EXPRESSION OF THE NERVE GROWTH FACTOR DURING EMBRYONIC GROWTH PERIOD OF JAPANESE QUAIL (COTURNIX COTURNIX JAPONICA)

Buket Bakir[1], Hakan Kocamis[2], Ebru Karadag Sari[3]

[1]Department of Histology-Embryology, Faculty of Veterinary Medicine, Namik Kemal University, Tekirdag, TURKEY,
[2]Department of Histology- Embryology, Faculty of Veterinary Medicine, Kırıkkale University, Kırıkkale, TURKEY,
[3]Department of Histology-Embryology, Faculty of Veterinary Medicine, Kafkas University, Kars, TURKEY

Geliş Tarihi: 11.11.2016 Kabul Tarihi: 21.12.2016
Makale Kodu: 5000207414

ABSTRACT

In this study, it was aimed to investigate expression of nerve growth factor (NGF) during embryonic growth period of Japanese quails (Coturnix coturnix japonica) using with immunohistochemical method. Quails embryos (5-6 eggs) were collected daily from 9th day until 16th day of incubation. The streptavidin-biotin-peroxidase complex method was used for immunohistochemical determination of NGF localization during embryonic growth development. NGF reaction was observed in intestine, muscle, kidney, liver, lung, spinal cord, blood vessels, and cartilage tissues from 9th day until 16th day of incubation. In conclusion, it was determination that NGF, which, especially in addition to its significant functions in development of sympathetic and sensory neurons, is active tissues take their final shape during embryonic growth by controlling growth, differentiation and apoptosis, is released from many primordial of tissues from 9th day until 16th day of embryonic period of Japanese quails.

Keywords: Embryo, immunohistochemistry, nerve growth factor, quail

Sinir Büyüme Faktörünün Japon Bıldırcın (Coturnix Coturnix Japonica) Embrıyolarının Büyüme Periyotları Boyunca Salınımı

ÖZET

Bu çalışmada, japon bıldırcın (Coturnix coturnix japonica) embriyolarının büyüme periyodu boyunca sinir büyüme faktörü (NGF) ekspresyonunun immünohistokimyasal yöntem kullanarak araştırılması amaçlandı. Bıldırcın embriyoları (5-6 yumurta) inkübasyonun 9. gününden 16. gününe kadar günlük toplandı. Embriyonik gelişim boyunca NGF lokalizasyonu immünohistokimyasal olarak belirlemek için streptavidin-biotin-peroxidase complex metodu kullanıldı. NGF reaksiyonu inkübasyonun 9. gününden 16. gününe kadar ince barsak, kas, böbrek, karaciğer, akciğer, omurilik, kan damarları ve kıkırdak dokularında tespit edildi. Sonuçta, özellikle sempatik ve duyu nöronlarının gelişiminde önemli görevlerine ek olarak, büyüme, farklılaşma ve apoptozisi kontrol ederek embriyonik gelişim boyunca dokuların son şekillerini almalarında aktif olan NGF'nin, japon bıldırcınlarının embriyonik periyotlarının 9. gününden 16. gününe kadar birçok doku taslağından salındığı tespit edildi.

Anahtar kelimeler: Embriyo, immünohistokimya, sinir büyüme faktörü, bıldırcın

İletişim / Correspondence
Namik Kemal University, Faculty of Veterinary Medicine, Department of Histology-Embryology, TR59000 TEKIRDAG TURKEY
+90 0282 250 47 15
buhal@hotmail.com

INTRODUCTION

Nerve growth factor (NGF) is a growth factor which plays an important role in the regulation of growth, differentiation and survival of neurons in both the central and peripheral nervous systems (4).

NGF has been implicated in several functions it can regulate hypertension (10), diabetes (19), Alzheimer (16), Parkinson (15) and elicit cardiovascular actions, including angiogenesis during fetal and neonatal stages (14). Also NGF plays a critical role in the regulation of both innate and acquired immunity (11) and induces ovulation (17).

Cell death (8) and proliferation (5) are a necessary process during embriyonic development and key to maintaining tissue homeostasis (9). NGF binds two different receptors: TrkA (Tropomyosine Receptor Kinase A) and LNGFR/p75NTR (Low-Affinity NGF Receptor) (7). TrkA stimulates the proliferation while LNGFR/p75NTR acts either survival or programmed cell death (12).

It was observed that NGF is present in chick embryos (2) and expressed in the central nervous system during embryonic development (6). It was explained NGF expressed firstly at stage 3 throughout in area pellucida in embriyonic development full gastrula and early neurula (3).

We preferred to work in the first primordial of tissues and we saw that first primordial of tissues were observed on 9th day in our previous study (1). This study was conducted in order to investigate that NGF is released from tissues of japanese quail (Coturnix coturnix japonica) embryos during mid and late stage of embryonic period. In this manuscript we reported for first time the localization of NGF in many primordial of tissues during mid and late stage of quail embryo development.

MATERIALS AND METHODS

Fresh fertilized Japanese quail's (Coturnix coturnix japonica) eggs were supplied from Kafkas University Education and Research Farm. Ethics approval was obtained from Kafkas University Local Ethics Committee for Animal Experiments (KAU-HADYEK/2016-001).

Animals and experimental design of our study was based on the study conducted by Bakir and Kocamis, (1). Japanese quail eggs were incubated at 37 0C and in humidified incubator. The eggs were collected daily from 9th day until 16th day (5-6 eggs). Embryos were fixed in 10% formol solution for 48 h and in Bouin's fixative for 24 h, and then routine procedures were applied and samples were embedded in paraffin. Serial sections of 5 µm thick were sliced from paraffin-embedded blocks.

For immunohistochemistry, the streptavidin-biotin-peroxidase complex method was used. Following deparaffinization and rehydration, sections were rinsed with Phosphate Buffer Solution (PBS) and incubated in 3% H202 (prepared in 0.1 M PBS) for 15 min in order to block the endogenous peroxidase activity. After rinsing with PBS, sections were put in citrate buffer solution and then processed in the microwave oven for 10 minutes in order to expose the antigenic sites. After rinsing with PBS again, sections were incubated in primary NGF antibody (ab6198), (1:100 dilution ratio) for 1 hour at room temperature. Negative control sections were incubated only in PBS. After rinsing with PBS, sections were kept at room temperature for 15 minutes with added streptavidin-horse radish peroxidase (HRP) (Invitrogen Histostain plus Broad Spectrum Ref. 85.9943). Sections were rinsed again with PBS and 3,3′-Diaminobenzidine tetrahydrochloride (DAB) (0.5 mg/ml; Dako Corp.) was used as chromogen followed by hematoxylin counterstaining. Sections were mounted with Immunmount and examined under research microscopy (Carl Zeiss Microscopy, Göttingen, Germany) and photographed.

NGF immunoreactivity in tissues was evaluated subjectively, determined semiquantitatively and graded from 0 to +3 (0: no reaction; 1: minimal reaction, 2: moderate

reaction; 3: strong reaction) according to intensity and extent of staining.

RESULTS

As a result of the evaluations, on the days of 9th, 10th and 11th on which primordia starts shaping, strong reaction (+3) in primordial of skeletal muscle, kidney, liver, spinal cord and intestine and minimal reaction (+1) was observed in primordial of cartilage tissue and lung (figure 1).

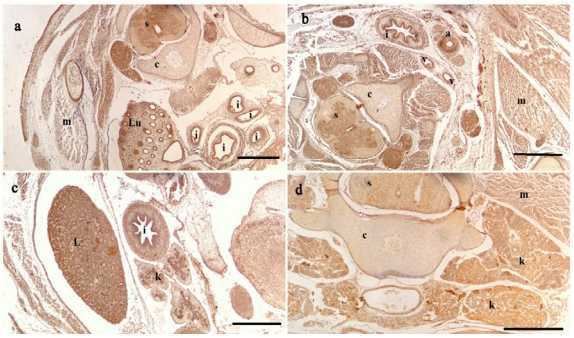

Figure 1. NGF immunoreactivity in Japanese quail (Coturnix coturnix japonica) on 9th day (a), on 10th day (b), on 11th day (c), on 12th day (d). s: spinal cord, m: muscular tissue, Lu: lung, i: intestine, c: cartilage, a: artery, v: vena, L: liver, k: kidney. Bar: 200 µm.

On the days of 12th, 13th, 14th, 15th and 16th, strong reaction (+3) was observed in villus and cyrpts epithelial cells, smooth muscle cells in intestine, skeletal muscle tissue cells, grey matter and central canal in spinal cord, proximal tubule cells in kidney, hepatocytes cells in liver, arterial and venous blood vessels and in ossification centers of cartilage tissues. Moderate reaction (+2) was determined white matter in spinal cord, distal tubule cells, and glomerulus of Bowman's space in kidney and minimal reaction (+1) was observed bronchus, bronchial tube and alveoli cells in lung and cartilage tissue cells (figure 2, 3, 4).

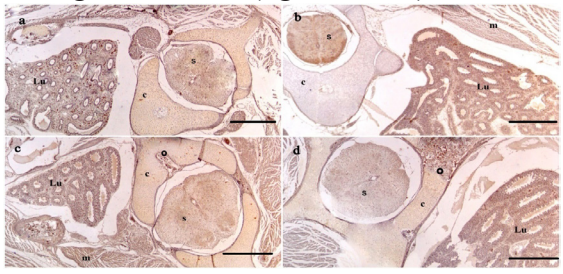

Figure 2. NGF immunoreactivity in Japanese quail (Coturnix coturnix japonica) on 13th day (a), on 14th day (b), on 15th day (c), on 16th day (d). s: spinal cord, m: muscular tissue, Lu: lung, c: cartilage, o: ossification center. Bar: 200 µm.

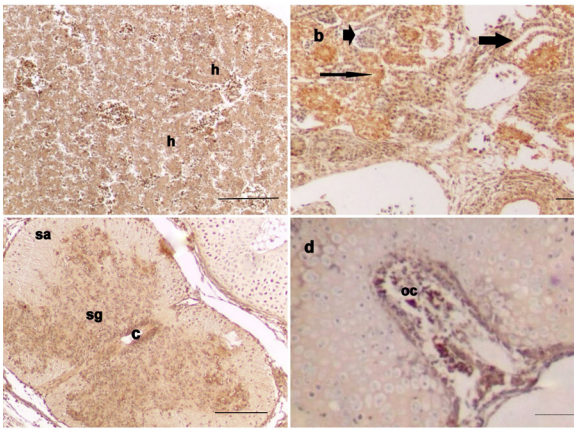

Figure 3. NGF immunoreactivity in Japanese quail (Coturnix coturnix japonica). Hepatocytes cells in liver on 13th day (h), Proximal tubule cell (long arrow), distal tubule cell (thick arrow) and glomerulus of Bowman's space (arrow head) in kidney on 14th day, grey matter (sg), central canal (c) and white matter (sa) in spinal cord on 15th day, ossification center of cartilage tissue (oc) on 16th day. Bar: 100 µm.

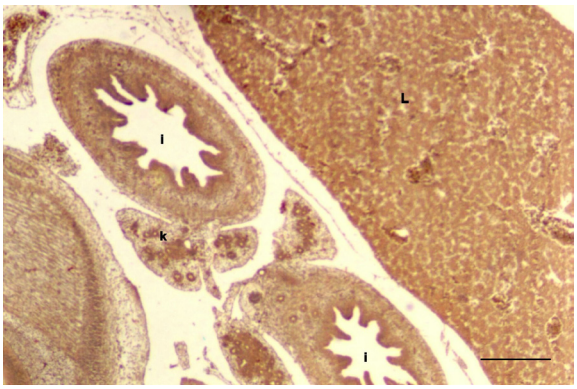

Figure 4. NGF immunoreactivity in Japanese quail (Coturnix coturnix japonica). Intestine (i), kidney (k), liver (L) on 12th day. . Bar: 100 µm.

DISCUSSION

NGF is a growth factor that is active in development and differentiation of neural system (4). In our study it was understood that NGF, whose release has been reported from certain tissues, especially from neural system in adult stage, releases from other tissues in addition to neural system during quail embryos' development.

Little is known about the presence or function of NGF in avian tissues. It was showed that optic tectum and cerebellum contained the highest levels of NGF mRNA in avian. It was reported that NGF expressed throughout the area pellucida (at stage 3), the initial primitive streak, the primitive groove, the primitive pit and Hensen's node (at stage 4), the notochord (at stages 5-7), the head fold

and the somites (at stages 7-8) (13).

Bhargava et al. (3) showed that NGF specific immunoreaction is first detected in the area pellucida stage localized to the cell surface of invaginated mesoblast and the cytoplasm in invaginated embryonic endoblast underlying the primitive streak (at stage 4), in the head fold (at stage 6), the neural crest (at stages 7-8), the neural tube, the neural cells, the head mesenchyme, the head ectoderm, the area pellucida endoderm, surrounding the anterior neuropore (at stages 9-10), also they showed NGF positive cells in the telencephelon, surround the neuropore, in the floor of the forebrain, the mid- and hindbrain (at stages 11-12). In our study, NGF release was determined from almost many tissues during embryonic process in japanese quails. NGF release from tissues during embryonic stage may be explained by its control on apoptosis for reproduction and differentiation and also for allowing organs take their final shape (3).

A study observed that NGF is required for the development of peripheral avian sensory and sympathetic neurones. They treated with the NGF antibody between 3th day and 11thday. NGF antibodies on sympathetic neurones was assessed by determining the levels of the adrenergic marker enzyme tyrosine hydroxylase (18).

Also Mancaa et al. (13), injected with the anti-NGF monoclonal antibody at stage of 11-12. They showed a defect in the axial rotation of the embryo fixed 48 h. In normal conditions, the head of the chicken embryo begins to rotate such that it comes to lie on its left side. External NGF administration or preventing NGF release has effect on embryonic growth (13).

As a result, it was observed that NGF, whose significant role in development of especially sympathetic and sensory neurons has been determined, is released from many tissues in organism according to recent studies. Current studies have displayed its effect on neural system development especially during embryonic stage (3, 13, 18). However, any study on its release from other primordial of tissues during embryonic period of avian was not seen. This study, which aimed to obtain data about NGF release in embryonic period in avians, showed that many tissues begin to have a shape similar to the structure in postnatal period from 9th day until 16th day. According to our investigations on Japanese quail embryos from 9th day until 16th day, NGF releases from many tissues like as smooth and skeletal muscle, kidney, liver, spinal cord, lung, intestine, blood vessels, center of ossification and cartilage tissue. Thus, it may be concluded that NGF is a growth factor, which releases from many tissue primordia and is active in growth and differentiation during this process.

REFERENCES

1. Bakir B, Kocamis H. The Effects of Insulin-Like Growth Factor-1 on Bone Growth During Japanese Quail Embriyonic Development. Kafkas Univ. Vet. Fak. Derg. 2011; 17:457-461.

2. Belew M, Ebendal T. Chick embryo nerve growth factor. Fractionation and biological activity. Exp. Cell Res. 1986; 167:550-558.

3. Bhargava S, Modak SP. Expression of nerve growth factor during the development of nervous system in early chick embryo. Dev. Brain Res. 2002; 136: 43-49.

4. Bibel M, Barde YA. Neurotrophins key regulators of cell fate and cell shape in the vertebrate nervous system. Genes Dev. 2000; 14:2919-2937.

5. Bothwell M. Functional interactions of neurotrophins and neurotrophin receptors. Annu. Rev. Neurosci. 1995; 18:223-253.

6. Escandon E, Chao MV. Developmental expression of the chicken nerve growth factor receptor gene during brain morphogenesis. Brain Res. Dev. 1989; 47:187-196.

7. Frade JM, Barde YA. Nerve growth factor: two receptors multiplefunctions, Bioessays. 1998; 20:137-145.

8. Glucksmann A. Cell death in normal vertibrate ontogeny. Biol. Rev. 1951; 26:59-86.

9. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell, 2000; 100:57-70.

10. Hennigan A, Callaghan CK., Kealy J, Rouine J, Kelly AM. Deficits in LTP and recognition memory in the genetically hypertensive rat are associated with decreased expression of neurotrophic factors and their receptors in the dentate gyrus. Behav. Brain Re. 2009; 197:371-377.

11. Lambiase A, Bracci-Laudiero L, Bonini S, Bonini S, Starace G, D'Elios MM, De Carli M, Aloe L. Human CD4+ T cell clones produce and release nerve growth factor and express high-affinity nerve growth factor receptors. J Allergy Clinic Immun. 1997; 100:408-14.

12. Lee R, Kermani P, Teng KK, Hempstead BL. Regulation of cell survival by secreted proneurotrophins. Sci. 2011; 294:1945-48.

13. Mancaa A, Capsonia S, Di Luzioc A, Vignonea D, Malerba F, Paolettia F, Brandia R, Arisia I, Cattaneo A, Levi-Montalcini R. Nerve growth factor regulates axial rotation during early stages of chick embryo development. PNAS, 2012; 109:2009-2014.

14. Meloni M, Caporali A, Graiani G, Lagrasta C, Katare R, Van L, Inthout S, Spillmann F, Campesi I, Madeddu P, Quaini F, Emanueli C. Nerve growth factor promotes cardiac repair following myocardial infarction. Circ. Res. 2010; 106:1275-1284.

15. Olson L, Backlund EO, Ebendal T, Freedman R, Hamberger B, Hansson P, Hoffer B, Lindblom U, Meyerson B, Stromberg I. Intraputaminal infusion of nerve growth factor to support adrenal medullary autografts in Parkinson's disease. One-year follow-up of first clinical trial. Arch. Neurol. 1991; 48:373-381.

16. Olson L, Nordberg A, von Holst H, Backman L, Ebendal T, Alafuzoff I, Amberla K, Hartvig P, Herlitz A, Lilja A. Nerve growth factor affects 11C-nicotine binding, blood flow, EEG, and verbal episodic memory in an Alzheimer patient (case report). J Neural Transm. Park Dis. Dement. Sect. 1992; 4:79-95.

17. Ratto MH, Leduc YA, Valderrama XP, van Straaten KE, Delbaere LT, Pierson RA, Adams GP. The nerve of ovulation-inducing factor in semen. Proc. Nat.l Acad. Sci. USA, 2012; 109:15042-47.

18. Rohrer H, Hofer M, Hellweg R, Korsching S, Stehle AD, Saadat S, Thoenen H. Antibodies against mouse nerve growth factor interfere in vivo with the development of avian sensory and sympathetic neurones. Dev. 1988; 103:545-552.

19. Sima AA. New insights into the metabolic and molecular basis for diabetic neuropathy. Cell Mol. Life Sci. 2003; 60:2445-2464.