 3 times with 3 ml of the assay buffer and counted on a Beckman counter. Protein concentrations were measured by the method of Lowry *et al.* (1951). Analysis of the binding data was performed using a computer method (Cressie and Keightley 1981).

Specific binding of [<sup>125</sup>I]iodomelatonin was already present in one-day-old hamster tissue. The affinity of the receptor for [<sup>125</sup>I]iodomelatonin was similar in both tissues tested, anterior pituitary and

pars tuberalis. The dissociation constant ( $K_d$ ) was in the range 21 to 54 pM (Figs 1 and 2) and it did not change significantly during postnatal development. The density of the receptors ( $B_{max}$ ) in anterior pituitary of 1-day-old hamsters was about 14 fmol/mg protein and it decreased thereafter. On the day 10 of age it was about 25 % of the neonatal value and declined even further in 25-day-old animals (Fig. 1). In adult

hamsters the density of the melatonin receptors in anterior pituitary was 0.8 fmol/mg protein, which is about 6 % of the neonatal values. The density of the melatonin receptors in pars tuberalis, however, ranged from 3 to 5 fmol/mg protein at all the ages tested and did not exhibit a systematic change in the course of postnatal development (Fig. 2).



**Fig. 1**

Developmental changes in affinity ( $K_d$ ) and density ( $B_{max}$ ) of [ $^{125}$ I]iodomelatonin binding sites in anterior pituitary of Golden hamsters.  $K_d$  and  $B_{max}$  were obtained from the linear Scatchard plot. Each point represents the computer-assisted estimate of the parameter ( $\pm$  S.E.) from an independent experiment.

In the adult hamsters, the highest density of melatonin receptors was found in pars tuberalis (Vaněček and Janský 1989). However, the present data show that in the neonatal hamsters, the highest density of melatonin receptors is in pars distalis of the pituitary. The postnatal decrease of melatonin receptor density was also described in the rat pituitary (Vaněček 1988), suggesting that it may be a general phenomena, at least in rodents.

The decrease of melatonin receptor density is due to the growth of the pituitary and the consequent increase of the total protein. When calculated per one pituitary (and not per mg of protein), the number of receptors is similar at all the ages tested. In this respect the postnatal development of melatonin receptors is different from that of other receptors in the pituitary. The number of GnRH receptors in the pituitary

increases more than 20 times during postnatal ontogenesis so that the receptor concentration per mg of protein in adults is about the same as in the neonates, despite pituitary growth (Chan *et al.* 1981). Moreover, in the early postnatal period the increase of the receptor number is more rapid than the growth of the pituitary. Thus the density of GnRH receptors per mg of protein is more than 3 times higher in 20-day-old rats than in the neonates. Furthermore, the density of oestrogen receptors in the rat pituitary shows a marked increase during postnatal development (MacLusky *et al.* 1979). Although the density of TRH receptors expressed per mg of protein in the adult pituitary is about 50 % of that in the neonates there is a several fold increase in the number of receptors per pituitary postnatally (Banerji and Prasad 1982).

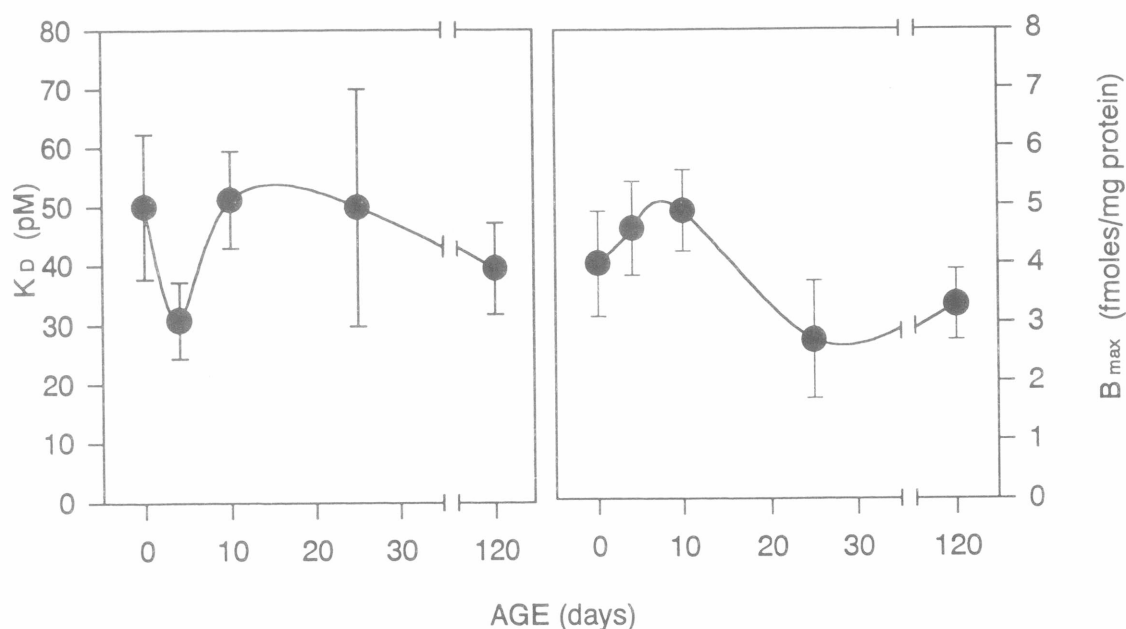


Fig. 2

Developmental changes in affinity ( $K_d$ ) and density ( $B_{max}$ ) of [ $^{125}$ I]iodomelatonin binding sites in pars tuberalis of Golden hamsters. For other details see Fig. 1.

The ontogenesis of melatonin receptors in the anterior pituitary is thus quite exceptional. This may imply a role for melatonin during foetal and early postnatal ontogenesis with the pituitary serving as the target. It is not known, however, which of the pituitary cells bears the melatonin receptors. Obviously, these cells are already present in the pituitary before birth and their percentage in the cell population decreases postnatally. Because melatonin inhibits LH release from the rat neonatal pituitary (Martin and Klein 1976), we suppose that the melatonin receptors are present on neonatal gonadotrophs. However, these

cells differ from adult gonadotrophs in several respects. One of them is that adult gonadotrophs have no melatonin receptors (Vaněček 1988), the other that their behaviour after stimulation with GnRH is different (Vaněček and Klein 1993). These cells may thus represent the immature precursors of pituitary cells.

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#### Reprint Requests

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