Ultrastructural Changes of the Neurohypophysis of the Rat after Castration*

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Summary. The infundibular processes of the neurohypophysis of male and female rats were studied after different periods of castration. After seven days an increase in neurosecretory granules was observed. Two types of neurosecretory nerve endings were identified: dark ones, with dense neurosecretory elementary granules of 1600 A, and clear ones, with lighter neurosecretory granules of 1800 A. Protoplasmatic pituicytes showed a large increase in lipid granules together with a general hypertrophy. After one week of castration but with hormonal therapy the protoplasmatic pituicytes appeared normal or even showed less lipid granules than in the controls.

With one month of castration the changes already mentioned in the nerve endings and pituicytes were more pronounced and after six months even more accentuated. Two types of neurosecretory nerve endings were clearly identified and the protoplasmatic pituicytes were loaded with lipid granules.

The probable significance of the two different neurosecretory axons was discussed in relation to recent studies on the isolation of neurosecretory terminals from the neurohypophysis. The changes in the protoplasmatic pituicytes were considered in relation to the possible significance of the lipid granules.

Studies on the ultrastructural and biochemical organization of the neurohypophysis have supported the view that the two hormones: i.e. vasopressin and oxytocin, present in this tissue, are stored in the elementary neurosecretory granules (NSG) contained within the unmyelinated axons originating in the supraoptic and paraventricular nuclei. Cell fractionation methods with electron microscope control have demonstrated that such polypeptide hormones are contained in the fraction rich in elementary granules (Heller, 1961; Weinstein et al, 1961; Schapiro and Stjärne, 1961; Barer et al, 1963; and La Bella, 1962, 1963). Furthermore specific stimuli to deplete vasopressin (Palay, 1957; Gerschenfeld et al, 1960; and Bodian, 1963) or oxytocin (Monroe, 1966) produce liberation of the content of the elementary granules into the perivascular spaces with loss of the dense cores, leaving empty limiting membranes inside the axons.

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Knowledge about the role of the pituicyte is practically lacking. The possible participation of this cell in the secretion of vasopressin postulated by several authors (Yamada, 1933; Itano, 1936; Gersh, 1939) was disproven by studies at the light microscope level (Hickey et al. 1941, De Robertis and Primavesi, 1942) and specially by the later work with electron microscopy.

The purpose of the present work is to describe the ultrastructural changes occurring in the infundibular process of the neurohypophysis of rats of both sexes after castration. With time a progressive change in the pituicytes occurs which mainly consists in cell hypertrophy and the accumulation of a material of probable lipid nature. Simultaneously there is an increase in the amount of elementary neurosecretory granules and two different types of neurosecretory axons and endings may be clearly differentiated. The probable nature of them will be discussed on the light of the current knowledge about the existence of vasopressinergic and oxytocinergic nerve endings in the neurohypophysis (Bindler et al., 1967).

Material and Methods

Groups of adult Wistar rats of both sexes, weighing 150—200 g were castrated and studied after one week, one and six months after the operation. Experiments of substitution therapy were carried on for one week in four male castrated rats, which were injected subcutaneously with 1 mg of testosterone propionate daily since the first day of operation and in four female castrated rats, which received 200 μg of 17-β-estradiol. The animals were anesthetized with sodium Pentobarbital (5 mg/100 gm) and extirpation of the neurohypophysis was done after 30 min to avoid the changes known to occur with manipulation of the animals.

Small pieces of the neurohypophysis cut with a razor blade were fixed by immersion at 0—4°C in a mixture of 3% glutaraldehyde and 2% formaldehyde in phosphate buffer 0.1 M at pH 7.4 for 24 hours. After a quick washing in phosphate buffer plus 10% of sucrose the pieces were “refixed” and trimmed in 1.5% osmium tetroxide in phosphate buffer 0.1 M pH 7.4. After 1 hour the pieces were immersed in uranyl acetate at 2% in water for 3 hours, dehydrated in ethanol and embedded in Epon 812. The sections were examined with a Siemens Elmiskop 1. At least seven animals from each lot of castrated animals were examined under the electron microscope.

For light microscopy, thick sections of 1 μ of the same blocks embedded in Epon were stained with toluidine blue according to Trump et al. (1961).

Results

Ultrastructural Changes in Pituicytes after Castration

a) In normal rats two types of pituicytes are observed: the reticular or fibrous and the protoplasmatic, the latter being by far the most common type. In 1 μ thick sections observed with the light microscope these cells have granules of about 0.7—1 μ around the clear nucleus and in the short processes (Fig. 1). The granules are stained with osmium tetroxide and also with toluidine blue.

Under the electron microscope these cells are clearly identified by their oval or indented nucleus with the chromatin condensed under the nuclear envelope and the well developed nucleolus (see Fig. 8). The cytoplasm contains clear mitochondria, cisternae of the granular endoplasmic reticulum, some Golgi complexes and lipid granules of low electron density. Sometimes these cells send processes which end near the pericapillary spaces. Some nerve endings making an apparent synaptic contact with the pituicytes may be observed.