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Original article

Morphology and immunohistochemical characteristics of the pterygopalatine ganglion in the chinchilla (*Chinchilla laniger*, Molina)

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Abstract

Histological and histochemical investigations revealed that the pterygopalatine ganglion (PPG) in the chinchilla is a structure closely connected with the maxillary nerve. Macro-morphological observations disclosed two different forms of the ganglion: an elongated stripe representing single agglomeration of nerve cells, and a ganglionated plexus comprising smaller aggregations of neurocytes connected with nerve fibres. Immunohistochemistry revealed that nearly 80% of neuronal cell bodies in PPG stained for acetylcholine transferase (CHAT) but only about 50% contained immunoreactivity to vesicular acetylcholine transporter (VACHT). Many neurons (40%) were vasoactive intestinal polypeptide (VIP)-positive. Double-staining demonstrated that approximately 20% of the VIP-immunoreactive neurons were VACHT-negative. Some neurons (10%) in PPG were simultaneously VACHT/nitric oxide synthase (NOS)- or Met-enkephaline (Met-ENK)/CHAT-positive, respectively. A small number of the perikarya stained for somatostatin (SOM) and solitary nerve cell bodies expressed Leu-ENK- and galanin-immunoreactivity. Interestingly about 5-8% of PPG neurons exhibited immunoreactivity to tyrosine hydroxylase (TH). Intraganglionic nerve fibres containing immunoreactivity to VACHT-, VIP- and Met-ENK- were numerous, those stained for calcitonin gene related peptide (CGRP)- and substance P (SP)- were scarce, and single nerve terminals were TH-, GAL-, VIP- and NOS-positive.

Key words: chinchilla, pterygopalatine ganglion, morphology, immunohistochemistry

Introduction

Most anatomical investigations on autonomic nerve structures of the head in small mammals have revealed substantial species dependent variations dealing with their morphology and topography (Gienc and Kuder 1985b, Gienc et al. 1990). Some studies performed in different species suggest the existence of a certain relation between morphological features and topography of parasympathetic ganglia of the head, and the taxonomic position of the animal species (Kuder 1983, Kuder 1985). Investigations dealing with

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immunohistochemical characteristics of the pterygopalatine ganglion (PPG) have been performed on a wide range of species, such as man (Motosugi 1993), gerbil (Shiotani et al. 1986), rat (Kuwayama et al. 1987, Kuwayama et al. 1988, Suzuki et al. 1990, Motosugi et al. 1992, Motosugi 1993), dog (Uemura et al. 1988, Nozaki et al. 1990) mouse (Ding et al. 2001, Ding et al. 2002) and pig (Podlasz et al. 2003). Neurons in the PPG are involved in the innervation of the lacrimal gland in monkey (van der Werf et al. 1996), cat (Kuchiiwa et al. 2000, Cheng et al. 2001) and also other structures of the eye (Kuchiiwa 1990, Elsas et al. 1994, Simons and Smith 1994). They also supply mucous glands of the nasal cavity (Kondo et al. 2000) as well as the palatum and vomeronasal organ (Matsuda et al. 1996). It is also possible that nerve fibres innervating the pineal gland originate from the PPG (Moller and Baeres 2002) and the ganglion is involved in the regulation of the cerebral blood flow (Sienkiewicz et al. 1995, Talman and Dragon 2000). Different biologically active substances (neurotransmitters and neurotransmitters synthesizing enzymes) have been detected in neurons of the PPG including nitric oxide synthase (NOS) found in the pterygopalatine neurons of humans (Uddman et al. 1999), rat (Warn et al. 1997) and cat, neuropeptides such as vasoactive intestinal polypeptide (VIP) (Elsas et al. 1994, Uddman et al. 1999), pituitary adenylate cyclase-activating peptide (PACAP) (Elsas et al. 1994, Uddman et al. 1999, Edvinsson et al. 2001) and peptide histidine-isoleucine (PHI) (Elsas et al. 1994). Some PPG neurons in the pig exhibit NADPH-diaphorase activity (Sienkiewicz et al. 1995). It is also suggested that in the rat neuropeptide Y (NPY)-positive parasympathetic nerve fibres may originate from the PPG (Elsas et al. 1994). The literature in the field contains some information on the immunohistochemical characteristics of the PPG in many mammalian species, but not in the chinchilla.

The present study deals with the morphology and immunohistochemical features of the pterygopalatine ganglion in the chinchilla. The results of these investigations provide comparative data on the autonomic nerve structures of the head in mammals.

Materials and Methods

The present study was designed in accordance with the international guidelines and protocol 3/2011 of the 1st Local Ethics Committee in Kraków (Poland). The reported investigations were carried out on twelve chinchillas of either sex (*Chinchilla laniger*, Molina). The animals (n=8) were killed by decapitation under the anaesthesia (they were anaesthetised with ether-anaesthesia and then injected intraperitoneally with 30 mg/kg of nembuthal to deepen anaesthesia) and the maxillary nerves were exposed under the stereomicroscope. The material was rinsed in physiological solution and fixed for 30 min. in 10% formalin. Four chinchillas were used for morphological studies. Further procedures followed "*in situ*" staining according to the thiocholine method modified for macromorphological investigations (Koelle and Friedenwald 1949, Gienc 1977). The other four animals were used for histological studies. Collected and fixed in formalin maxillary nerves were embedded in paraffin (Paraplast plus, Sigma, Poland), cut with a microtome HM335E (Microm International GmbH, Germany) for 3-5 µm slides and stained according to the Gomori and hematoxylin and eosin methods. The last four animals were used for immunohistochemical (IHC) studies.

Anaesthetized chinchillas (as previously described) used for IHC were transcardially perfused with 0.4 l of 4% ice-cold buffered paraformaldehyde (pH 7.4) and the tissues were collected as described above. They were then postfixed by immersion in the same fixative for 2 hours, rinsed with phosphate buffer (pH 7.4) and transferred to and stored in 30% buffered sucrose solution (pH 7.4) until further processing. The ganglia were cut into 12 µm-thick cryostat sections, which were processed for the double immunofluorescence method on slide mounted sections. The sections were washed 3x10 min. in PB, incubated 45 min. in 10% normal horse serum (NHS, Cappel, Warsaw, Poland) or normal goat serum (NGS, Cappel, Warsaw, Poland) in saline phosphate buffer (PBS) containing 0.25% Triton X-100 (Sigma, USA) and then incubated overnight at room temperature (RT) with antibodies (Table 1) diluted in phosphate buffer (PB) containing 0.25% Triton X-100. After incubation with primary antiserum, the sections were washed 3x10 min. in PB and further incubated with secondary antisera (1h; RT). After incubation the sections were washed 3x10 min. in PB, coverslipped with buffered glycerol and examined under a confocal microscope (Zeiss, LSM 700). Control of specificity of staining was performed by preabsorbtion of a diluted antiserum with 20 µg/ml of an appropriate antigen [except tyrosine hydroxylase (TH), vesicular acetylcholine transporter (VACHT) and choline acetyltransferase (CHAT)], which abolished the specific immunoreaction completely. In addition, experiments were carried out in which the primary antiserum was replaced by non-immune serum or by PBS, in order to verify the specificity of particular immunoreactions. Additionally, to verify results of immunohistochemical staining against VACHT and CHAT some sections of the PPG were stained using acetylcholinesterase

Table 1. Antisera used in the study.

Antigen	Host	Type	Dilution	Cat. No.	Lot/Batch	Supplier
Primary antisera						
CHAT	goat	polyclonal	1:50	AB144P	LV1480420	Milipore
VACHT	rabbit	polyclonal	1:4000	V5387	095K4751	Sigma
TH	mouse	monoclonal	1:50	T2928	Clone TH-16	Sigma
VIP	mouse	monoclonal	1:500	MaVIP	91278	East Acres Biologicals
CGRP	rabbit	polyclonal	1:2000	11535	2659F	Cappel
SP	rat	monoclonal	1:150	8450-0505	NC134	ABD Serotec, UK
GAL	rabbit	polyclonal	1:2000	RIN 7153	990921-2	Peninsula Lab.
NOS	mouse	monoclonal	1:100	N2280	081K4815	Sigma-Aldrich
Leu-5-Enk	rabbit	polyclonal	1:500	RPN 1552	5	Amersham
Met-5-Enk	rabbit	polyclonal	1:500	RPN 1562	11461	Amersham
Secondary antisera						
Host		Fluorochrom	Dilution	Code	Lot	Supplier
Donkey-anti-goat IgG (H+L)		Alexa Flour 546	1:500	A11056	399681	Invitrigen
Donkey-anti-rabbit IgG (H+L)		Alexa Fluor 488	1:500	A21206	57542A	Invitrogen
Donkey-anti-mouse IgG (H+L)		Alexa Fluor 488	1:500	A21202	536050	Invitrogen
Goat-anti-mouse IgG (H+L)		Alexa Flour 488	1:500	A11001	632115	Invitrogen
Goat-anti-rabbit IgG (H+L)		Alexa Flour 568	1:500	A11011	623962	Invitrogen

(ACHE) method according to Karnovsky and Roots (Karnovsky and Roots 1964).

Counting of neurons: to determinate percentages of particular neuronal populations (presented as means ± SEM), at least 200 of neuronal profiles from every animal, investigated for each combination of antisera were counted. The sections were collected from different, characteristic regions of the ganglion (from its upper, middle and lower one-third). To avoid double-counting of the same neurons, appropriate distance (minimum five sections = 60 μm) between the sections was maintained. The number of immunolabelled profiles was calculated as a percentage of immunoreactive neurons in regard to all perikarya counted.

The density of ganglionic nerve fibres immunoreactive to substances studied was assessed visually according to the following semi-quantitative scale: absent (-), single (+), moderate (++), numerous (+++) and very numerous (++++).

Results

The pterygopalatine ganglion (PPG) of the chinchilla is topographically closely connected with the maxillary nerve (Fig. 1, 2). It is situated on the dorsal and medial surface of this nerve in the pterygopalatine fossa. Histochemical investigations revealed that the PPG consists of elongated and intensely stained agglomerations of nerve cells, about 8 mm long and 0.5 mm wide. The caudal part of the structure was elongated and thickened, but the nasal part was irregular or the star shaped (Fig. 2). The middle part was significantly narrower (about 0.25 mm) and less intensely stained. The study revealed two main morphological forms of the ganglion: an elongated stripe (Fig. 1) and a plexo-ganglion (Fig. 2). The elongated uniform stripe was placed at the beginning of the maxillary nerve. The petrosal major nerve (the parasympathetic radix) was associated with the caudal part of the PPG. The plexo-ganglionic structure consisted of several agglomerations of neurocytes connected with nerve fibres. Usually there were 3-7 aggregations of nerve cells and the total length of this complex reached from 8 to 11 mm. One elongated agglomeration at the caudal part and two aggregations, often star shaped, at the nasal part were observed (Fig. 2). Numerous and intensely stained postganglionic fibres left the PPG. The most numerous and thickest nerve bundles directed towards the palate and nasal cavity were observed at the nasal part of the ganglion (Fig. 1, 2). Emerging from the caudal part, delicate postganglionic fibres ran upwards to the orbit and along the nasal direction (Fig. 1, 2). Relatively few nerve fibres left the middle part of the ganglion and they mostly ran towards the palate mucosa.

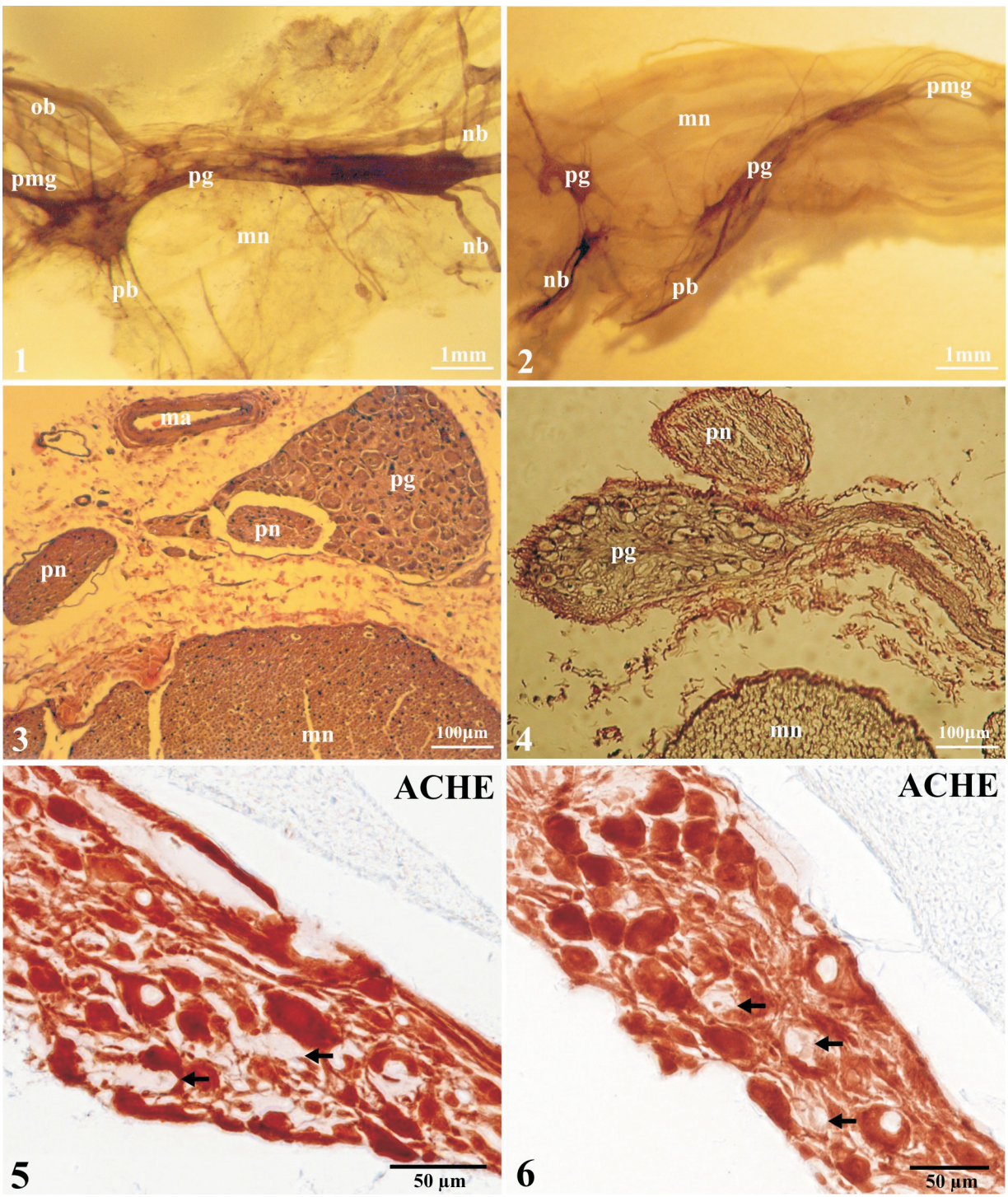


Fig. 1-2. Morphology of the pterygopalatine ganglion in the chinchilla. Tissues stained with thiocholine method; Fig. 3. Cross-section of the central part of the pterygopalatine ganglion in the chinchilla. Section stained with hematoxylin and eosin method; Fig. 4. Cross-section of the pterygopalatine ganglion in the chinchilla. Section stained with silver method; Figs. 1-4. mn – maxillary nerve, pg – pterygopalatine ganglion, pmg – petrosal major nerve (parasympathetic radix), pb – palatine branches, nb – nasal branches, ob – ophthalmic branches, ma – maxillary artery, pn – pterygopalatine nerves; Fig. 5-6. Section from the pterygopalatine ganglion stained with ACHE-method. Arrows indicate neurons negative for ACHE-activity.

Histological observations confirmed the described topography of the PPG. Cross-sections of the maxillary nerve showed the connective tissue capsule with a thickness of about 0.6 µm surrounding the ganglion (Fig. 3). In terms of size the neurocytes could be divided into two groups: small cells with a diameter of 8-18 µm and large cells with a diameter of 19-27 µm. All the ganglionic nerve cells had a large clear nucleus

with a well-outlined nucleolus. The largest cross-sections contained on average about 70 cells. Precise cytoarchitectonic analysis showed evenly distributed neurocytes (90% nerve cells and 10% fibres) on the cross-sections of the elongated uniform tape and irregular cell distribution with a predominance of fibres in the plexo-ganglionic structure (Fig. 3, 4). Histochemical staining using ACHE-method revealed the presence of neurones lacking the activity of acetylcholinesterase (Figs. 5, 6).

Immunohistochemistry revealed that nearly 80% of neuronal cell bodies in PPG stained for CHAT (79.5±2.5%) (Fig. 9, 10) but only about 50% (50±1.83%) contained immunoreactivity to VACHT (Fig. 7, 8, 11, 12). These nerve cells belonged mainly to the subset of large neurons.

Many neurons (39.75±1.93%) were vasoactive intestinal polypeptide (VIP)-positive and represented mainly a subpopulation of small and medium sized nerve cells (Fig. 7). Double-staining demonstrated that approximately 20% of the VIP-immunoreactive neurons were VACHT-negative (Fig. 7). VACHT-IR intraganglionic nerve fibres were very numerous (++++) forming "basket-like structures" surrounding all PPG perikarya (Fig. 7, 8, 12). Only single VIP-positive nerve terminals were encountered (+). Double-staining using anti-VACHT and anti-NOS antibodies revealed that VACHT/NOS-positive nerve cells comprised about 10% of the PPG neurons and NOS-positive only perikarya were much more numerous (32.5±1.55% of the PPG neurons; Fig. 8). Only few intraganglionic (+) NOS-positive nerve fibres were observed. Immunoreactivity to enkephalins was determined in both the nerve cell bodies and intraganglionic nerve fibres. Staining against Met-ENK showed that 10% (9.75±0.85%) of the neuronal somata were immunoreactive for this peptide (Fig. 9). They were simultaneously CHAT-positive (Fig. 9). Intraganglionic Met-ENK-IR nerve fibres were moderate in number (++) (Fig. 9). Leu-ENK-immunoreactivity was exhibited by single neurons (1 or 2 neurons per section). These perikarya were simultaneously VACHT-positive. Leu-ENK-IR nerve fibres were very scarce (+). Only single GAL-IR neurons were encountered (2-3 per section), and they were CHAT-negative (Fig. 10). GAL-positive intraganglionic nerve fibres were very scarce and delicate (+). Immunoreactivity to SOM was expressed by few nerve cell bodies (Fig. 11) and about half of them also contained immunoreactivity to VACHT (Fig. 11). Intraganglionic SOM-positive nerve terminals were moderate in number (+) (Fig. 11). CGRP-positive intraganglionic nerve fibres were numerous (+++). The majority of them simultaneously stained for SP. SP-immunoreactivity was found within nerve fibres which were less numerous (++) than those containing immunoreactivity to CGRP but most of them were also CGRP-positive; only single fibres stained for SP only. SP-immunoreactivity was also expressed by single (1-2 per section) neurons.

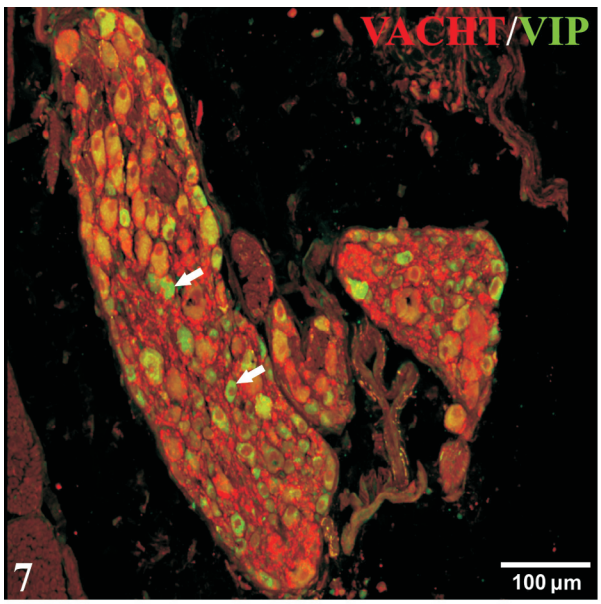
Interestingly about 6.25±1.03% of PPG neurons exhibited immunoreactivity to thyrosine hydroxylase (TH). They formed a small cluster of intensely stained cells (Fig. 12), but some neurons were also found to be dispersed throughout the ganglion. Smooth TH-IR nerve fibres (+) were observed only in a close vicinity of the TH-positive perikarya (Fig. 12).

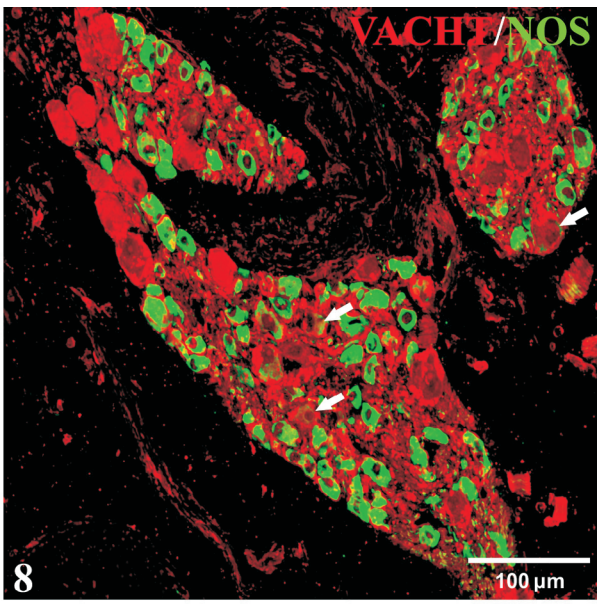
Discussion

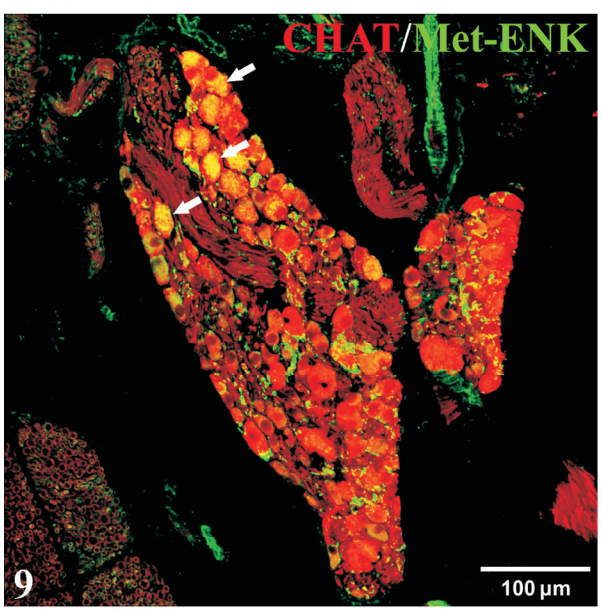
According to the literature in the field, the counterpart of the mammalian PPG in reptiles is the agglomeration of nerve cells which receive preganglionic fibres through the palatine branch of the facial nerve (Berger and Burnstock 1979). On this basis several ganglia have been distinguished: palatine ganglion, spheno-ethmoidal ganglion and infraorbital ganglion (Bellaris and Shute 1953, Oerlich 1956, Soliman 1964, Guide 1970, Nilsson 1983). These structures are topographically connected with the facial nerve. The most primitive form of the PPG representing a delicate net distributed on the surface of the Harderian gland has been found in the Greek turtle (Gienc 1984).

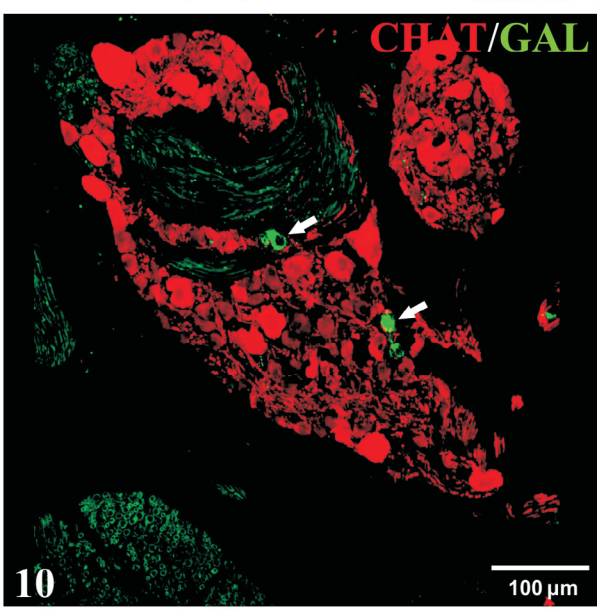
Similar anatomical relations have been observed in birds. The same type of nomenclature was used in case of hens, where ethmoidal and spheno-palatine ganglia were distinguished (Watanabe and Yasuda 1970). Using histochemical technique many differently sized agglomerations of nerve cells have been found in three species of birds: hen, goose and pigeon, and the terminology of the dorsal and ventral *pterygopalatine ganglion* was adapted to the investigated structure (Goller 1972). Subsequent studies have revealed a considerable spread of the neurocytes in PPG (Gienc and Zaborek 1984, 1985, Gienc and Kuder 1985b). In most cases there were two large agglomerations of nerve cells on the superior and inferior part of the Harderian gland and several small aggregations of neurocytes localized among nerve fibres in the palatine branch of the facial nerve. All the ganglia investigated were topographically connected with the Harderian gland. This kind of comparative anatomical relations were observed in the hen, goose and pigeon (Gienc and Zaborek 1984, Gienc and Kuder 1985a, b).

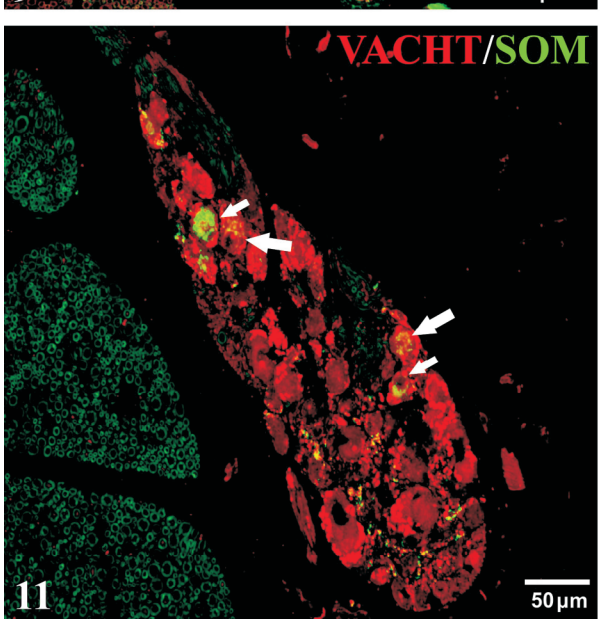
Among the mammalian species investigated only the PPG in the guinea pig is similar to that in birds, where it forms a plexoganglionic structure connected with the Harderian gland (Gienc and Kuder 1982).

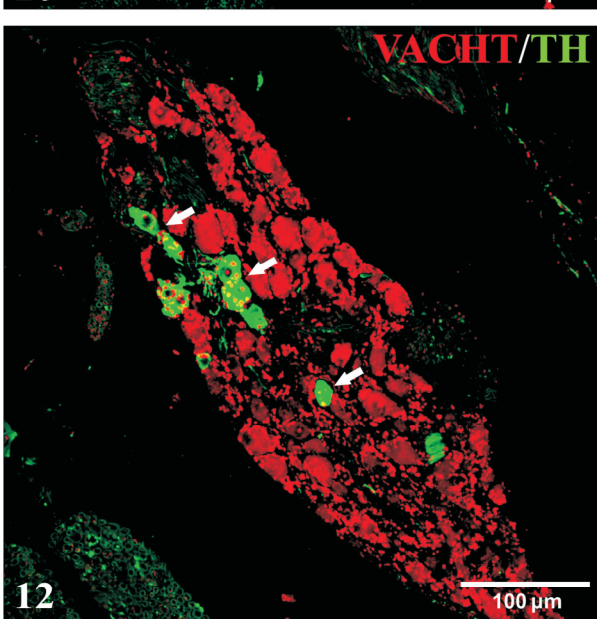

VACHT/VIP
7
100 µm


VACHT/NOS
8
100 µm


CHAT/Met-ENK
9
100 µm


CHAT/GAL
10
100 µm


VACHT/SOM
11
50 µm


VACHT/TH
12
100 µm

In most investigated mammals, including the species investigated here, the PPG comprises single, elongated agglomerations of neurocytes located on the trunk of the maxillary nerve (Ruskell 1965, 1970, Kuder 1983, 1985, 1990a, 1990b, Kosierkiewicz and Szczurkowski 1992).

In the second topographic variant found in man, macaque and dog, the PPG is situated below the maxillary nerve and connected with the pterygopalatine nerve (Tanaka 1932, Sadowski et al. 1970, Nitschke 1976).

The precise comparative anatomical analysis allows to distinguish three forms of the PPG. The first form represents the single, closed agglomeration of neurocytes observed in man (Żabotyński 1953), monkeys (Ruskell 1970, Sadowski et al. 1970), rodents (Kuder 1983, 1985, 1990a, 1990b, Kuder and Szczurkowski 1996, Szczurkowski et al. 2002) and hedgehog (Gienc et al. 1990). The second form found in ruminants and boar represents the ganglion consisting of some small cell aggregations connected with nerve fibres (Godinho 1968, Petela 1979). Generally the two forms represent a single, larger ganglion and several smaller, additional agglomerations of neurocytes. Considering mammals, the most dispersed form of the PPG has been found in the guinea pig (Gienc and Kuder 1982). In this species it is a widely spread plexoganglionic structure composed of about twenty different in size aggregations of neurocytes topographically connected with the pterygopalatine nerve located on the medial surface of the Harderian gland. The third, intermediate form of the PPG found in the rabbit is represented by one large, single agglomeration of nerve cells and some small ganglionic structures (Ruskell 1965). In the chinchilla, two forms of the PPG can be distinguished: a compact – tape form, and a dispersed form, composed of several aggregations of neurocytes. It therefore represents the third form of the PPG. The even distribution of the nerve cells in this structure seems to be specific to this species.

As was already mentioned, systematic studies on the neurochemical coding of the neurons in the PPG are scarce and related exclusively to laboratory animals and pigs (for references, see Podlasz et al. 2003). The presence of cholinergic neurons in the ganglion has been confirmed histochemically (Hara et al. 1985) and immunohistochemically (Hardebo et al. 1992, Ding et al. 2001, Bleys et al. 2001) in rodents and in the pig (Podlasz et al. 2003). It has been reported that in mice all the neuronal somata are VACHT-positive (Ding et al. 2001); similarly in pigs all the neurons express immunoreactivity to CHAT and VACHT (Podlasz et al. 2003). In rats the majority of PPG nerve cells are VACHT-immunoreactive (Bleys et al. 2001). Surprisingly, we found that only 50% of the PPG neurones in the chinchilla contained immunoreactivity to VACHT, whereas 80% of the perikarya were immunopositive for CHAT (in our study the presence of non-cholinegic neurones was additionally confirmed by histochemical staining with ACHE-method). Such dissimilarities can be explained by interspecies differences. As mentioned above, in pigs all the neurons exhibited immunoreactivity to CHAT and VACHT, whereas in chinchillas only some of the CHAT-positive parikarya also contained VACHT. CHAT is an enzyme synthesizing acetylcholine, whereas VACHT is involved in the transportation of the neurotransmitter from perikarya to nerve endings. The incomplete overlap of the CHAT and VACHT immunoreactivities found in the studied chinchillas can suggest that in these animals not all the neurons were actively involved in the innervation of the target organs. In humans more than 90% of PPG neurons contain VIP and NOS (approx. 80%) (Uddman et al. 1999) whereas in the rat only 40% of the nerve cell bodies are NOS-positive (Warn et al. 1997) however VIP is present in 99% of the perikarya (Motosugi 1993). In pigs all the neurons in the PPG are VIP- and NOS-positive (Podlasz et al. 2003). Nevertheless data concerning NOS are inconsistent because Sienkiewicz et al. (1995) have reported that in the porcine PPG not all the neurons display NADPH-activity. In the chin-

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Fig. 7. Section from the pterygopalatine ganglion stained with antibodies against VACHT (red) and VIP (green). The majority of the neurons exhibited immunoreactivity to VACHT, and a number of them also stained to VIP. Some neurons (approx. 8%) displayed immunoreactivity to VIP only (examples indicated by arrows). Very numerous VACHT-immunoreactive nerve fibers formed "basket-like structures" surrounding perikarya; Fig. 8. Section from the pterygopalatine ganglion stained with antibodies against VACHT (red) and NOS (green). Less than half of the neurons contained NOS-immunoreactivity, whereas double stained nerve cell bodies comprised approx. 10% of all the neurons (examples indicated by arrows); Fig. 9. CHAT- (red) and Met-ENK-positive (green) nerve structures in the pterygopalatine ganglion. The vast majority of ganglionic cells were CHAT-positive. About 10% of the neurons contained Met-ENK-immunoreactivity (arrows) and all these neuronal somata were simultaneously CHAT-positive. Note the presence of Met-ENK-IR ganglionic nerve fibers; Fig. 10. CHAT- (red) or GAL-positive (green) neurons in the pterygopalatine ganglion. GAL-immunoreactivity was restricted to single perikarya (arrows) and fine nerve fibers; Fig. 11. VACHT- (red) and/or SOM-positive (green) neurons in the pterygopalatine ganglion. Immunoreactivity to SOM was present in single only SOM-positive neurons (small arrows) or simultaneously VACHT-positive nerve cells (large arrows). Note the presence of moderate in number SOM-positive nerve fibers; Fig. 12. VACHT- (red) and/or TH-positive (green) neurons in the pterygopalatine ganglion. Immunoreactivity to TH was present in a small number (approx. 5-8%) of the perikarya.

chilla only 40% and 45% of PPG nerve cell bodies contain immunoreactivity to VIP and NOS, respectively. Thus it seems that there are profound species-dependent differences in the number of neurons displaying immunoreactivity to VIP and NOS in this ganglion. The presence of opioid peptides has been studied in the rat and human PPG (Motosugi et al. 1992, Motosugi 1993). It has been established that 10.5% of neurons within the ganglion in rats contain immunoreactivity to enkephalins. This observation confirms our findings with regard to Met-enkephalin, but we found that only single neurons were immunoreactive to Leu-enkephalin. In humans, immunoreactivity to enkephalin was detected only in varicose nerve fibres associated with the nerve cell bodies (Motosugi 1993).

In our study only single GAL-positive neurons were found. This finding confirms previous data regarding the porcine PPG (Podlasz et al. 2003) in which a relatively low number of neurons (approx. 6%) contain immunoreactivity for GAL. Galanin is a neuropeptide considered to be closely associated with cholinergic neurons. In contrast to the results obtained by Podlasz et al. (2003), galaninergic neurons in the chinchilla's ganglion were VACHT- or CHAT-immunonegative. There is also a study which, in contrast to our findings, reports that in the cat approximately 80% of neurons in the PPG contain immunoreactivity to GAL (Grimes et al. 1994). Podlasz et al. (2003) have reported the presence of somatostatin (SOM) in neurons of the porcine PPG. They found that SOM-positive nerve cell bodies, which comprise approx. 11% of the total neuronal population of the ganglion, belonged exclusively to the subpopulation of small nerve cells. The present study partly confirms these results; nevertheless in the chinchilla PPG SOM-positive neurons were less numerous and only half of them were simultaneously VACHT-positive. Nerve structures positive to SOM were also found within the avian PPG (Schrodl et al. 2005). SOM-immunoreactivity was absent from the perikarya which were densely surrounded by SOM-positive nerve fibres. In our study moderate numbers of SOM-positive intraganglionic nerve fibres were found.

In pigs, no neurons of the PPG stained for SP or CGRP, while immunoreactivity to these peptides was determined in a small population of nerve fibres (Podlasz et al. 2003). All SP-positive nerve fibres contained simultaneously immunoreactivity to CGRP, but only a fraction of CGRP-positive nerve fibres contained immunoreactivity to SP. Generally, this information is in agreement with our findings, but we found also single SP-positive neurons within the chinchilla PPG. The available literature contains very limited information on the presence of neurons immunoreactive to TH in PPG. Podlasz et al. (2003) have reported the presence of residual immunoreactivity to TH in single neurons of the porcine PPG. Kuwayama et al. (1988) have revealed the existence of smaller, clustered adrenergic cells (resembling small intensely fluorescent cells) in the rat PPG with the use of TH immunohistochemistry and glyoxylic acid-induced fluorescence for catecholamines. In the present study we found that some neurons in the chinchilla PPG were intensely stained for TH. This observation corresponds with the information on the occurrence of a small population of TH-positive neurons in the ciliary ganglion of species such as the monkey, dog, cat, rat and pig (Uemura et al. 1987, Hardebo et al. 1992, Kaleczyc et al. 2005).

The present results compared with the previous findings have revealed the existence of profound interspecies differences concerning immunohistochemical characteristics of PPG nerve structures, however, further studies must be undertaken to clarify their functional significance.

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