VIA MEDICA

SHORT COMMUNICATION

Folia Morphol.
Vol. 62, No. 3, pp. 239–242

ISSN 0015–5659
www.fm.viamedica.pl

Ontogeny of the expression of catecholamine synthesising enzymes in the female porcine median eminence arcuate nucleus complex (MEARC)

Waldemar Sienkiewicz, Mirosław Łakomy

Department of Animal Anatomy, Faculty of Veterinary Medicine, University of Warmia and Mazury, Olsztyn, Poland

[Received 4 June 2003; Accepted 30 June 2003]

The ontogeny of the catecholaminergic system of the median eminence (ME) arcuate nucleus (ARC) complex (MEARC) has been studied in various animal species but so far, nothing has been learnt about the development of catecholaminergic structures in the porcine MEARC. To study this problem the hypothalami from animals at different ages (six groups) were collected. Nerve structures immunoreactive (IR) for the substances studied [(tyrosine hydroxylase (TH), dopamine β-hydroxylase (DβH) and phenylethanoloamine-N-metylthransferase (PNMT)] were found in the pigs at different age periods. In MEARC, TH-IR structures appeared before the 70th day of foetal life, DβH-IR before the 10th week of postnatal life and PNMT-IR only in sexually mature sows.

key words: ontogeny, catechoalmines, pig, median eminence, arcuate nucleus

INTRODUCTION

It has been shown that dopamine [4], noradrenaline and adrenaline [1] regulate the release of LH-RH. There are several hypotheses explaining the mechanisms for the influence of catecholamines on the release of LH-RH. One of these suggests the regulation of the secretion of LH-RH by catecholaminergic nerve fibres (mainly dopaminergic) projecting to the median eminence and modulating the release of LH-RH from adjacent nerve fibres [6]. Regulation of LH liberation from secretory cells of the hypophysis can be also influenced by catecholamines released from nerve fibres directly into the bloodstream of portal vessels in the median eminence and hypophysis [6]. Morphological studies focused on the hypothalamus have so far been performed mainly on laboratory animals. Data on this in farm animals are only fragmentary [2, 4, 5, 7, 8]. Studies have been performed on the pig using quantitative methods [2] or single-labelling immunohistohemistry [5, 6] and nothing is yet known about the coexistence of different catecholamine-synthesising enzymes in the same neuronal structures of the MEARC. In view of this, it is very important to understand some developmental aspects of the catecholaminergic system of the porcine MEARC: the onset of catecholamine synthesis, changes in the content of catecholamines or their markers, the ontogeny of the catecholaminergic system and its final form.

MATERIAL AND METHODS

The hypothalami from female foetal and postnatal pigs at different ages were used. The female foetuses at different ages (112 days, n = 4, 84 days, n = 8 and 70 days, n = 9) were removed from the uterus by caesarean section. The pregnant sows

Address for correspondence: Waldemar Sienkiewicz, University of Warmia and Mazury, Faculty of Veterinary Medicine, Department of Animal Anatomy, ul. Oczapowskiego 14, Bldg 105J, 10–957 Olsztyn, Poland, tel. +48 89 523 39 53, e-mail: sienio@moskit.uwm.edu.pl

were deeply anaesthetised with propionylopromazine and sodium pentontobarbital. After extraction, the foetuses were perfused with 4% paraformaldehyde in phosphate buffer. The postnatal animals (1-day-old n = 4, 10-week-old piglets, n = 7 and 8-month-old sows, n = 3) were deeply anaesthetised as described above. They were then perfused with cold 4% paraformaldehyde. The hypothalami were dissected out, postfixed in the same fixative and then transferred into 30% buffered sucrose. Serial cryostat sections were mounted on chromalum coated slides and immunostained using double-labelling according to the Wessendorf and Elde method [10]. Antibodies raised in mice (TH, code 2/40/15, source Boehringer, FRG, dilution 1:50) or rabbits (DβH code TE 103, source ETI, USA, dilution 1:600, PNMT code TE 104, source ETI, USA, dilution 1:600) were used. Omission, replacement and preabsorption tests proved the specificity of the immunostaining.

RESULTS AND DISCUSSION

In ARC of 70-day-old foetuses only small (about 10 μm in a diameter) single neurones (Fig. 1), and in 84-day-old foetuses single neurones (Fig. 2) but also single fine nerve fibres, solely TH-IR, were disclosed. In the oldest foetuses the number of IR nerve fibres increased. In 1-day-old animals a small increase in the number of nerve cells (Fig. 3) but a decrease in the number of nerve fibres was observed. In ARC of 10-week-old piglets numerous TH-IR nerve cell bodies were observed (Fig. 4). Nerve fibres were very numerous and contained TH-, DβH-, TH/ /DβH- and PNMT-IR. TH-IR fibres were the most numerous, whereas PNMT-IR nerve terminals were only occasionally encountered. In the sexually mature sows, the immunoreactive structures resembled those found in 10-week-old pigs (Fig. 5). In the ME of 70-day-old foetuses, single smooth and varicose nerve fibres, immunoreactive only to TH, were found (Fig. 6). The number of TH-IR nerve fibres was higher in ME of 84 (Fig. 7) and 112-day-old foetuses. In 1-day-old animals, ME contained numerous TH-IR (Fig. 8) and only single DβH-IR nerve fibres. In 10-week-old and sexually mature animals a higher number of TH (Fig. 9) and DβH-IR (Fig. 10) nerve terminals was observed. The vast majority of these fibres were located in the external zone of ME. Larger numbers of nerves supplied intrinsic blood vessels. The density of the nerve fibres increased with advancing age. In the ME TH-IR nerve terminals appear before the 70th day, whereas DβH-IR nerve

Figure 1. ARC in the 10-week-old foetus. TH-IR neuron.

Figure 2. ARC in the 12-week-old foetus. TH-IR neuron.

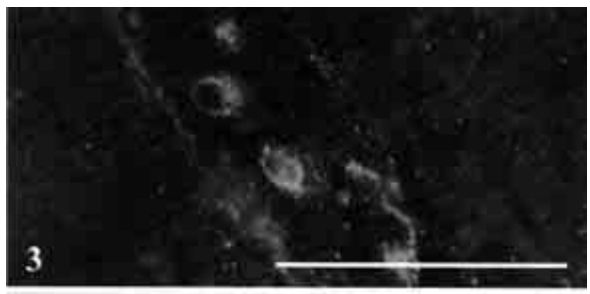

Figure 3. ARC in the 1-day-old piglet. TH-IR neurones.

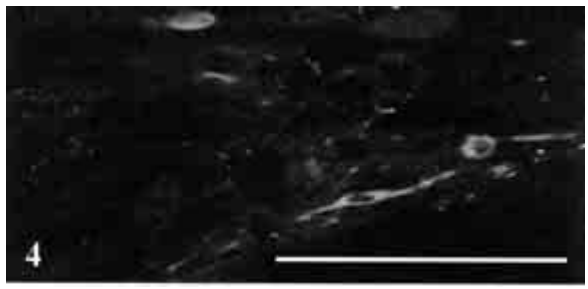

Figure 4. ARC in the 10-week-old pig. TH-IR neurones.

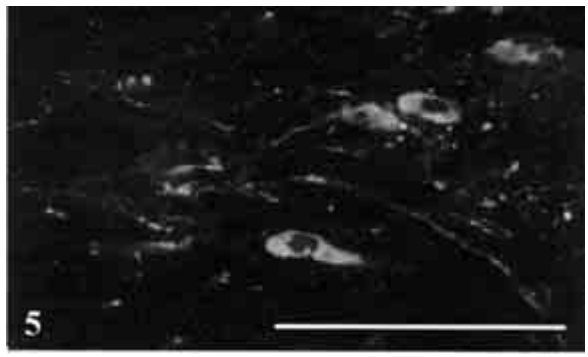

Figure 5. ARC in the sexually mature sow. TH-IR neurones.

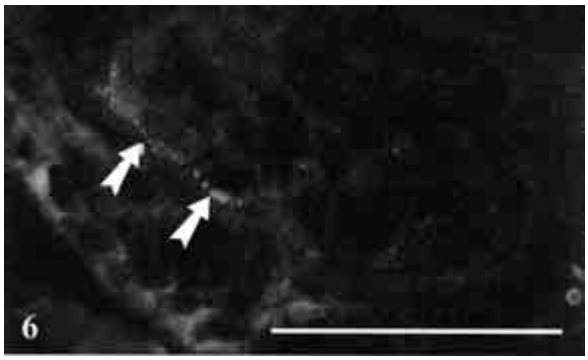

Figure 6. ME in the 10-week-old foetus. Fine varicose TH-IR nerve fibres.

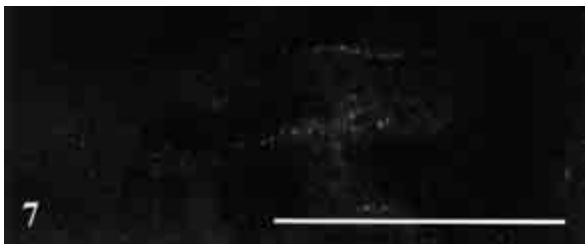

Figure 7. ME in the 12-week-old foetus. Fine varicose TH-IR nerve fibres.

Figure 8. ME in the 1-day-old piglet. TH-IR nerve fibres.

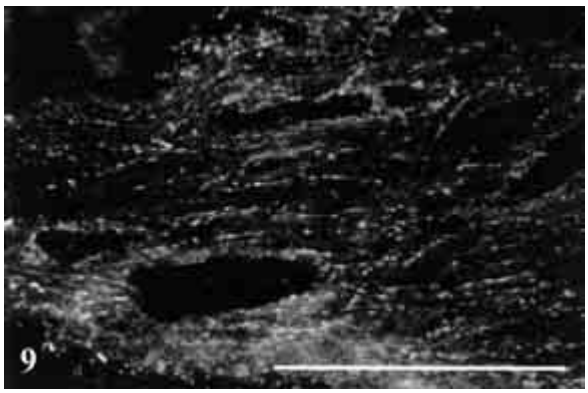

Figure 9. ME in the sexually mature sow. Very dense network of TH-IR nerve fibres.

terminals appear for the first time on the 1st day of postnatal life. The time and order of appearance of

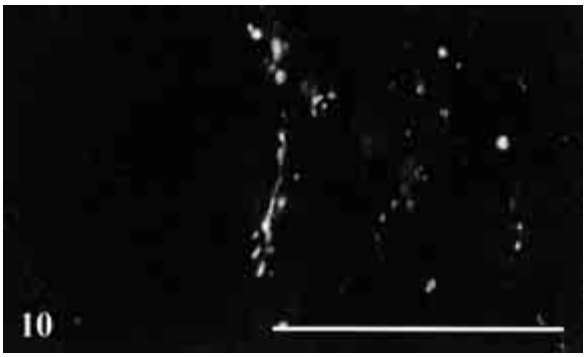

Figure 10. ME in the sexually mature sow. DßH-IR nerve fibres. All the bars = 50 μm.

different catecholamine synthesising enzymes in the catechoalminergic structures in MEARC was similar to those described previously in the porcine preoptic area [7].

In the rat ME TH-IR nerve fibres appear in the 18th day of foetal life [3, 9]. In the rat ME the number of IR nerve terminals increased up to the 9th day of postnatal life, when they reached a value characteristic for mature animals. A simple comparison of the duration of pregnancy suggests that the 70th–71st day of foetal life in the pig corresponds to the 13th day of foetal life in the rat. Thus it can be concluded that the first ME nerve structures appear earlier in the pig than in the rat.

Previous studies performed on sexually mature pigs revealed dense catecholaminergic innervation of ME [5]. This finding corresponds well with our results. The first dopaminergic neurones were already found in ARC of 70-day-old foetuses. During the postnatal life of the pig an increase in the number of these neurons was observed. The most numerous nerve structures were observed in the sexually mature pigs. The first dopaminergic neurones were found in the ARC of the rat on the 13th [9] and 15th day of foetal life [3]. Both the above-mentioned papers reported an increasing number of catecholaminergic structures. Noradrenergic (DβH-IR) and adrenergic (PNMT-IR) fibres appeared in the porcine ARC around the 10th week of postnatal life, much later than in the rat, in which adrenergic and noradrenergic nerve structures in ARC were already observed in the 16th and 21st day of foetal life respectively.

In the sexually mature pigs numerous catecholaminergic nerve cell bodies and fibres were found. This observation is in agreement with previous data on TH-, and DβH-IR structures in large farm animals [5, 7, 8].

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