Annals of Anatomy 207 (2016) 38–46

Contents lists available at ScienceDirect

Annals of Anatomy

journal homepage: www.elsevier.com/locate/aanat

Neurotrophins and specific receptors in the oviduct tracts of Japanese quail (*Coturnix coturnix japonica*)

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ARTICLE INFO

Article history:
Received 29 October 2015
Received in revised form 21 March 2016
Accepted 22 April 2016

Keywords:
Neurotrophins
Trk receptors
Oviduct
Quail

ABSTRACT

Neurotrophins (NGF, BDNF and NT-3) and their specific receptors (TrkA, TrkB and TrkC) were studied in the oviduct of egg laying quails. Neurotrophins (NTs) are mainly involved in the development and maintenance of neuronal populations in the central and peripheral nervous system, but also in reproductive system. In this survey, we first studied the morphological organization of the quail oviduct, distinguished in infundibulum, magnum, isthmus, uterus and vagina, and then we analyzed the expression and localization of NTs and Trks receptors in the whole tracts. By western blotting we detected that the investigated NTs and Trks receptors are expressed in all oviductal tracts. By immunohistochemistry we were able to define the distribution of NTs and Trks. Specifically, NGF, BDNF and NT3 were localized in lining and ductal epithelial cells, and NGF was also detected in secretory cells of tubular glands and in nervous fibers of vessel wall. TrkA and TrkB were present in the lining and ductal epithelium; TrkA and TrkC were present in nervous fibers of vessel wall in all oviductal tracts. Furthermore, we also observed NGF and BDNF co-localized with TrkA and TrkB in cells of the lining and ductal epithelium, suggesting an autocrine mechanism of action.

1. Introduction

Neurotrophins (NTs) are a family of growth factors including nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), NT3, NT4. They control the development and maintenance of neuronal populations in the central and peripheral nervous system, but they can also act on non neuronal cells in different organs and systems, such as the mammalian reproductive system (Ibáñez et al., 1991; Dissen et al., 1995, 2002; Anderson et al., 2002; Abir et al., 2005; Seifer et al., 2006).

The neurotrophin sequences are highly conserved in vertebrates (Lanave et al., 2007; Tettamanti et al., 2010). However, in birds, NT4 has not been identified (Hallbook et al., 1995). All neurotrophin genes code glycosylated precursors (31–35 kDa), that are cleaved by convertases to give rise to mature neurotrophins (13.2–15.9 kDa) (Roux and Barker, 2002; Chao, 2003). The action of NTs is mediated by highly specific tyrosine kinase receptors (Trks) (Teng and Hempstead, 2004), and by a pan-neurotrophin receptor, the $p75^{NTR}$ (Barret, 2000), that binds each neurotrophin with low affinity. Among Trk receptors, TrkA is the preferred receptor for NGF, TrkB for BDNF/NT4, and TrkC for NT-3 (Patapoutian and Reichardt, 2001; Deinhardt and Chao, 2014). In addition, TrkA and TrkB receptors can be activated, even if less efficiently, by NT3, as well (Skaper, 2012). The Trk receptors are generally characterized by full-length and truncated isoforms. Full-length kinase isoforms (140–145 kDa) comprise the tyrosine kinase signaling domain, whereas truncated isoforms (50–95 kDa) lack the domain (Klein et al., 1990; Barbacid et al., 1991; Lamballe et al., 1993). It has been hypothesized that the truncated forms of receptors are expressed by non neuronal tissue (Barbacid, 1995).

In the present survey, we investigate the expression and localization of NTs and Trks receptors in the oviduct of quail *Coturnix coturnix japonica*, species used as animal model in reproductive biological studies (Hosseini et al., 2016). The morphology and physiology of oviduct in birds are much more complex than in mammals, making this organ particularly challenging. The left oviduct is the site of the second meiotic division of the oocyte, and sperm storage and oocyte fertilization occur similarly to that in mammals (Buhi, 2002). Here the formation of the different components of the egg (i.e., albumen, shell membranes, shell and cuticle) (Bell

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http://dx.doi.org/10.1016/j.aanat.2016.04.033
0940-9602/

and Freeman, 1971), necessary for embryonic development, takes place. To our knowledge, there are no data on the presence of NTs and Trks in the avian genital tract. Previous studies have been carried out in mammalian species, documenting the involvement in the oocyte transport and fertilization, in spermatozoa capacitation (Weng et al., 2009), and in development of preimplantation embryos (Kawamura et al., 2007; Mirshokraei et al., 2013).

2. Materials and methods

2.1. Animals and tissue preparations

The Japanese quail (*Coturnix coturnix japonica*) used in this study came from the intensive growing system belonging to the University of Agricultural Science and Veterinary Medicine of Iasi. Three month old quails in the period of egg laying were used. All animals were euthanized by an overdose of sodium pentobarbital. Immediately, the genital tract of 6 quails was collected. Oviduct tracts from 3 animals were separately identified (infundibulum, magnum, isthmus, uterus and vagina) and stored at −80 °C for Western blotting (WB) analysis; and the same tracts from 3 animals were fixed in Bouin's fluid for 24 h for histological and immunohistochemical techniques. The fixed samples dehydrated and embedded in paraffin, were serially sectioned at 7 μm and stained with hematoxylin-eosin (HE), for a general histological description, and used for immunohistochemistry.

2.2. Western blotting analysis

For WB analysis of NGF, BDNF and NT-3, three samples of infundibulum isthmus, magnum, uterus and vagina for each antibody, respectively, were homogenized using Tissue Lyzer (Qiagen) in 100 μl of ice-cold lyses buffer (50 mM Tris pH 7.5; 150 mM NaCl; 1 mM EDTA; 0.25% deoxicolic acid, 1% Triton X 100) in addition to 20 mM sodium pyrophosphate, 0.1 mg/ml aprotinin, 2 mM phenylmethylsulphony fluoride (PMSF), 10 mM sodium orthovanadate (Na2VO3), and 50 mM NaF. The quantity of total proteins was determined by use of a protein assay kit (Bio-Rad Laboratories). Equal amounts of lysate samples (80 μg) were boiled and loaded on bis/acrylamide gel and electrophoresed. The proteins were blotted from the gel onto nitrocellulose membranes. The membranes were blocked with 5% bovine serum albumin (BSA) in TBS-0.1% Tween buffer (10 mM Tris–HCl, pH 7.4, 165 mM NaCl, 0.1% Tween) at room temperature (RT), washed with TBS-0.1% Tween, and incubated with antibodies (Table 1). Each antibody was diluted 1:200 in TBS-0.1% Tween. After appropriate washing steps in TBS Tween 0.1%, anti-rabbit peroxidase-conjugated secondary antibody (Amersham, Gel Health Care) was applied 1 h at RT at 1:2000 dilution. The blots were stripped and reprobed against mouse anti-actin antibody (CP01, Calbiochem), 1:5000, to ensure equal amounts of proteins for each sample.

For WB analysis of receptors TrkA, TrkB and TrkC, three samples of infundibulum isthmus, magnum, uterus and vagina for each antibody, were homogenized using Tissue LyzerII (Qiagen, Chatsworth, CA) in 100 ml of lysis buffer (50 mM Tris–HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.5% NP40, 1 μg/ml Leupeptid, 1 μg/ml Aprotimin, 1 μg/ml Pepstatin A, 0.25% Sodium deoxycholate, 1 mM NaF) homogenized at 13,200 rpm at 4 °C. The quantity of total proteins was determined by use of a protein assay kit (Bio-Rad Laboratories). Samples containing 50 μg of lysated protein were denatured by incubation at 95 °C in 0.1% sodium dodecyl sulfate (SDS) 1 mM Beta mercaptoethanol in Tris–HCl pH 6.8 and separated on a 15% SDS-polyacrylamide gel electrophoresis (SDS PAGE) gel in a mini-Protean Tetra Cell chamber (BioRad, Hercules, CA) at 4 °C with 90 V for 2 h. Proteins were transferred onto Hybond-ECL (Amersham, Arlington Heights, IL) nitrocellulose membranes using the Mini Protean system (BioRad) for 45 min using 400 mA at 4 °C. Ponceau red was used to assess homogenous transfer. Prestained ladder (Thermo Scientific PageRuler Prestained Protein Ladder) was used. The proteins were blotted from the gel onto nitrocellulose membranes. The membranes were blocked with 5% bovine serum albumin (BSA) in TBS-0.1% Tween buffer (10 mM Tris–HCl, pH 7.4, 165 mM NaCl, 0.1% Tween) at RT, washed with TBS-0.1% Tween, and incubated with antibodies (Table 1). Each antibody was diluted 1:200 in TBS-0.1% Tween. After appropriate washing steps in TBS Tween 0.1%, anti-rabbit peroxidase-conjugated secondary antibodies (Amersham, Gel Health Care) were applied 1 h at RT at a dilution of 1:2000. The blots were stripped and reprobed against mouse anti-actin antibody (CP01, Calbiochem), 1:5000, to ensure equal amounts of proteins for each sample. As positive controls, two mouse submandibular glands were employed for NGF antibody, and PC12 whole cell lysates for BDNF, NT-3, TrkA, TrkB and TrkC. For TrkA and TrkC, the image was adjusted, by re-organizing the lanes according to the anatomical organization of oviductal tracts.

2.2.1. Morphological stainings and single immunohistochemistry

Hematoxilin-eosin staining was carried out on microtome sections adjacent to those utilized for immunohistochemical stainings.

Immunohistochemistry was carried out using the EnVision system + horse radish anti-peroxidase (HRP) (Dako, Santa Barbara, CA,

Table 1
List and features of antibodies.

Antibody	Antigen	Dilution	Code and source
NGF	N-terminus of the mature chain of NGF of human origin	1:500 IHC 1:50 IF 1:200WB	sc-548 S. Cruz Biotecnology, CA, USA
BDNF	Internal region of BDNF of human origin aa.100-150	1:500 IHC 1:50 IF 1:200WB	sc-546 S. Cruz Biotecnology, CA, USA
NT-3	Internal region of NT-3 of human origin	1:500 IHC 1:50 IF 1:200WB	sc-547 S. Cruz Biotecnology, CA, USA
TrkA	Human COO-domain 763-777	1:400 IHC 1:40 IF 1:200WB	sc-118 S. Cruz Biotecnology, CA, USA
TrkB	Human COO-domain 794-808	1:200 IHC 1:20 IF 1:200WB	sc-12 S. Cruz Biotecnology, CA, USA
TrkC	Human COO-domain 798-812	1:200 IHC 1:20 IF 1:200WB	sc-117 S. Cruz Biotecnology, CA, USA

IHC, immunohistochemistry; IF, immunofluorescence; WB, western blotting.

USA, cod.K400311-2) protocol. In order to block the activity of endogenous peroxidase, sections were treated with 3% hydrogen peroxide 30 min, and rinsed in PBS (phosphate buffered saline, pH 7.4). Successively, the sections were pre-incubated in the humid chamber with normal goat serum (NGS) (Vector Lab. Inc., Burlingame CA, USA, cod. S-1000), 30 min at RT, and incubated overnight at 4 °C with the primary polyclonal rabbit antibody (Table 1). Subsequently, the sections were rinsed in phosphate buffered saline (PBS) 5 min, incubated with En Vision system HRP, 30 min at RT, and washed again in PBS. The sites of immunoreaction were visualized by using 10 μg 3,3′-diaminobenzidine tetrahydrochloride (DAB) (Sigma-Aldrich Corporation, St Louis, MO, USA, cod.D5905) as chromogen in 15 ml Tris buffer 0.5 M pH 7.6 containing 1.5 ml 0.03% H_2O_2. After dehydration and mounting, sections were examined and photographed using a Nikon Eclipse 90*i* microscope.

2.2.2. Double immunohistochemistry

For the immunofluorescence reaction, red fluorochrome, cianin 3 (cy3) conjugated with a signal amplifier, tiramide (TSA) (PERKIN ELMER SAT 704 A 001EA) was used for the first antibody, and Alexafluor 488 conjugated with streptavidin (Jackson Immunoresearch, 016-540-084) for the secondary antibody. For the working protocol: the rehydrated sections were treated with H_2O_2 for 20 min, to block endogenous peroxidase activity, then they were incubated for 30 min with primary polyclonal antibody (Table 1), diluted in normal donkey serum (NDS) (Jackson Immunoresearch, 017-000-121), and, after 2 rinses in PBS, incubated for 30 min with secondary donkey anti rabbit antibody IgG (H + L) conjugated with HRP (Jackson Immunoresearch, 711-035-132). After rinsing in PBS, the sections were again incubated for 10 min with Cy3 conjugated with TSA and washed again in PBS. Successively, they were incubated for 30 min with NGS, treated with avidin-biotin blocking kit (Vector Laboratories SP-2001), and then incubated in humid chamber at 4 °C overnight with the primary antibody (Table 1). The sections were incubated for 2 h with goat anti-rabbit as secondary antibody biotinilated (Vector laboratories BA-100), washed in PBS, incubated for 2 h with Alexafluor 488 Streptavidin and rinsed in PBS. Finally sections were mounted with a solution containing PBS and glycerol (1:1), for observation with microscope Nikon Eclipse 90*i*. The acquired images were cataloged using the software NIS-elements 4.20.

2.2.3. Controls

Controls of antibody specificity were performed by absorbing each primary antiserum with an excess of the relative blocking peptide (NGF sc-548P; BDNF sc-546P; NT-3 sc-547P; TrkA sc-118P; TrkB sc-12P; TrkC sc-117P); and by substituting primary or secondary antisera with PBS or normal serum in the specific steps.

3. Results

3.1. Western blotting

The WB results (Fig. 1) showed that: NGF antibody recognizes two protein bands of ~15 kDa and ~27 kDa, in all tracts of oviduct; BDNF antibody recognizes three protein bands of ~14 kDa, ~25 kDa and ~38 kDa, in all tracts; NT-3 antibody recognizes a single protein band of ~35 kDa in all tracts; TrkA antibody recognizes a protein band of ~80 kDa, in all oviduct tracts; TrkB antibody recognizes two protein bands of ~95 kDa and ~140 kDa, in all oviduct tracts; TrkC antibody recognizes three protein bands of ~90 kDa in all oviduct tracts, ~70 kDa in infundibulum, isthmus and vagina and ~55 kDa in infundibulum, uterus and vagina.

β-actin, used as internal marker, was detected in all examined oviduct tracts as a band of about 43 kDa.

3.2. Morphological observations

The different oviductal tracts share the same basic structure consisting in mucosa, submucosa, muscular layer and serous membrane.

The infundibulum, divided in a funnel toward the ovary and the caudal tubular part has broad highly branched mucosal folds, carrying numerous small secondary folds, lined with ciliated and non ciliated simple columnar epithelium, without mucosal glands.

The magnum shows high primary mucosal folds and numerous branched tubular glands. The luminal epithelium is simple, columnar and consists of alternating ciliated and non-ciliated cells. The isthmus shows primary and numerous secondary mucosal folds, containing tubular glands. The lining epithelium consists of ciliated columnar and non ciliated cells.

The uterus is characterized by the leaf aspect of the mucosal folds, and the branched tubular glands. The lining epithelium consists of both ciliated and non-ciliated cells.

The vagina is characterized by a thin mucosa, composed mainly of primary folds, without glands. The lining epithelium is mainly formed of columnar ciliated and non ciliated cells and appears pseudostratified.

3.3. Immunohistochemistry

Immunoreactivity (IR) to NTs and Trks receptors in all oviduct tracts is reported (Table 2). NGF IR is observed in epithelial cells of all oviduct tracts (Fig. 2a–e), and in the tubular glands of magnum, isthmus and uterus (Fig. 2b–d). Intense IR is detected in the apical cytoplasm of the epithelial cells of infundibulum, magnum and isthmus, and sometimes in the whole cytoplasm of magnum, isthmus and uterus epithelium. Moreover, in the uterus, NGF IR is more evident at the apical pole of ductal cells. Only in the vagina is NGF IR mainly localized in the basal cytoplasm of epithelial cells (Fig. 2e). Positive nervous fibers are also observed in vessels wall of all oviduct tracts (Fig. 2f).

BDNF IR is found only in magnum (Fig. 3a) and isthmus. In these tracts BDNF positivity is detected in surface and ductal epithelial cells. The BDNF cell distribution appears mainly in the basal cytoplasmic area and sometimes in the whole cytoplasm (Fig. 3a).

NT-3 IR is found in magnum (Fig. 3b) and isthmus. Numerous NT-3 immunopositive cells are detected in the surface epithelium and in ductal cells of tubular glands (Fig. 3b). IR is detected in the apical cytoplasmic region and sometimes in the whole cytoplasm .

TrkA IR is found in the epithelial cells of all oviduct tracts, except for infundibulum (Fig. 4b–e) and in tubular glands of magnum and isthmus (Fig. 4b and c). The positivity is localized in the apical cytoplasm and sometimes in the whole cytoplasm. Moreover, positivity to TrkA is observed in fibers of vessels wall in all oviduct tracts (Fig. 4a).

TrkB IR is found only in the lining epithelium of infundibulum and magnum, and in ductal cells of magnum (Fig. 3c). The positivity is mainly distributed in apical region of epithelial cells.

TrkC IR is only localized in nervous fibers of vessels wall in all oviduct tracts (Fig. 3d).

The double immunostaining reveals that NGF and TrkA IR are colocalized in magnum (Fig. 5a–a″) and isthmus (Fig. 5b–b″), especially in surface epithelial cells and ductal cells of tubular glands. Moreover also BDNF IR is colocalized with TrkB IR in surface epithelial and ductal cells of magnum (Fig. 6).

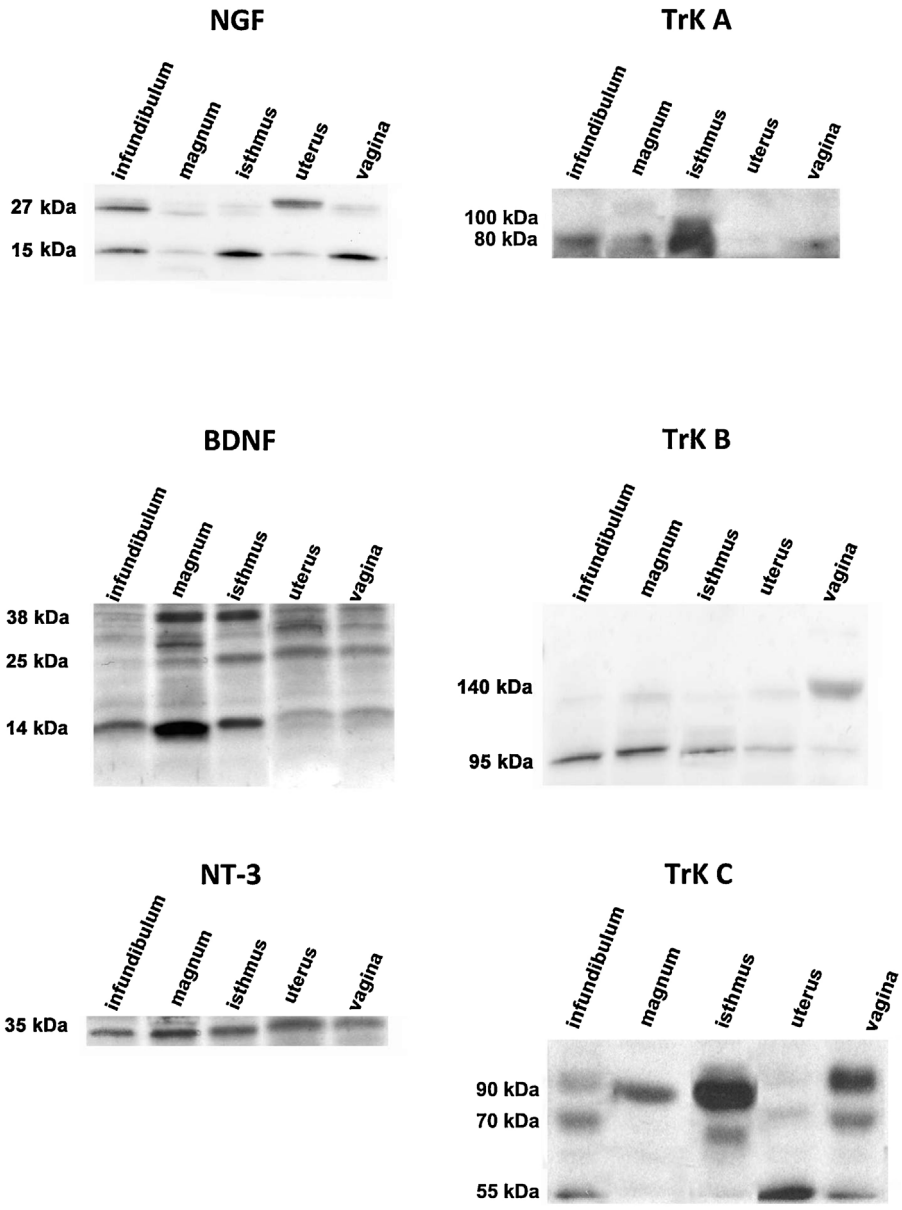

Fig. 1. Western blotting analysis of neurotrophins and Trk family receptors in quail oviduct homogenates. The NGF antibody recognized two protein bands of ~15 kDa and ~27 kDa; the BDNF antibody recognized three protein bands of ~14 kDa, ~25 kDa, and ~38 kDa; the NT-3 antibody recognized a single protein band of ~ 35 kDa; the TrkA antibody recognized a protein band of ~80 kDa; the TrkB antibody recognized two protein bands of ~95 kDa and ~140 kDa; the TrkC antibody recognized three protein bands of ~90 kDa, ~70 kDa in infundibulum, isthmus and vagina and ~55 kDa in infundibulum, uterus and vagina.

Table 2
Distribution of neurotrophins and specific receptors in quail oviduct.

Tracts	NGF			TrkA			BDNF			TrkB			NT-3			TrkC		
	E	TGDC	TGSC	E	TGDC	TGSC	E	TGDC	TGSC	E	TGDC	TGSC	E	TGDC	TGSC	E	TGDC	TGSC
Infundibulum	+	/	/	–	/	/	–	/	/	+	/	/	–	/	/	–	/	/
Magnum	+	+	+	+	+	–	+	+	–	+	+	–	+	+	–	–	–	–
Isthmus	+	+	+	+	+	–	+	+	–	–	–	–	+	+	–	–	–	–
Uterus	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Vagina	+	/	/	+	/	/	–	/	/	–	–	–	–	/	/	–	/	/

E, luminal epithelium; TGDC, tubular glands duct cells; TGSC, secretory cells of tubular glands. +, positive; –, negative; /, the structure is missing.

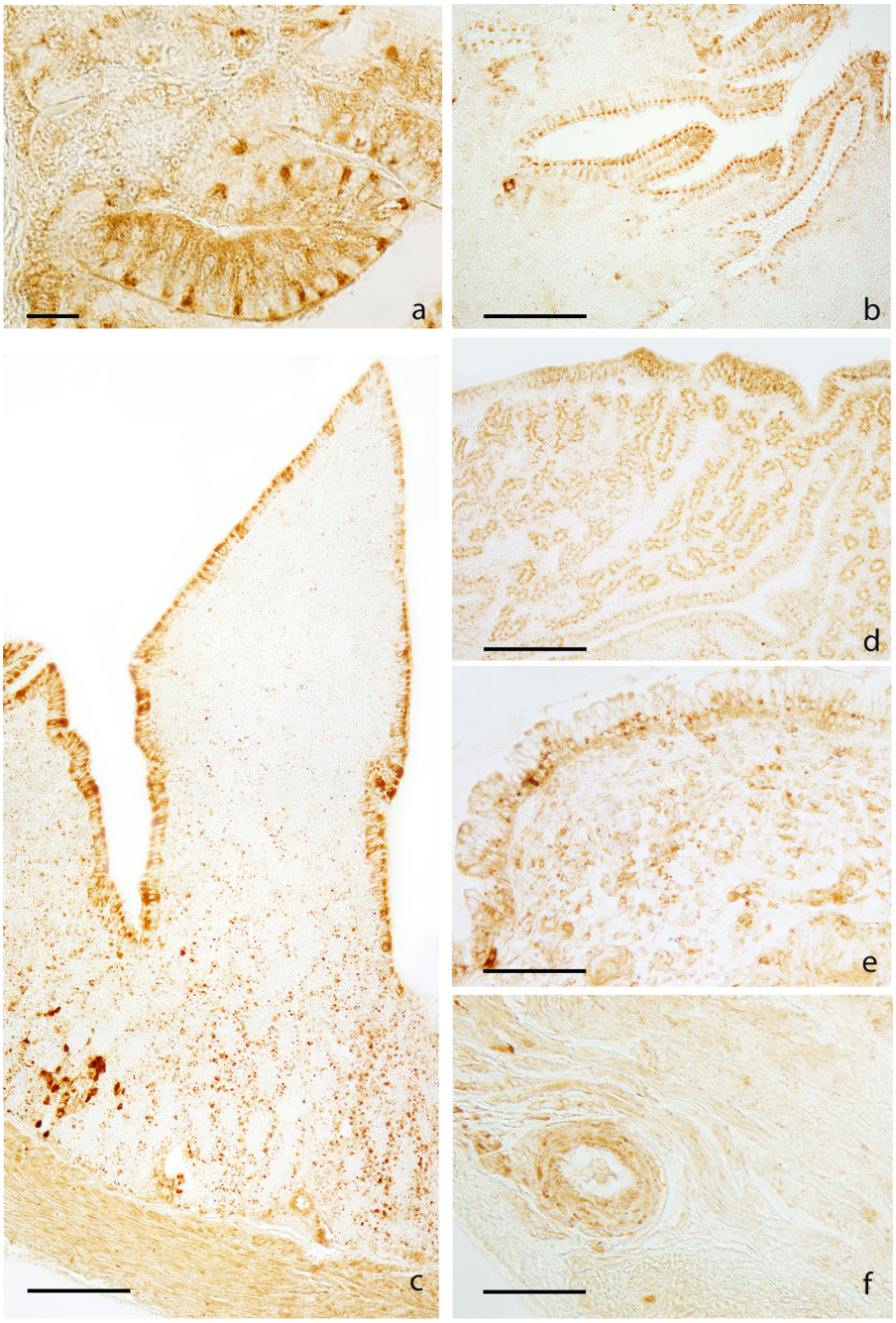

Fig. 2. NGF in quail oviduct tracts. Immunopositive lining epithelial cells and/or ductal cells of infundibulum (a), magnum (b), isthmus (c), uterus (d) and vagina (e). Immunopositive nervous fibers in the vessel wall of infundibulum (f). Scale bars: a = 20 μm; b–d = 50 μm; e–f = 30 μm.

3.4. Controls

No reaction has been observed in controls performed by substituting the primary antibodies with PBS, normal serum or antibodies adsorbed by their homologous antigens.

4. Discussions

The current study reveals, for the first time, the presence of both NTs and Trk receptors in the female genital tract of adult Japanese quail. Moreover, this is the first description of NT-3 in vertebrate female genital tract.

Western blotting analysis revealed the presence of pro and mature forms of NGF and BDNF in all tracts, similarly to results reported in the uterus of mammals (Li et al., 2012; Wessels et al., 2014), and only one band corresponding to proNT-3, as described in pineal glands, extracerebral blood vessels and external carotid (Randolph et al., 2007). With regard to Trks, in the oviduct TrkA showed a molecular weight of 80 kDa, consistent with the molecular weight of non-glycosylated full length receptor. As reported more than a decade ago, human TrkA receptor contains four potential N-glycosylation sites that are highly conserved within the Trk family of neurotrophin receptors, and nine additional sites that are less well conserved (Watson et al., 1999). In addition, quite recently, it was reported that *TRKA* gene in mammals is more complex than previously thought, with an intricate splicing pattern involving exons that encode TRKA isoforms. Thus, a larger variability has been assumed among TRKA proteins with different

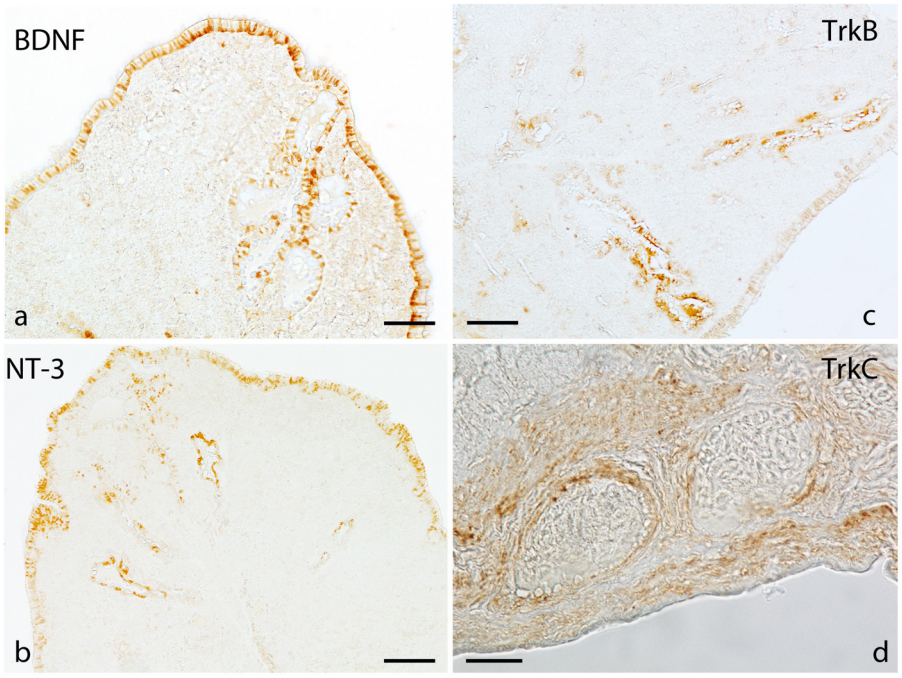

Fig. 3. Neurotrophin and receptors in quail magnum. Immunopositivity to BDNF (a), NT-3 (b), and TrkB (c) in lining epithelial and ductal cells. Immunopositivity to TrkC in nervous fibers of vessel wall (d). Scale bars: a–c = 100 μm; d = 20 μm.

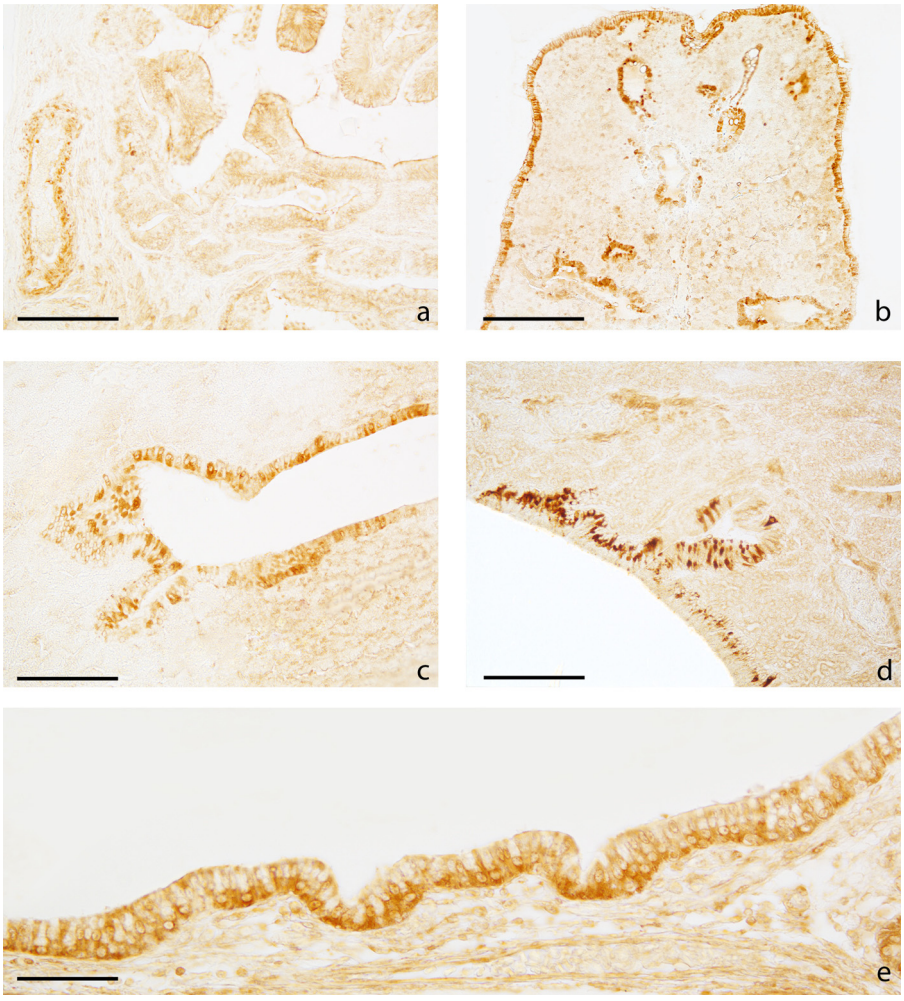

Fig. 4. TrkA in quail oviduct tracts. Immunopositivity in nervous fibers of vessel wall of infundibulum (a). Immunopositive lining epithelial and/or ductal cells in magnum (b), isthmus (c), uterus (d) and vagina (e). Scale bars: a–d = 100 μm; e = 50 μm.

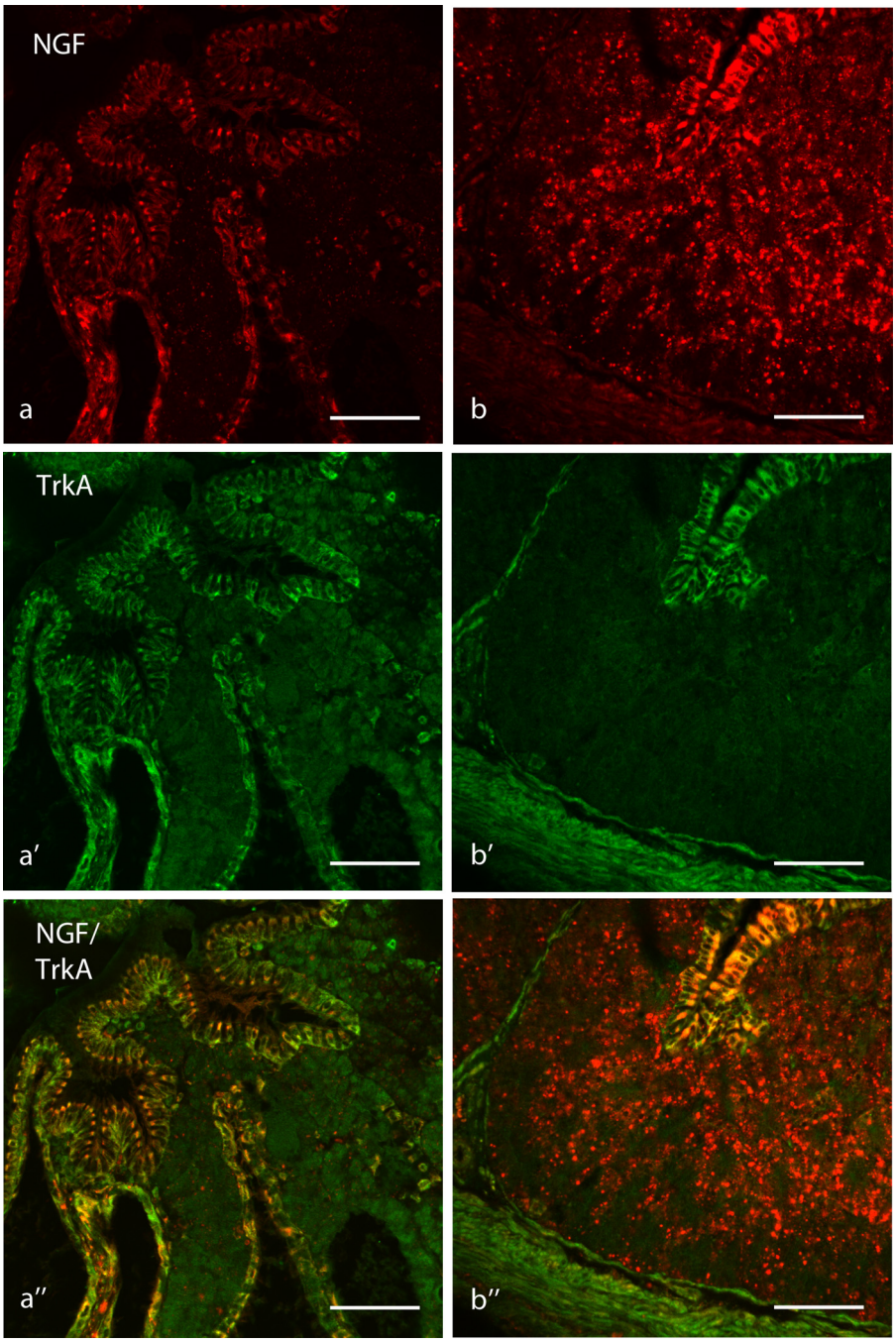

Fig. 5. NGF and TrkA double immunostaining. Colocalization of NGF (red) and TrkA (green) in lining epithelial and ductal cells of magnum (a, a′, a″), and isthmus (b, b′, b″). Scale bars: a–a″ = 50 μm; b–b″ = 40 μm.

properties than was previously known (Luberg et al., 2015). With regards to TrkB, we identified two bands of about 95 and 140 kDa, likely corresponding to the splice variants. The results of TrkB are coherent with the mammalian isoforms in the reproductive system (Li et al., 2012; Wessels et al., 2014). Concerning TrkC, three bands were detected in oviduct homogenates. The antibody employed in this study recognizes TrkC isforms A, C and D of mammals. Thus, it would be presumable that multiple isoforms of different weight are also expressed in the quail.

The morphology of quail oviduct matches with the study by Berg et al. (2001) and is quite similar to descriptions reported in other bird studies (Yoshimura and Ogawa, 1998; Mohammadpour and Keshtmandi, 2008).

Regarding NTs' distribution, the main topics of the study are: (a) NGF, BDNF and NT-3 were localized in lining and ductal epithelial cells. In particular, NGF was diffused to all oviduct tracts, while BDNF and NT3 were limited to magnum and isthmus; (b) NGF was detected in secretory cells of tubular glands of magnum, isthmus and uterus; (c) NGF was detected in nervous fibers of vessel wall.

The presence of NTs in the surface and ductal epithelium could play a role in oviductal transport, fertilization, capacitation of spermatozoa and early embryonic development, as documented in some mammalian species (Lommatzsch et al., 1999; Ren et al., 2005; Shi et al., 2006; Weng et al., 2009; Li et al., 2012). Moreover, only NGF, taking into account its presence in glands of magnum, isthmus and uterus, might have an influence on the secretion of egg

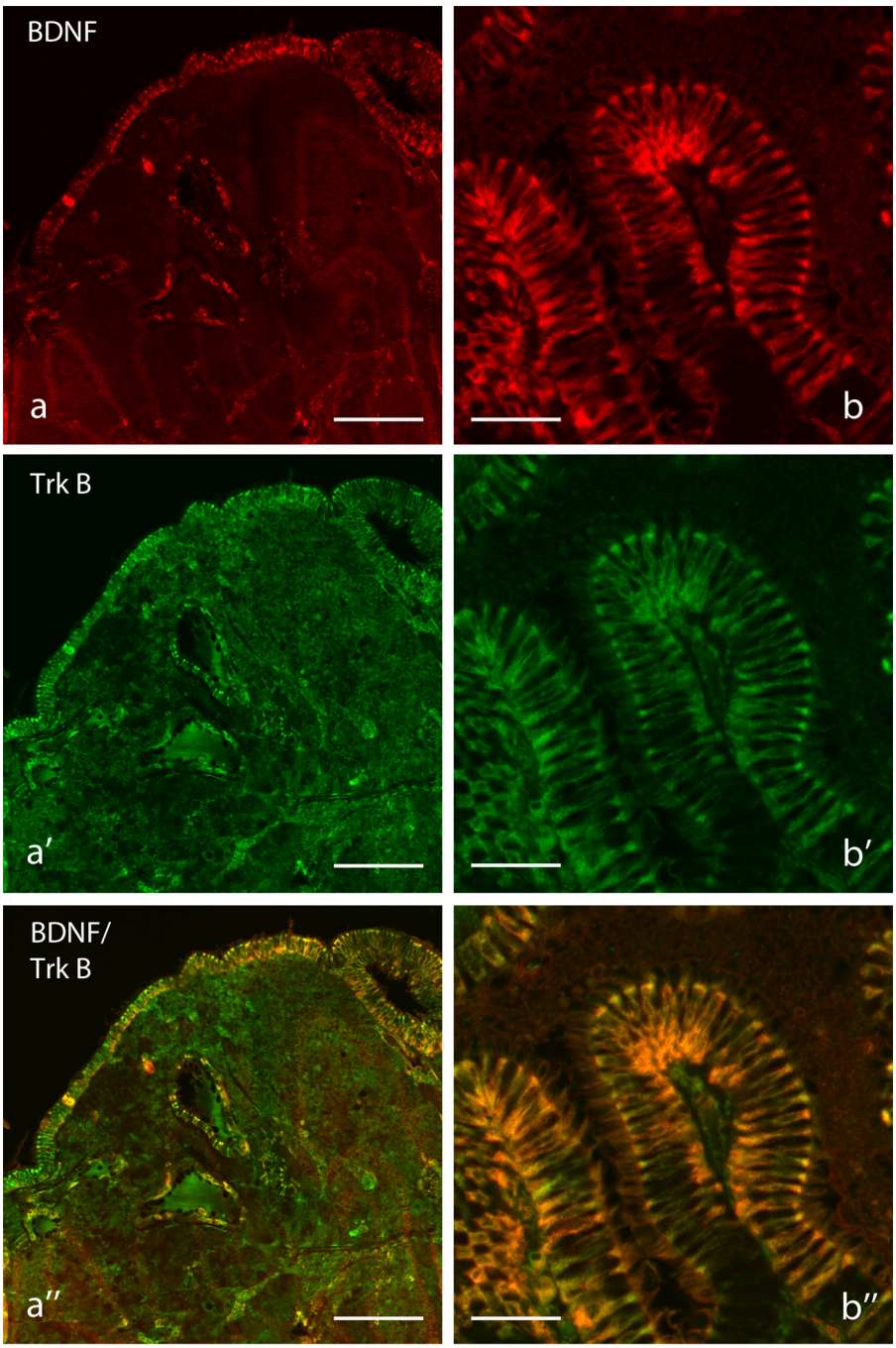

Fig. 6. BDNF and TrkB double immunostaining. Colocalization of BDNF (red) and TrkB (green) in lining epithelial and ductal cells of magnum (a, a′, a″) and (b, b′, b″). Scale bars: a–a″ = 100 μm; b–b″ = 20 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

components. Based on the observations of the presence of NGF in chicken embryo amniotic fluid (Mashayekhi et al., 2011), it could also be hypothesized that NGF accumulates in the albumen and then is transferred in the amniotic fluid.

Regarding NT3, the western blotting analysis has shown the presence of NT3 pro-peptide. It is known that, in mammals, proneurotrophins preferentially bind to p75/sortilin, eliciting apoptotic response (Hempstead, 2014). Thus, also considering that in quail oviductal mucosa TrkC was not detected, it could be arguable that NT3 might bind to the other neurotrophin receptor p75.

Concerning Trks' distribution, the mainly significant observations are that (a) TrkA and TrkB were present in the lining and ductal epithelial cells; (b) TrkA and TrkC were present in vessel wall nervous fibers of all oviductal tracts. The results obtained for TrkA and TrkB agree with those reported in mammalian oviduct, although the due anatomical differences (Lommatzsch et al., 1999; Ren et al., 2005; Shi et al., 2006; Weng et al., 2009; Li et al., 2012).

Our results show that, in the tracts where NGF/TrkA and BDNF/TrkB are present, they were colocalized in the majority of cells, suggesting that ligand and receptor can modulate their effect by an autocrine mechanism of action. In the female genital tract of several mammals, BDNF colocalized with TrkB, in epithelial and ductal cells of magnum and isthmus, demonstrating their involvement in the physiology of reproduction (Dissen et al., 1995; Kawamura et al., 2009; Mirshokraei et al., 2013; Wessels et al., 2014). However, we also observed a wider distribution of the ligand

and its receptor in different cell populations in the same oviductal segment. This could reinforce the hypothesis that the main mechanism of action in the genital tract of Japanese quail might be paracrine (Kawamura et al., 2007; Weng et al., 2009; Mirshokraei et al., 2013).

In conclusion, all investigated NTs were present in the mucosa, mainly in the luminal epithelium of all oviduct tracts. In particular, NGF and BDNF seem to act in a paracrine manner through their specific receptors TrkA and TrkB. These findings thus suggest the involvement of NTs in finely regulated mechanisms of fertilization, egg formation and sperm storage, transport and selection exerted by quail oviducts.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aanat.2016.04.033.

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