Int. J. Devl Neuroscience 40 (2015) 76–84

Contents lists available at ScienceDirect

International Journal of Developmental Neuroscience

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

Development of non-catecholaminergic sympathetic neurons in para- and prevertebral ganglia of cats

Petr M. Masliukov[a,*], Andrey I. Emanuilov[a], Konstantin Moiseev[a], Alexandr D. Nozdrachev[b], Svetlana Dobrotvorskaya[c], Jean-Pierre Timmermans[d]

[a] Department of Normal Physiology, Yaroslavl State Medical Academy, Revoliucionnaya 5, Yaroslavl 150000, Russia
[b] Department of Physiology, St. Petersburg State University, St. Petersburg, Russia
[c] Kazan (Volga Region) Federal University, Kazan, Russia
[d] Laboratory of Cell Biology and Histology, Department of Veterinary Sciences, University of Antwerp, Antwerp, Belgium

ARTICLE INFO

Article history:
Received 26 November 2014
Accepted 5 December 2014
Available online 6 December 2014

Keywords:
Autonomic nervous system
Sympathetic ganglia
Cholinergic neurons
Nitric oxide
Vasoactive intestinal peptide
immunohistochemistry
Postnatal development

ABSTRACT

Expression of vasoactive intestinal peptide (VIP), neuronal nitric oxide synthase (nNOS), choline acetyltransferase (ChAT) and calcitonin gene-related peptide (CGRP) in the sympathetic ganglia was investigated by immunohistochemistry in the superior cervical ganglion (SCG), stellate ganglion (SG) and celiac ganglion (CG) from cats of different ages (newborn, 10-day-old, 20-day-old, 30-day-old and 2-month-old). Non-catecholaminergic TH-negative VIP-immunoreactive (IR) and nNOS-IR sympathetic ganglionic neurons are present from the moment of birth. In all studied age groups, substantial populations of VIP-IR (up to 9.8%) and nNOS-IR cells (up to 8.3%) was found in the SG, with a much smaller population found in the SCG (<1%) and only few cells observed in the CG. The percentage of nNOS-IR and VIP-IR neurons in the CG and SCG did not significantly change during development. The proportion of nNOS-IR and VIP-IR neuron profiles in the SG increased in first 20 days of life from 2.3 ± 0.15% to 8.3 ± 0.56% and from 0.3 ± 0.05% to 9.2 ± 0.83%, respectively. In the SG, percentages of nNOS-IR sympathetic neurons colocalizing VIP increased in the first 20 days of life. ChAT-IR and CGRP-IR neurons were not observed in the sympathetic ganglia of newborn animals and did not appear until 10 days after birth. In the SG of newborn and 10-day-old kittens, the majority of NOS-IR neurons were calbindin (CB)-IR, whereas in the SCG and CG of cats of all age groups and in the SG of 30-day-old and older kittens, the vast majority of NOS-IR neurons lacked CB. We conclude that the development of various non-catecholaminergic neurons in different sympathetic ganglia has its own time dynamics and is concluded at the end of the second month of life.

1. Introduction

The vast majority of sympathetic ganglionic neurons are catecholaminergic and contain specific synthetic enzymes including tyrosine hydroxylase (TH), aromatic amino acid decarboxylase and dopamine β hydroxylase. Some sympathetic neurons lack catecholamines and mostly use acetylcholine as their main neurotransmitter. Cholinergic sympathetic neurons are present in the stellate ganglion (SG) and other thoracic sympathetic chain ganglia, but are rare in the superior cervical ganglion (SCG) and prevertebral ganglia (Weihe et al., 1996; Schäfer et al., 1998; Anderson et al., 2006). In mammals, these neurons innervate sweat glands and the periosteum (Asmus et al., 2001). In cats and dogs, but not in rodents, monkeys or humans, cholinergic sympathetic neurons also innervate arterial blood vessels in skeletal muscle (Bolem and Fuxe, 1970; Järhult et al., 1980; Klimaschewski et al., 1996).

All cholinergic sympathetic neurons also express vasoactive intestinal peptide (VIP). In rats, sudomotor neurons contain VIP and calcitonin gene-related peptide (CGRP) and always lack calbindin D28 K (CB). Cholinergic neurons innervating the periosteum contain VIP and sometimes CB, but always lack CGRP (Anderson et al., 2006). Two subclasses of VIP-positive cells are found in cat sympathetic ganglia: scattered sympathetic ganglion neurons expressing immunoreactivity (IR) for CGRP in addition to VIP and acetylcholinesterase (AChE), and clustered VIP and AChE-positive cells lacking CGRP IR (Lindh et al., 1989).

In cats, some cholinergic postganglionic neurons also express neuronal nitric oxide synthase (nNOS), which is detected in 99% of the presumptive sudomotor neurons exhibiting CGRP and VIP IR and in 70% of the presumptive muscle vasodilator neurons containing VIP but not CGRP (Anderson et al., 1995).

* Corresponding author. Tel.: +7 0852 305763, fax: +7 0852 305013.
E-mail address: mpm@yma.ac.ru (P.M. Masliukov).

http://dx.doi.org/10.1016/j.ijdevneu.2014.12.004
0736-5748/

Current data about the development of cholinergic sympathetic ganglionic neurons are conflicting. Despite considerable insight into this critical developmental process, key features of cholinergic neuronal development are still subject to debate. It has been hypothesized that cholinergic sympathetic innervation of sweat glands in rat and mouse is the result of a postnatal switch from a fully functional noradrenergic phenotype to a cholinergic phenotype under the influence of target-derived factors, and IR for ChAT, VIP and CGRP is not detectable during the first postnatal week (Landis, 1988). On the other hand, some cholinergic sympathetic neurons are already present prenatally and hence long before target innervation, i.e., their neurochemical phenotype developed target-independent (Schäfer et al., 1997; Masliukov et al., 2006; Schütz et al., 2008). In newborn rodents, the vast majority of cholinergic sympathetic ganglionic neurons are initially noradrenergic and display IR for both ChAT and TH. TH IR disappears in the first 20 days of life (Masliukov et al., 2003). Thus, cholinergic sympathetic differentiation seems to be complex, involving either both target-independent and target-dependent control or only target-induced differentiation, according to the specific neuronal subpopulation and target (Ernsberger and Rohrer, 1999).

In rodents, nNOS-positive neurons are not present in sympathetic neurons from birth onwards. In cats, we previously found some changes in the number of sympathetic neurons expressing NADPH-d. In the SG, the proportion of NADPH-d-positive cells increased in the first 20 days and then decreased again between the first and the second month of life (Emanuilov et al., 2008). NADPH-d histochemistry is often used to label nNOS-containing neurons in the nervous system (Hope et al., 1991; Santer and Symons, 1993). However, if was found that a slight mismatch between nNOS-immunostaining and NADPH-d staining may occur and that some nNOS-positive structures can be labeled in the absence of NADPH-d (Spessert et al., 1994). In this respect,no data about the postnatal development of sympathetic ganglionic nNOS-IR neurons are available.

In kittens, many sympathetic neurons in the SG contain CB. During development, the number of CB-IR neurons rapidly decreases in the first two months of life, and only scattered CB-IR neurons are found in the sympathetic ganglia of older cats (Masliukov et al., 2012). CB is a member of the EF-hand family of calcium-binding proteins, which are involved in numerous functions, including cell signaling, calcium uptake and transport, cell motility and intracellular calcium buffering (Andressen et al., 1993; Schwaller, 2012). Obviously, CB plays an important role in the development of sympathetic neurons but the colocalization of CB with different types of neurotransmitters in the sympathetic ganglia of mammals at different stages of ontogenesis remains to be elucidated.

Taking together, the purpose of this study was to gain further insight into the neuroplasticity of sympathetic neurons during early postnatal ontogenesis by comparing the development of non-catecholaminergic neurons expressing different markers in para- and prevertebral sympathetic ganglia. In the current work, we performed our experiments on cats since NOS-IR neurons are absent in rodents (Emanuilov et al., 2008; Masliukov et al., 2014) and because the highest numbers of nitrergic neurons are observed in the cat and human sympathetic ganglia (Klimaschewski et al., 1996).

2. Experimental procedures

2.1. Animals

All animal procedures were approved by the Institutional Animal Care and Use Committee of the Yaroslavl State Medical Academy and were conducted in accordance with the "Guide for the Care and Use of Laboratory Animals" (NIH Publication No. 85–23, revised 1996) as well as the relevant Guidelines of the Russian Ministry of Health for scientific experimentation on animals.

Newborn, 10-day-old, 20-day-old, 30-day-old cats and 2-month-old (5 groups each containing 5 animals) were used in this work to study the localization and morphological parameters of ChAT-, nNOS-, VIP-, CGRP-IR neurons in sympathetic ganglia. All animals were kept in an acclimatized room (12/12 h light/dark cycle; 22 ± 3 °C) and were provided with water and pellets ad libitum.

2.2. Tissue preparation

All animals were sacrificed with a lethal dose of sodium pentobarbital (Nembutal®, 300 mg/kg, i.p.), after which they were perfused transcardially with 500 ml of physiological saline and 1 ml heparin followed by a similar volume of fixative composed of 4% paraformaldehyde (PF) in 0.1 M phosphate buffer. After perfusion, the superior cervical (SCG), stellate (SG) and celiac ganglia (CG) from each side were dissected out, rinsed in physiological solution and immersed in 4% PF for 1–2 h at room temperature. Following fixation, they were washed in three 30-min changes of phosphate-buffered saline (PBS; 0.01 M; pH 7.4), cryoprotected by overnight immersion in 20% buffered (pH 7.4) sucrose solution at 4 °C, mounted in TissueTek (Sakura Finetek Europe, the Netherlands) on a cryostat chuck and frozen. Twelve-μm-thick cross sections were cut with a cryostat, mounted on poly-L-lysine-coated slides and air-dried for 1 h.

2.3. Immunohistochemistry

Serial sections of the SCG, SG and CG were processed for immunohistochemistry. The sections were pre-incubated for 30 min at room temperature with blocking buffer containing 5% normal donkey serum (Jackson ImmunoResearch Laboratories, USA) and 0.3% Triton X-100 (Sigma, USA) in PBS to prevent non-specific binding of secondary antibodies. In order to visualize ChAT, CGRP, VIP, nNOS, TH, CB, single or double immunostaining with antibodies (raised in different host species; see Table 1a) was performed.

Subsequently, the sections were incubated in the primary antisera for 24 h at room temperature, rinsed in PBS and further incubated in the corresponding secondary antisera for 2 h at room temperature (see Table 1b). In some experiments, sections were

Table 1a
Primary antisera used for immunohistochemistry.

Primary antisera	Host species	Dilution	Source
nNOS	Goat	1:300	Abcam, ab1376
nNOS	Rabbit	1:20	LifeSpan BioSciences, LS-B8696
VIP	Rabbit	1:200	Abcam, ab43841
TH	Sheep	1:1000	Abcam, ab113
ChAT	Goat	1:50	Millipore, AB144P
CGRP	Goat	1:200	Abcam, ab36001
CB	Rabbit	1:500	Abcam, ab11426
CB	Mouse	1:300	Abcam, ab82812

Table 1b
Secondary antisera used for immunohistochemistry.

Secondary antisera	Dilution	Source
Donkey anti-goat IgG FITC	1:200	Jackson immunoresearch
Donkey anti-goat IgG CY3	1:200	Jackson immunoresearch
Donkey anti-rabbit IgG FITC	1:200	Jackson immunoresearch
Donkey anti-rabbit IgG CY3	1:200	Jackson immunoresearch
Donkey anti-mouse IgG CY3	1:200	Jackson immunoresearch
Donkey anti-sheep IgG CY3	1:200	Jackson immunoresearch

CY3 – cyanine 3, FITC – fluorescein isothiocyanate.