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Substance P and neuron-specific enolase in mouse testis. An immunocytochemical study

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An investigation was undertaken to visualize the two markers of neuroendocrine cells: neuron-specific enolase (NSE) and substance P (SP) in the mouse testis during ontogenesis. Mouse testes from neonatal, prepubertal and adult animals were studied. For immunocytochemical visualization of substance P (SP) and neuron-specific enolase (NSE), a combination of avidin-biotin-peroxidase complex (ABC) and PAP-techniques was applied. The SP and the NSE were observed to be present in the mouse testes at all stages studied, e. g. in both foetal and adult generations of srteroidogenic cells. The majority of Leydig cells showed a moderate to intense cytoplasmic immunostaining. The findings presented here confirm and extend the investigations concerning the nature of the human and some rodents Leydig cells. Together with other literature data our results suggest that the neuropeptides under study are demonstrated in the Leydig cells of almost all Mammalian species.

Key words: substance P (SP), neuron-specific enolase (NSE), Leydig cells, testis, mouse.

In the last few years numerous studies report that neuroactive substances such as renin [6], β-endorphin [14], methionin — enkephalin [11] and oxytocin [7, 8] were detected in gonads. The presence of a neurotransmitter, namely, substance P (SP) and of neuron specific enolase (NSE), a marker of neuroendocrine cells was demonstrated immunocytochemically in Leydig cells from different mammalian testes (human, hamster, mouse, guinea-pig) [1, 2, 3, 5, 9, 10, 11, 12]. The substance P has not been established in the rat testis, providing evidence for the existence of species — specific differences [2].

In mice, data about distribution of SP and NSE in the Leydig cells during ontogenesis are not available.

The aim of our study was to elucidate the localization of SP and NSE in the mouse testes during ontogenesis.

Materials and methods

Mouse testicular tissue was obtained from neonatal, prepubertal and adult animals, kept under standard conditions. The animals were sacrificed by decapitation under ether narcosis. Testes were rapidly removed in Bouin's solution for 24 to 48 h at room temperature (20 °C). After fixation, tissues were dehydrated and embedded in paraffin. Histological sections 6 to 9 μm thick were mounted on chromalum/gelatine precoated slides. For immunohistochemical visualization of SP and NSE, a combination of the peroxidase antiperoxidase (PAP) [13] and the avidin-biotin-peroxidase complex (ABC) — method according to D a v i d o f f and S c h u l z e (1990) was applied [4].

The SP-antiserum was obtained from INCSTAR (USA) and diluted 1:800. The NSE-antiserum was supplied by Dakopatts (Denmark) and diluted 1:200. A biotinylated anti-rabbit IgG, 1:250 (Vector, USA) was applied in the second step of the immunostaining. Then, a PAP complex (1:200, Dakopatts, Denmark) and an ABC-complex (1:250, Vector, USA) were used. Development of the peroxidase activity was performed with a solution containing 20 mg/100 ml 3,3'-diaminobenzidine-4 HCl (Sigma, USA) and H_2O_2 at a final concentration of 0,01 %. Controls: Sections were incubated with the primary antisera at their working dilutions, previously preabsorbed for 24 h at 4 °C with the corresponding antigens synthetic SP (Sigma) and human NSE (Polysciences) at a concentration of 20 or 100 μg/ml. No SP or NSE-like immunoreactivity was observed in the control sections.

Some histological sections were stained with haematoxylin eosin to compare the immunocytochemical findings with the histological structure of the gonads at the same stages of development.

Results and discussion

In the neonatal mouse testis the first foetal generation of Leydig cells was observed in the interstitium. At birth in the intertubular tissues the Leydig cells usually occur in groups or nests (Fig. 1). At this stage SP-like immunoreactive cells, single or in groups were situated between the primary tubules (Fig. 3).

The second proliferation of Leydig cells appeared from the 10th day after birth. Fully differentiated Leydig cells were found on the 15th day post partum. In the interstitium SP-immunoreactive cells, showing location and morphology of Leydig cells were identified (Fig. 4).

On day 30th after birth as in adult animals Leydig cells were present as cords or groups of large cells, situated between the seminiferous tubules (Fig. 2). A strong SP-like immunoreactivity in their cytoplasm was observed. Early spermatids exhibited an unspecific staining.

In all stages studied, the Leydig cells of both foetal and adult generations demonstrated a well expressed immunoreactivity for SP as well for neuron-specific enolase. The reaction product was localized in the cytoplasm of the steroidogenic cells, single, or in groups (Figs. 5, 6, 7).

Substance P and neuron-specific enolase negative staining in the testis were found during the first postnatal week, when a regression of Leydig cells was reported by some authors.

The results presented here confirm and extend previous investigations concerning man and some rodents (S c h u l z e, D a v i d o f f, 1987, 1991; A n g e l o v a et al.,

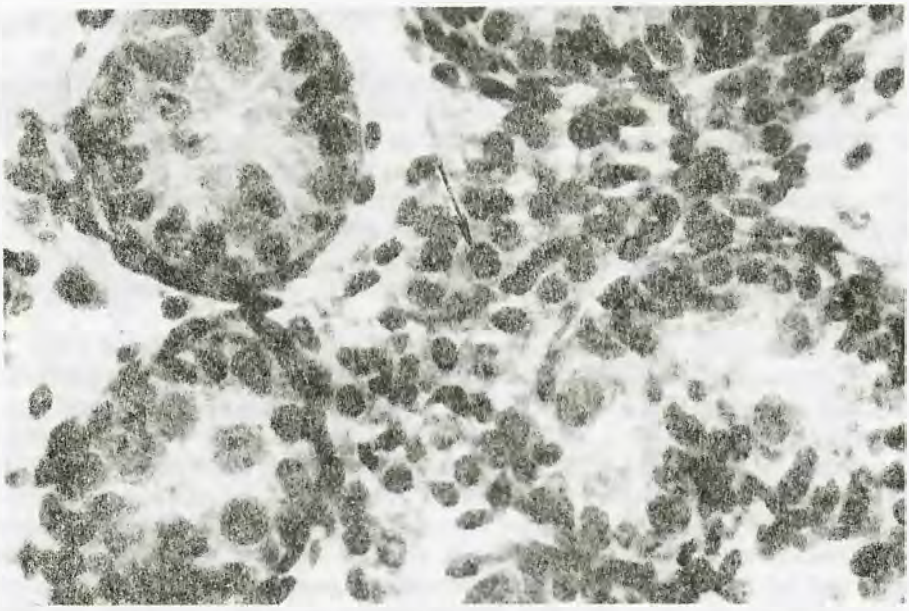

Fig. 1. Histological section: testis of a new-born mouse. Well differentiated single Leydig cells are present beween the sex cords (→). 160 ×

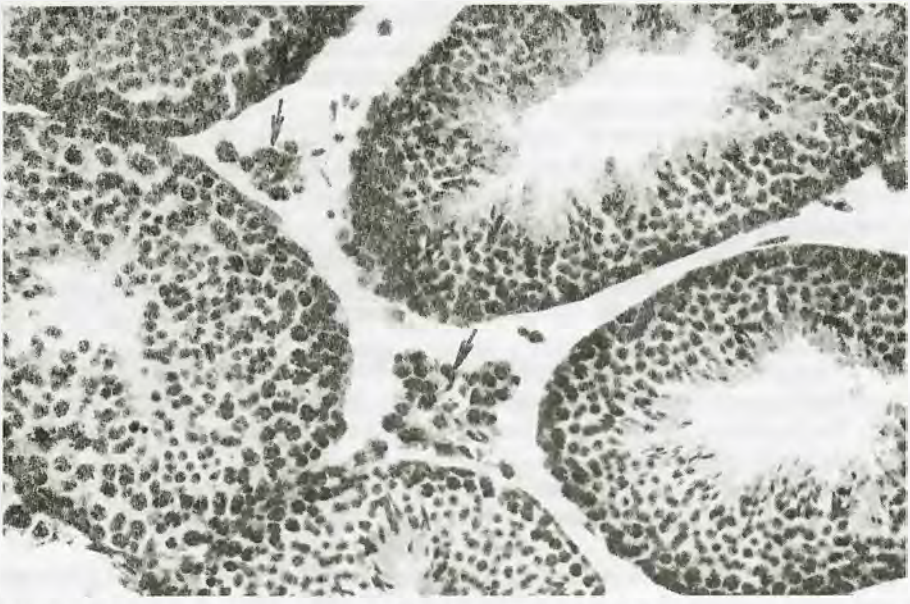

Fig. 2. Histological section: group of Leydig cells in the testis of an adult animal (→). 160 ×

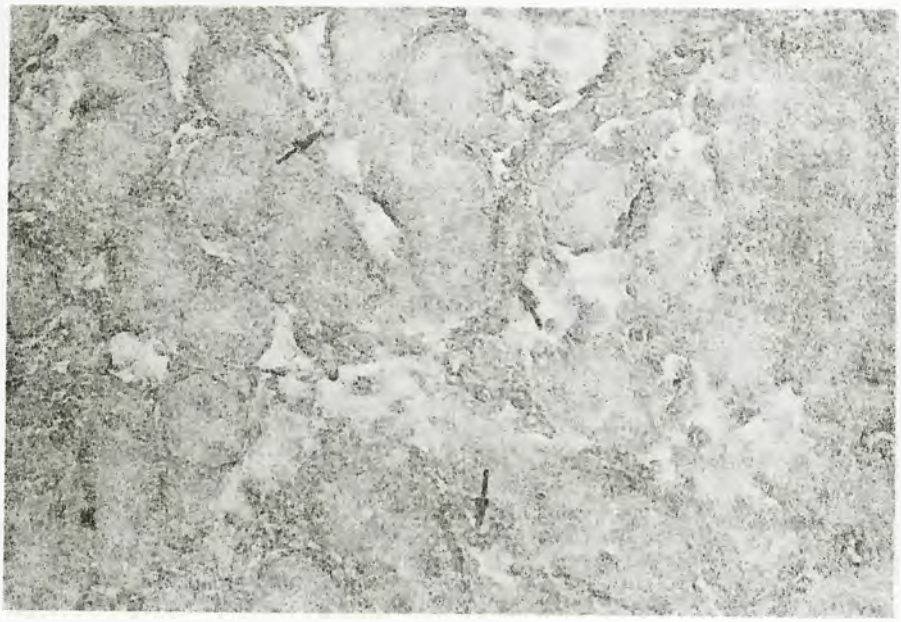

Fig. 3. SP-immunoreactive cells (→) in the testicular tissue of a new-born animal. 160 ×

Fig. 4. SP-immunostaining of the second generation of Leydig cells (10th day post partum) (→). 160 ×

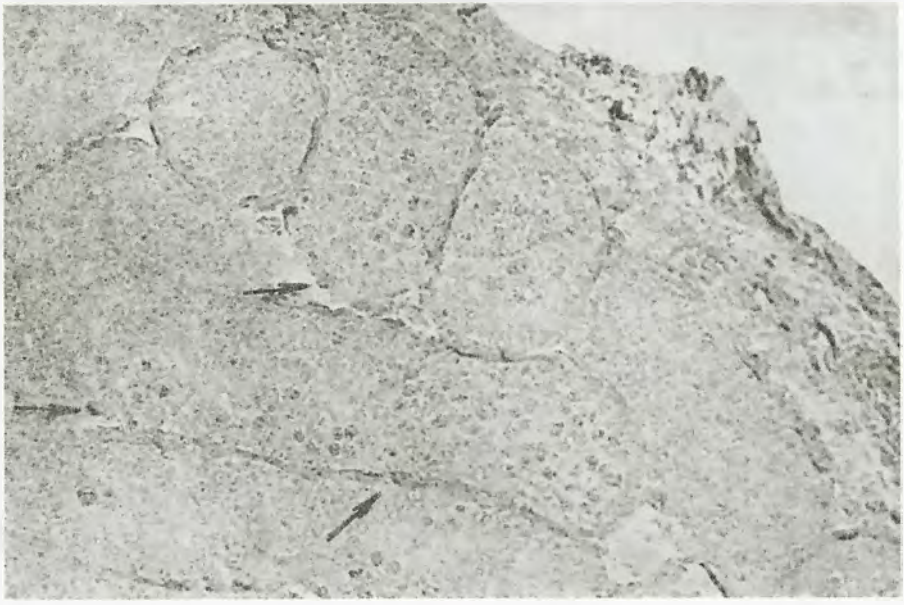

Fig. 5. NSE-like immunoreactive cells (→) of the second generation of Leydig cells (10th day post partum). 160 ×

Fig. 6. Testis from a 15-day-old animal. NSE-immunoreactivity in the Leydig cells (→). 160 ×

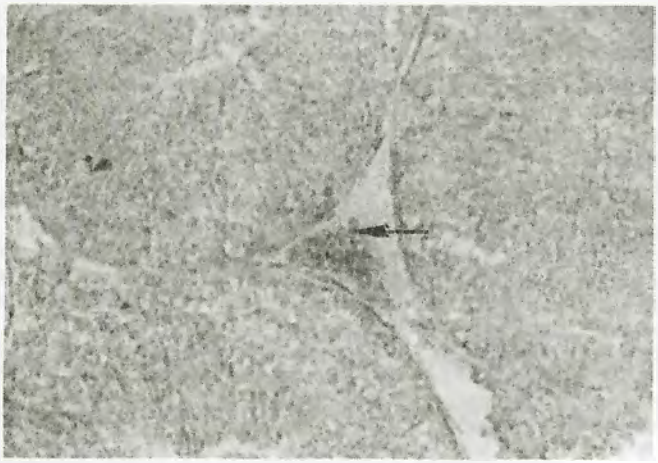

Fig. 7. NSE-immunoreactive Leydig cells (→) in the testis of an adult animal. 160 ×

1991). Our data suggest that the neuropeptides under study are demonstrated in the Leydig cells of almost all Mammalian species.

Our findings show a well expressed SP- and NSE-immunoreactivity in mouse Leydig cells during testicular development thus, completing their characteristics as neuroendocrine cells. Our data provide additional evidence for the neuroendocrine nature of Mammalian Leydig cells and confirm the data concerning their possible para- and autocrine function [3, 5, 9].

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