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The Innervation of the Regenerated Thymus after the Application of LHRH

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The innervation of the regenerated thymus after the application of LHRH applied a thiocholinmethod of cytologic evidence AchE. The distribution and density of acetylcholinesterase (AChE) positive nerve fibres of the regenerated thymuses resembled those observed in the pre-involution period. In particular, there was marked innervation within the functional parenchyma. AChE-positive nerve fibres were clearly visible, containing a high amount of reaction products. The morphological changes of thymuses treated with an analogue of LHRH lead to the regeneration of the AChE-positive innervation to the level of pre-involution period.

Key words: AChE-positive nerves, innervation of the thymus, regenerated thymus the rats, LHRH

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Introduction

The regeneration of an involuted thymus can be seen as a result of an orchidectomy (Fitzpatrick *et al.*, 1985; Gomboš *et al.*, 1992, 1993). This surgical castration has caused a marked increase in the number of lymphocytes in the blood taken from the thymuses of the experimental animals compared with non-operated animals (Fitzpatrick *et al.*, 1985). A regeneration of the thymus in old rats was found after treatment with a stable analogue of LHRH also (Greenstein *et al.*, 1987), while the testes of these animals were reduced in weight substantially.Monroe *et al.*, 1987 investigated canine thymuses and they confirmed that these were regenerated after treatment with growth hormone.

There is also evidence of increases in the weight of thymus after a prepubertal gonadectomy (Lee and Meade, 1992). In the adults, this type of surgery prevents decreases in weight of the thymus (Lee and Meade, 1992). Utsuyama and Hirokawa, 1989 also confirmed a hypertrophy of the thymus in gonadectomized old rats. There are other data concerning to the regeneration of the thymus after both surgical and chemical castration (Kendall *et al.*, 1990; Gomboš *et al.*, 1992, 1993; Sirotáková and Škardová, 1999). Therefore, the study was conducted to investigate whether or not the AChE-positive innervation of regenerated thymuses would be affected by a long-time treatment with LHRH-analogue.

Materials and Methods

Thymuses from eleven male Wistar rats were used in this study. Animals were kept four or five to a cage under conditions of controlled lighting and heating (lights on 08.00-22.00 h, temperature 21-23 °C). Rats weighed 480-530 g, and ranged from 12 to 14 months of age. There were two groups of rats. In the first group of six rats each animal received daily injections of 0.5 ml of LHRH-analogue (Hoechst AG, LH-RH 0.1 mg, Reg .-Nr. 10800, PZN-1983387). The second group of five rats not given any S.C. treatment. After 28 days, animals were deeply anesthetized with ether and the thorax was exposed. Thymuses were removed and weighed after the animals were killed. Organs were prepared for an AChE-positive nerve visualization by the histochemical method of El Badawi and Schenk (1967) in which the iso-OMPA was used as an inhibitor of unspecific cholinesterase.

Results and Discussion

A regeneration of the thymus in intact animals was produced after using the LHRH-analogue. In gross-anatomical appearance these organs resembled thymuses at the prepubertal age. They were bi or multilobular with a minimum fatty tissue surrounding them. LHRH-analogue treated thymuses and had increased in weight.

A light microscopy revealed that a relatively broad band of cortex, densely packed with thymocytes, and well vascularized. The distribution of AChE-positive innervation in regenerated thymuses was similar to that in the pre-involution period. AChE-positive nerve fibers were entered in the thymus close to blood vessels. They were running along branching vessels in trabecular septas, and seen as a dense perivascular AChE-positive plexuses in a medulla of thymic lobules.

Fine, solitary AChE-positive nerves entered in the cortex of regenerated thymus from the subcapsular perivascular trabecular and medullar perivascular plexuses (Figs. 1,3).

The thymuses of non-treated animals showed advanced involution. The parenchyma was replaced by high amount of fatty tissue. From this histological evidence, it was estimated

Fig. 1: Regenerated rat thymus after treatment with an analogue of LHRH. Solitary nerve fibres are separated from perivascular AChE-positive plexuses and penetrating medulla and cortex. C-cortex, M-medulla, PP-perivascular plexus, sNF-solitary nerve fiber. (x200)

Fig. 2: Abundant perivascular and also solitary AChE positive nerve fibres running in parenchyma of the rat thymus treated by LHRH-analogue. PAP-periarteriolar plexus, NF-nerve fiber, C-cortex. (x200)

Fig. 3: Solitary nerve fibres are present in the parenchyma of the rat thymus after treatment with an analogue of LHRH sNF-solitary nerve fiber. (x200)

Fig. 4: An involuted thymus of an old rat with a weakly stained AchE - positive nerve fibres seen only in the perivascular location. C-cortex, M-medulla, PP-perivascular plexus. (x160)

that at least two-thirds of the tissue was removed as fat. The thymuses were poorly organized and had a reduced cortex and an indistinct corticomedullary region.

AChE-positive nerve fibres of involuted thymuses contained a low amount of reaction material and therefore, weakly stained. Moreover, they could be identified only in the perivascular location (Fig. 4) and no solitary AChE-positive nerves were seen in the parenchyma of involuted thymuses.

The regeneration of the thymus as a result of surgical and chemical castration has been well known for many years (Castro, 1974; Fitzpatrick *et al.*, 1985; Greenstein *et al.*, 1987; Ojeda *et al.*, 1996; Kendall *et al.*, 1990; Gomboš *et al.*, 1992, 1993; Škardová *et al.*, 1999). However, we have not found any reference to the regeneration of a thymus in response to the AChE innervation.

The literature has not include data on the innervation of thymus after treatment with an LHRH-analogue. Our experiments showed that, while in involuted thymuses AChE-positive nerve fibres were found only in the perivascular location, the AChE- positivity in the functional parenchyma was observed in regenerated thymuses. Therefore, there is evidence that thymuses with increased lymphopoesis showed an abundant amount of AChE-positive innervation. The reversibility of macro and microscopical alterations of involuted thymuses is accompanied by the restoration of AChE-positive innervation. Our results are confirmed by the findings of Singh and Fatani, 1988. It also can be suggested that cholinergic component of thymic autonomic innervation has a stimulating effect on the lymphopoesis in this organ.

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