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Developmental appearance of the nitric oxide synthase during postnatal gonadal development in the rat

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Nitric oxide is an intra- and intercellular messenger in physiological and pathophysiological processes in mammalian cells. NADPH-diaphorase staining histochemistry was applied to study the gonadal localization of nitric oxide synthase (NOS) in rat gonads. Ovaries and testes removed from neonatal, pubertal (15- and 30-day-old) and adult animals were investigated. In the testis the appearance of NOS-activity was closely correlated to active testosterone production. Its persistence in both foetal-type and adult-type Leydig cells (LC) at all stages was shown. In the ovary, the NOS-activity is located in theca cells, follicle cells, luteal and interstitial cells. The positive reaction was observed to be present in primary follicles at postnatal day 15. In adult ovary all steroidogenic cells were stained, the intensity of the reaction being most prominent in luteal and interstitial cells. These results suggest that NOS is expressed differently during gonadal development in the rat in close correlation to the steroid hormone production.

Key words: nitric oxide synthase, steroidogenic cells, gonads, development, rat.

Introduction

Recent investigations suggest that nitric oxide (NO) is of wide occurrence as a major messenger molecule regulating immune function and blood vessel dilatation and serving as a neurotransmitter in the brain and peripheral nervous system (S n y d e r, B r e d t, 1991; L o w e n s t e i n, S n y d e r, 1992). Moreover, NO is thought to represent an intra- and intercellular signal molecule in various mammalian tissues including brain, blood vessels, kidney, immune system etc (N a t h a n, 1992). Neuronal nitric oxide synthase (NOS) was recently shown to be a 150 kDa enzyme identical to NADPH-diaphorase (D a w s o n et al., 1991; H o p e et al., 1991). At present there is no specific method for the visualization of NOS using its catalytic properties. So far NO and its reaction products, NO_2 and NO_2^-, could not be used in a suitable procedure as could not the other final reaction product, citrulline. A suitable possibility offers the third final product, NADPH, i. e. using the NADPH-diaphorase activity of the enzyme (G o s s r a u, 1994). A one-to-one correlation between NOS-immunoreactivity and NADPH-diaphorase staining in some neurons was revealed indicating that NADPH-diaphorase histochemistry may be a good marker for cells containing NOS (D a w s o n et al., 1991; Y o u n g et al., 1992).

In this study we focused on the histochemical visualization of NOS in steroidogenic cells of developing rat gonads (Leydig cells, follicle cells, theca cells) using NADPH-diaphorase activity of the enzyme.

Material and methods

In this study Wistar rats at different developmental stages were used: neonates, pubertal (15 and 30-day-old) and adult animals. They were killed by decapitation. Gonads, ovaries and testes, were removed and tissue blocks were frozen in liquid nitrogen. Ten micron-thick cryostat tissue sections were fixed in 0,7 % formaldehyde in 0,1 M phosphate buffer, pH 7,6. The sections were then incubated for 1 h at 37 °C in a solution containing the fixative with addition of 1 mg/ml nitro-blue-tetrazolium (NBT) and 1 mg/ml NADPH. The sections were rinsed with distilled water and coverslipped with glycerol-gelatine. The sites of the NOS-activity were marked by formazan granules.

Results

I. Testis

In testicular sections at all developmental stages studied the NOS-activity was always present in the interstitium.

In neonates, the fetal-type LC appeared stained and the reaction product was localized in the cell body (Fig. 1).

In rats, the second proliferation of testosterone producing cells appeared between days 12 and 15 after birth and lasted from puberty to adulthood during maturation of adult testis and testicular functions (L o r d i n g, D e K r e t s e r, 1972). Histochemically

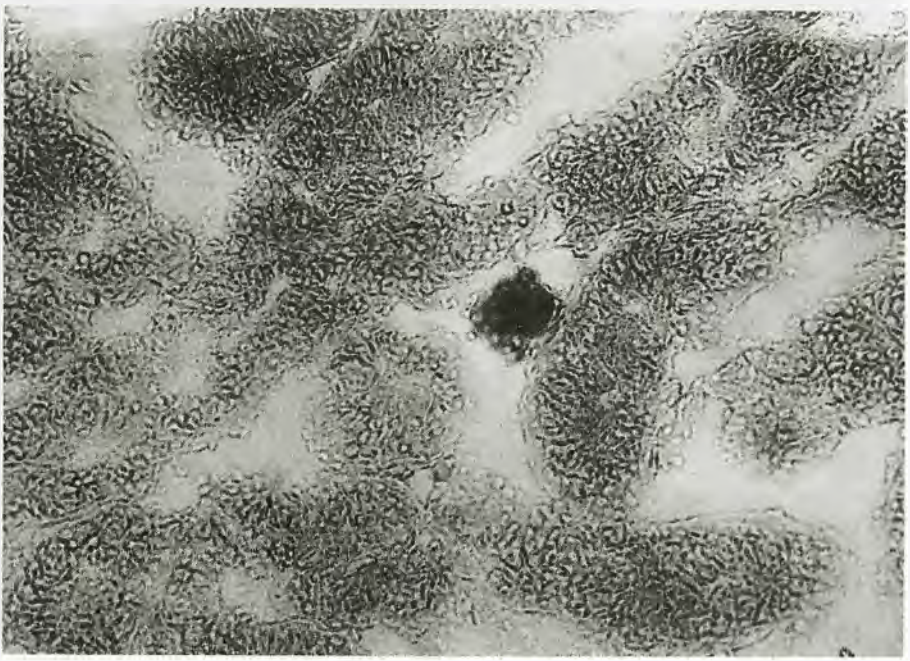

Fig. 1. Neonatal testis. A group of Leydig cells between seminiferous cords is strongly diaphorase-reactive. ×160

Fig. 2. 15-day old testis. A moderate NOS-activity in the second generation of Leydig cells is visible (→). ×160

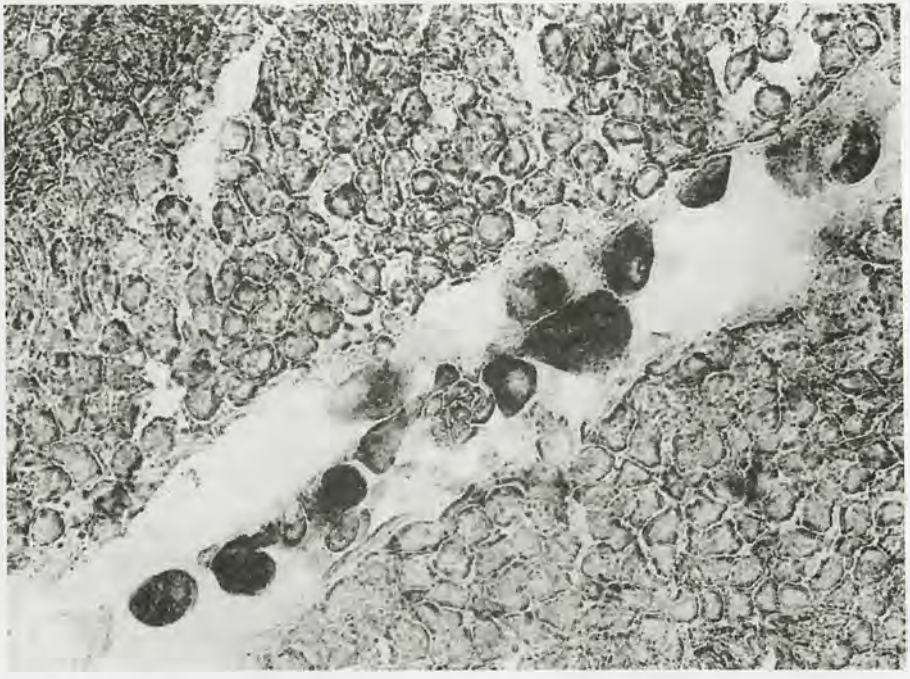

Fig. 3. Testis from an adult animal. Formasan granules in the cytoplasm of Leydig cells are observed pointing to a strong NOS activity. ×400

NOS-activity was also observed in the LC. Located between seminiferous tubules they contained formazan granules in their cytoplasm. Nuclei remained unreactive at all developmental stages under study.

In the interstitial tissue of the 15-day-old testis LC were faintly diaphorase stained (Fig. 2). Later, on day 30 post partum,a more intense reaction was visualised.

In adult testis a strong NOS-activity was present in well differentiated Leydig cells (Fig. 3). No staining was observed in seminiferous tubules.

II. Ovary

In the ovary, the principal sites of steroid hormone production are follicle cells, theca cells and luteal cells. The interstitial tissue is the main source of ovarian androgens.

In neonatal rat ovary, the oocytes were in diplotene stage of meiotic prophase. In cryostat sections, the diaphorase reaction was negative.

In 15-day-old pubertal animals, the follicle formation was completed. The primary follicles showed a moderate positive NOS-reaction in the follicle cells. Later, in growing and antral follicles, both follicle and theca cells were stained. Oocytes remained negative at all developmental stages.

An increase in diaphorase staining was noted in the adult ovary. Besides in follicles, strong staining was demonstrated in luteal and interstitial cells (Figs. 4, 5, 6).

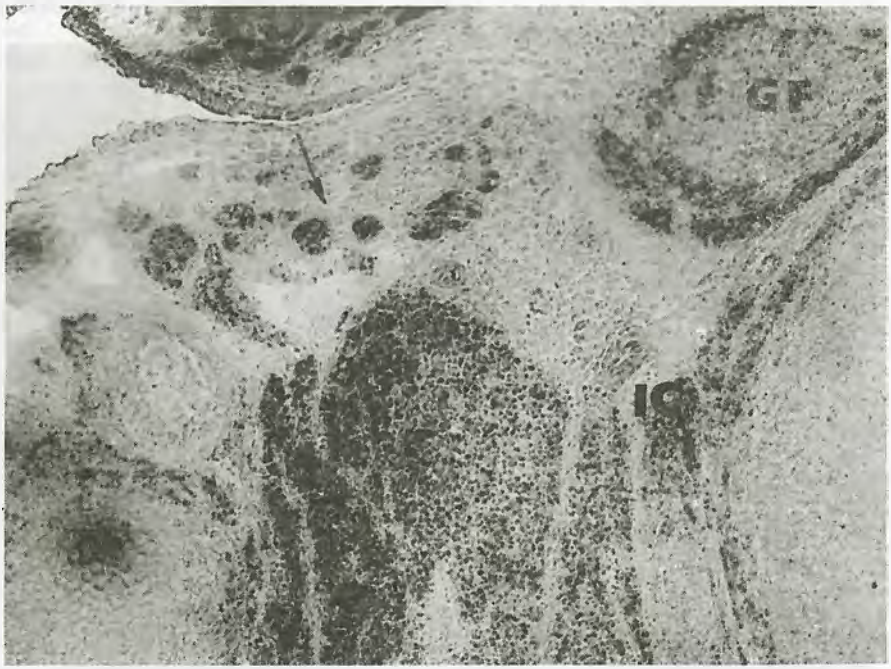

Fig. 4. Adult ovary NOS-reactivity in the follicle cells of primary follicles (→) and growing follicles (GF) as well as in the interstitial cells (IC) is present. ×160

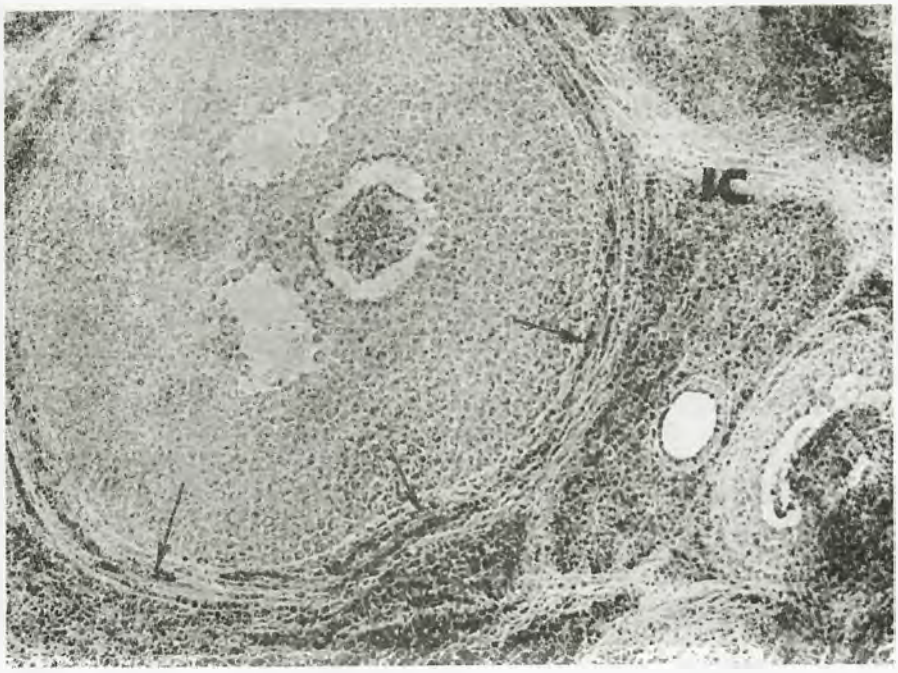

Fig. 5. Adult ovary theca cells (→) in an antral follicle and interstitial cells (IC) are diaphorase-reactive. ×160

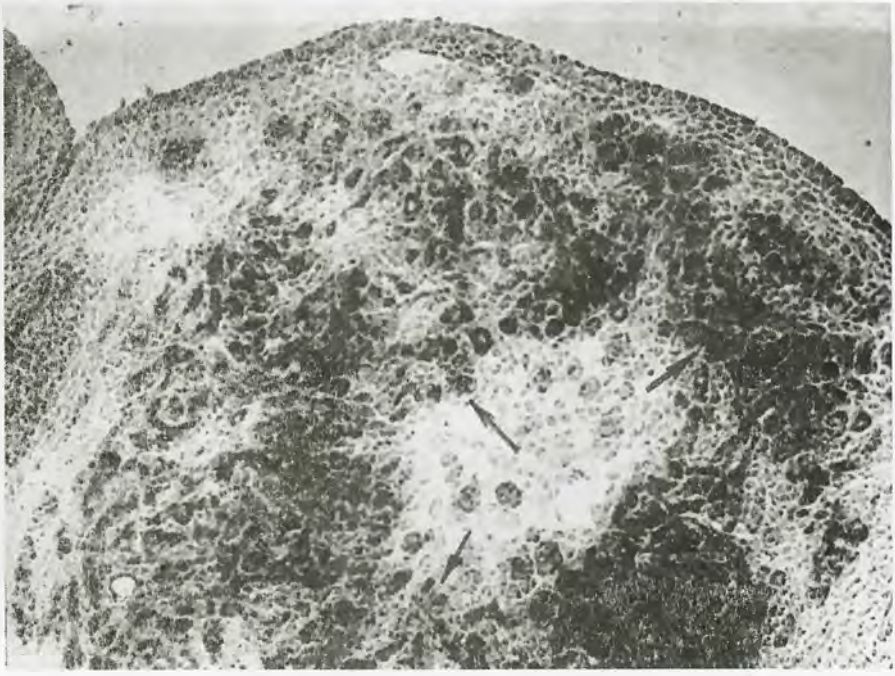

Fig. 6. A strong NOS-reaction is found in the luteal cells (→). ×160

Discussion

At present it is known that NOS is capable to exhibit a so-called NADPH-diaphorase activity which can easily be detected histochemically at the light microscope level.

In this study using NADPH-diaphorase activity of the enzyme, we were able to demonstrate the histochemical localization of NOS in developing rat gonads. It is interesting to note that the cellular diaphorase staining is correlated to the appearance and the differentiation of steroidogenic cells both in ovary and testis.

A diaphorase staining was present in fetal-type LC in neonates as well as in the second generation LC in pubertal and adult animals. In the ovary, the NOS was localized in all sreroidogenic cells: follicle cells in primary folicles, theca cells in growing and antral follicles, luteal and interstitial cells. High NOS-activity was observed at stages of high steroid production: in neonatal period for LC and starting from puberty to reach a maximum in adulthood for both testicular and ovarian steroidogenic cells.

Our results are consistent with previously reported data about immunocytochemically detected NOS-activity in rodent LC (A n g e l o v a et al., 1995, in press).

Nitric oxide is membrane permeable and may, therefore, have effects in surrounding cells, as well as in the cells in which it is formed (H o p e et al., 1991). Being an intra- and intercellular messenger, NO is evidently involved in steroidogenic process. Recently it was shown that all three NO synthase isozymes display significant sequence homology to only one other mammalian enzyme, cytochrome P-450 reductase (see L o w e n s t e i n, S n y d e r, 1992). Perhaps NOS and the cytochrome P-450 enzyme were linked in evolution, which fits with NOS displaying P-450 properties (M c M i l l a n et al., 1992)

In conclusion our results revealed the localization of NOS in gonadal steroidogenic cells thus suggesting a possible role for NO as a regulator of steroid hormone production and secretion.

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