Differential displacement of cells from the median eminence into the arcuate nucleus during puberty. Effects of melatonin administration

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Summary. A displacement of catecholaminergic-positive and catalase-containing cells from the median eminence into the arcuate nucleus at puberty has been described previously. This study reports on the displacement phenomena after postnatal administration of melatonin. Catalase-positive cells undergo a delayed displacement from the median eminence into the arcuate nucleus. However, part of this cell population lags behind within the median eminence. This differential reaction of cell displacement by catalase-positive cells is considered as a special reaction of these cells to melatonin administration.
Key words. Melatonin; puberty; hypothalamus.

'Puberty can be defined as a maturation process of the hypothalamo-pituitary-gonadal axis resulting in growth and development of the genital organs and concomitantly physical and physiological changes towards adulthood leading to the capacity to reproduce'[1]. Most studies have been focussed on the hormonal aspects of puberty. Only a few studies take into consideration the morphological changes in the medio-basal hypothalamus that occur around puberty[2,3].

On the basis of histochemical methods the displacement of catalase-containing 1-µm granula around puberty, as well as the migration of catecholaminergic fluorescence from the median eminence towards the arcuate nucleus in the hypothalamic nucleus, could be described[4-6]. To ascertain that these displacements are correlated to the pubertal physiological events, several experiments have been carried out in which puberty was manipulated. These experiments included administration of monosodium-glutamate[7], elevation of prolactin levels[8], destruction of the suprachiasmatic nucleus[9], as well as neonatal castration[10]. They all supported the assumption that the histochemical parameters mirrored pubertal changes in the medio-basal hypothalamus.

Melatonin is known to delay sexual maturation and to disrupt regular estrous cyclicity after vaginal opening[11,12]. In female rats, vaginal opening, which occurs approximately 10 days[13] later after melatonin administration, occurs at day 42–45 postnatally. In this experiment melatonin was administered to newborn female rats, inducing a pubertal retardation. Simultaneously the changes in catalase activity of the various medio-basal hypothalamic areas were analyzed.

Material and methods. Litters of female Wistar rats were housed in light, temperature and humidity controlled rooms and, after weaning, fed ad libitum with standard laboratory chow (LD12:12, light on: 03.00 h, light out 15.00 h). Melatonin (1g/l NaCl 0.9%: ethanol 9:1) was administered s.c.[13] at 13.00 h (10 h after onset of light) starting at day 5 of age. From day 17 after birth until days 42–45, every other day two animals were decapitated and the brains dissected. The brains were frozen[14] and used for enzyme histochemistry (catalase[9]; acetylcholinesterase[15]; 5'-nucleotidase[16]) and Nissl staining[17]. Whole brains were sectioned (4 out of every 10 successive sections, 14-µm thick) and stored at − 40 °C for a few days.

Results. In the normal stained and normal enzyme-histochemical treated parallel series of the experimental animals no changes in the mediobasal hypothalamus were found.

In normal female rats catalase activity appeared in the median eminence at day 20–21 after birth. The first groups of catalase 1-µm granula could be noticed at day 27 after birth in melatonin-treated rats. The shift of catalase particles into the intermediate area just below the ventromedial hypothalamus took place at day 35 after birth (fig. 1). At this age the catalase particles have disappeared from the median eminence. In the experimental animals the catalase granula were present in the intermediate area of the ventromedial hypothalamus[4,9] but also in the median eminence at days 33 after birth (fig. 1). In the median eminence these granula occupied the superficial layers and were bigger than in normal rats at days 20-21 after birth. In normal rats the catalase particles started to enter the arcuate nucleus at days 41–45 after birth, leaving the intermediate area and median eminence free from catalase granula. In melatonin treated rats these granula entered the arcuate nucleus. However, the median

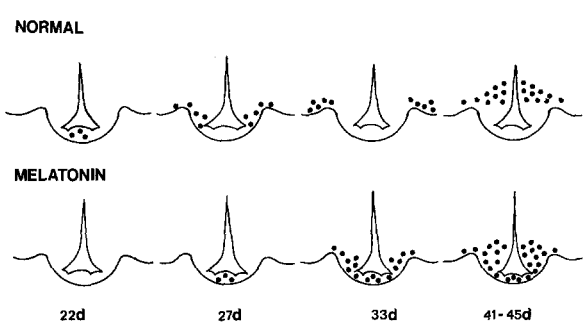

Figure 1. Summary diagram of the displacements of catalase positivity in normal medio-basal hypothalamus and melatonin treated medio-basal hypothalamus.

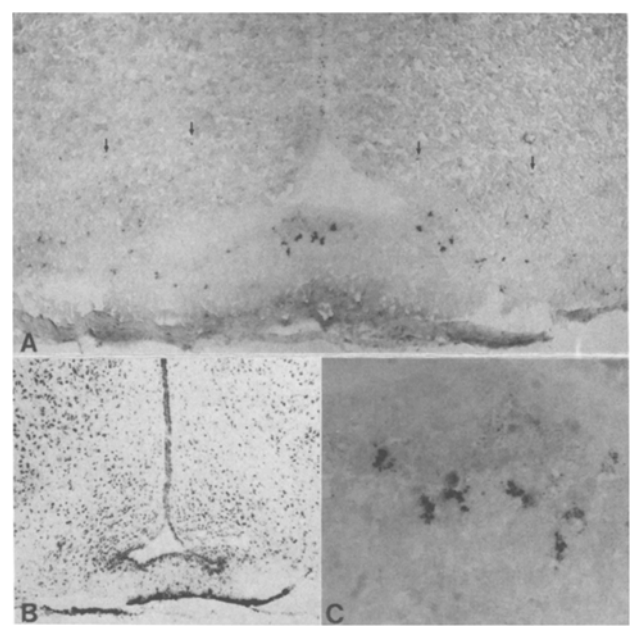

Figure 2. *A* shows a transverse cryostat section from a melatonin-treated rat, aged 41 days postnatally. The section is through the median eminence and the arcuate nuclei. Dark stippling represents catalase clusters of 1-µm granula. Arrows indicate the normal sized clusters. *B* is the Nissl successive section of *A;* note the periventricular arrangement of the arcuate cells. *C* demonstrates an enlargement of the catalase granula clusters in the superficial layers of the median eminence.

eminence still contains clusters of big catalase granula in its superficial layers (fig. 2).

Discussion. In previous studies on the histochemical characteristics of puberty in the mediobasal hypothalamus it was found that prolactin administration[8], castration[10] and suprachiasmatic nuclei destruction[9] in one way or another resulted in a retardation of the described cell displacements[2-6] but in all cases the arrival of catalase granula at day 45 in the arcuate nucleus was completed. No catalase granula remained present in the intermediate area or median eminence in these cases except in the prolactin administration study. On the other hand, administration of monosodium glutamate postnatally from day 4 until day 11 accelerated the displacement[7].

Administration of melatonin did block the displacement of catalase granula, but only partially. Such a differential answer to melatonin administration in prepubertal rats is unexpected. A retardation of the neuronal migratory events could be expected on the basis of descriptions of a delayed sexual maturation by others[13]. These results suggest that melatonin indeed causes a delay of maturation of the hypothalamus-pituitary axis.

Melatonin action not only contributes to the termination of phasic LH secretion, but also participates in the entrainment of the LH surge to the photoperiod in the female rat[18]. Melatonin performs an acute inhibitory effect on pituitary LH and FSH responses to LHRH[19,20]. Since binding of melatonin to a cytoplasmic receptor was found in several gonadal and endocrine tissues[21], as well as in brain tissue[22], it cannot be concluded whether a direct or an indirect action of melatonin is involved. The differential effect of melatonin administration on the displacement of catalase positive cells is under investigation now.

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Crowding during pregnancy delays puberty and alters estrous cycles of female offspring in mice

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Summary. Chronic crowding of mice during late pregnancy resulted in offspring of lowered birthweights and, in the females, delayed puberty and altered estrous cycles. Plasma corticosterone in the crowded dams was elevated acutely, lending some support to the hypothesis of adrenocortical mediation of prenatal stress effects.

Key words. Crowding stress; pregnancy; puberty; estrous cycle; corticosterone; birthweight.

Exposure of rodents during pregnancy to stressful environmental conditions has been shown to produce a variety of effects on the endocrine systems of male offspring, including accelerated fetal testosterone surge[1], underdevelopment of adrenal and testis[2], lower testosterone secretion[3] and lower stress-induced corticosterone and prolactin secretion[4]. All these endocrine effects have been reported in the rat but impairment of sexually-differentiated behavior in prenatally stressed males has been reported in both rats[5] and mice[6,7]. Of the few studies examining the effects of stress during pregnancy upon the female offspring the following consequences have been reported: impairment of sexual receptivity in rats[8] and mice[9], lengthening of the combined estrus-metestrus stages of the vaginal cycle in rats[10], and delay of vaginal opening in mice[11]. However, to date, there has been no thorough study of the effects of stress during pregnancy upon reproductive development of the female offspring. It has been suggested that the effects of stress during pregnancy upon male offspring are mediated by exposure of the fetus to maternal pituitary-adrenal products[2]. We have recently examined this suggestion giving experimental evidence for this hypothesis in both male[6] and female[12] offspring. The hypothalamo-pituitary-gonadal axis develops prenatally in both rats and mice; LH, FSH and PRL are all secreted by the fetal pituitary[13-15] and, estrogen receptors are present in fetal rat hypothalamus[16]. Although development of hypothalamo-pituitary function is not completed until early postnatal life[17], it is clear that any abnormality in the maternal endocrine profile during late pregnancy is likely to have some impact on the development of these systems in the fetus. The purpose of this study was to examine the effects of chronic crowding during pregnancy upon the onset of puberty and the estrous cycle of female offspring, to provide a more complete documentation of the effects of prenatal stress on