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WHY THE TERM ACROSYRINGIUM IS NOT USED SO FAR BY HISTOLOGY?

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Abstract

Sweat glands can be involved in various inflammatory processes which can trigger both benign and malignant tumours. Microscopic examination of the malignant skin lesion revealed nests of tumour cells and foci of ductlike lumina invading the dermis. The cells were positive for carcinoembryonic antigen (CEA), epithelial membrane antigen (EMA) and Ck 18, Ck 19, so that porocarcinoma was diagnosed. The knowledge, the epidermal portion of the sweat gland duct can be a source of a particular group of tumours showing typical immunohistochemical features, leads us to suggestion that the acrosyringium is not only a simple spiral canal running between the cells of the epidermis, although being so characterized in the textbooks of histology. This directs us to suppose, it could be useful to incorporate the term acrosyringium, used until now only in dermatopathology, into the terminology of general histology.

Key words: sweat glands, tumours, acrosyringium

INTRODUCTION

Sweat glands can be involved in various inflammatory processes that can lead to a large number of both benign and malignant tumours. The skin and its appendages, including sweat glands, show typical morphological and immunohistochemical characteristics (1). Microscopic examination of epithelial adnexal tumours showed interesting differences between tumours arising from the epidermis and those originating from the structures developing from epidermis. This instigated us to summarise the today’s knowledge about the structure and development of the sweat glands and to put it to the correlation with that provided by modern morphological methods.

METHODS

A malignant skin lesion 12x12 cm in the fronto-temporal region was examined. Within a period of two years it was the third surgical intervention, each time of a different effectiveness. Preceding histology revealed a spinocellular carcinoma. The lesion underwent radiotherapy, however, it recurred and grew larger. Biopsy material was examined with the aid of classical staining methods and a wide array of immunohistochemical methods – H&E, Van Gieson, CEA, EMA, Ck18, Ck19, S-100 protein, PAS. All the antibodies used, except for S – 100 protein (Biogenex), were produced by the firm DAKO.

RESULTS

Hematoxylin and eosin (H&E) staining showed the tumour was made up of solid nests of atypical cells, containing abrupt keratinization in places, elsewhere in the form of narrow ducts with two layers of epithelium (Fig. 1,2). The tumour parenchyma was buried in a denser, even hyalinized tumour stroma and infiltrated the epidermis as well as the dermis. Immunohistochemically, the cells displayed CEA, EMA, Ck 18, 19, S-100, rarely a PAS-positive substance (Fig. 3,4). Sialomucins were absent. On the basis of the results presented, malignant tumour stemming from the acrosyringium, most probably a porocarcinoma, was diagnosed.

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Fig. 1. Marginal part of the tumour. In the epidermis hyperplastic intraepidermal part of duct is found (arrows) – acrosyringium. Van Gieson, x 120

Fig. 2. Hematoxylin and eosin staining shows a part of the porocarcinoma containing ductlike lumina (arrows) located immediately near the solid nests. H&E, x 120
**Fig. 3.** In solid nests of porocarcinoma epithelial membranous antigen positivity is manifest. EMA, x 120

**Fig. 4.** Sweat glands localised in the subcutis show S-100 positivity (arrows), while in the nests of the tumour S-100 protein is absent. S-100 protein, x 120
DISCUSSION

The sweat glands are derivatives of epidermis. They begin to develop during the fourth month initially as solid downgrowths of epidermis into the dermis (2, 3).

Fully developed sweat gland comprises secretory and ductular portions. In microscopic structure the secretory portion consists of pseudostratified epithelium, composed of clear and dark cells and myoepitheliocytes. Dermal sweat duct is lined by two layers of basophilic cuboidal epitheliocytes. Epidermal portion of duct – the acrosyringium – is a spiral canal running between the cells of the epidermis (see the schema 1).

Bearing in mind that the epidermal portion of sweat gland duct – the acrosyringium – can be a source of a particular group of benign and malignant tumours, we can suggest that this part of sweat gland is not a simple spiral canal surrounded by concentrically arranged epidermocytes, however, generally believed, the ductular cells within the epidermis migrate and keratinize in the same way as the neighbouring keratinocytes.

Different microscopic picture of porocarcinoma and squamous cell carcinoma (although both tumours arise from epidermal cells) indicates there must be a structural and functional difference between epithelia of epidermal part of sweat gland duct and other cells of epidermis. Porocarcinoma and squamous cell carcinoma differ also in immunohistochemical characteristics (as shown in the table 1) – squamous cell carcinoma, in contrast to the porocarcinoma, is not positive for EMA and CEA.

As revealed by another studies, acrosyringium shows special immunohistochemical features even under general conditions. (4, 5).

Although in most of the textbooks the epidermal part of the sweat gland duct is described as being surrounded by concentrically arranged epidermal cells, these cells are not regarded to be principally different from other epidermocytes, and the term acrosyringium is not used in general histology, even if it is not found in the official histological nomenclature (6, 3, 7).

Table 1. Characteristics of some malignant cutaneous tumours

<table>
<thead>
<tr>
<th>Kind of tumour</th>
<th>Histogenesis</th>
<th>Location</th>
<th>Morphology</th>
<th>Immunohisto-chemistry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porocarcinoma</td>
<td>ectoderm acrosyringium</td>
<td>epidermis dermis</td>
<td>tubular lumens nests of cells PAS +</td>
<td>Ck 5,7,8,15, 18,19 EMA, CEA</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>ectoderm epidermis (stratum spinosum)</td>
<td>epidermis dermis</td>
<td>stratum spinosum similarity, various degree of differentiation keratinizing, nonkeratinizing</td>
<td>mainly Ck8,13,17,19,</td>
</tr>
<tr>
<td>Another sweat glands carcinomas</td>
<td>ectoderm glandular and ductular structures</td>
<td>epidermis and/ or dermis</td>
<td>ducts, glands, solid, cystic, PAS + mucins +</td>
<td>EMA, CEA (in lesser degree) Vm, S-100, NSE,GFAP</td>
</tr>
</tbody>
</table>
On the other hand, the term acrosyringium is generally used in dermatology and dermatopathology which is evidenced by a number of studies (8, 9, 10, 11, 12, 13). Most of them concentrate on the pathology of acrosyringium, but the reports directed on the normal structure and development are also met (14, 15, 16, 17, 18, 19).

Generally speaking, the effort of dermatopathologists to make the diagnosis of sweat glands tumours more precise brought a new knowledge about the normal structure of sweat glands. This directs us to suppose that it should be useful to incorporate the term acrosyringium into the terminology of general histology.

In fact, this is about the structure being the source of a particular group of tumours showing specific immunohistological features under both normal and pathological conditions and it is assumed to be a really overpowering reason.

REFERENCES


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ANATOMIC VARIATIONS OF NERVE ROOTS, TRUNKS AND FASCICLES OF THE BRACHIAL PLEXUS

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Abstract
Starting point: variations detected during operations stimulated us to start the study concerning the variations of the formation of the brachial plexus.
File: we have examined 100 brachial plexus in 50 adult cadavers in order to find out the incidence of neural variations. We have observed the contribution of the C4 root and possibly Th2 root as well as various anomalies in the origination of each plexiform root and its branches from the spinal cord, variations of the formation of the neural trunks, fascicles and peripheral branches. We have focused our attention on their course, anastomosis, thickness and possible absence. Motor innervation particularities in relation to the diagnosis are emphasized as well, apart from the anatomic variability. Attention is focused on the mechanism and the morphologic reasons of particular types of injuries.
Results: the prefixed type was present in 24 cases (48 %), postfixed in one case. We have not observed any particular anomaly in the formation of the plexus in only 6 cases. We have observed 75 anomalies in 44 cases (88 %), there was one anomaly present in 19 cases and in 25 two or more anomalies in the same time. The anomalies were present most frequently on the left side – in 29 cases, and bilaterally in 15 cases. The anomalies were usually of anatomic character. Least number of anomalies was present in the area of neural roots.
Conclusion: this study allowed us to find out and describe rare and until now not described anatomic variations in the formation of neural roots, trunks, fascicles up to the origin of terminal branches of the brachial plexus. The formation of the axillary nerve as a continuation of the posterior branch of the superior trunk.

Key words: brachial plexus, supraclavicular part, infraclavicular part, variations

INTRODUCTION
The brachial plexus develops from a connection of anterior branches of the last four cervical spinal nerves (C5,C6,C7,C8) and the first thoracic nerve (Th1). These five plexiform roots are connected in order to form three plexiform trunks:
- truncus superior made up from anterior branches of C5 and C6
- truncus medius made up from anterior branch of C7
- truncus inferior made up from anterior branches of C8 and Th1
Each of these trunks is divided into two branches, anterior and posterior, which then connect in order to form three plexiform fascicles:
- fasciculus lateralis made up from a connection of anterior branches of the superior and middle trunk
- fasciculus medialis made up from the inferior trunk
- fasciculus posterior made up from a connection of the posterior branches from the three trunks of the plexus.

The brachial plexus ensures sensory and motor innervation of the upper limb except of the trapezius muscle, which is the only muscle of the upper limb girdle not innervated from the brachial plexus. The total amount of myelinated fibers in the brachial plexus in adults is between 120 000 to 150 000 and up to 25% of which innervate the upper limb girdle. The fifth cervical and first thoracic nerves contain the least amount of myelinated fibers – between 15 000 – 20 000. The eighth nerve is the biggest and contains around 30 000 myelinated fibers. The greatest amount of motor fibers is in C5 and then in C8, the smallest amount is in C7 and Th1. The greatest
amount of sensory fibers is in C7, then in C6 and finally in C8. The basic nerves forming the plexus with the whole mass of their anterior branches are C6, C7 and C8.

Most branches of the brachial plexus come out from fascicles. Some supraclavicular branches may originate in roots or trunks. The branches from fascicles do not necessarily have to contain innervation from all roots forming the fascicle.

Our study has topographically specialized in anatomic variations of the formation of the brachial plexus from the neural roots up to the origin of the terminal branches.

We have found only rare studies concerning this problem in the literature available to us (1,2,3,4,5,6,7).

**METHODS**

The study is concerning 50 cadavers, in which the brachial plexus was observed bilaterally. The body was in a lying position with limbs slightly pulled out. The skin incision was performed from the upper third of the lateral side of sternocleidomastoid muscle to the median third of the clavicle and from there along with the clavicle to its lateral part and through the deltoid-pectoral sulcus to the medial surface of the shoulder to its inferior third. Skin and subcutaneous tissues were moved to side. In the beginning we dealt with the supraclavicular part of the plexus.

The visualization of the spinal nerves in the intraforaminal level up to the border of dura mater was performed using a resection of the endings of the transverse processes.

When it was necessary to isolate the inferior plexus as C7 spinal root – and C7 root as the middle trunk or C8 and Th1 of the inferior trunk, we have removed the insertion of the anterior scalene muscle up to the anterior tubercle or we have cut a part of the scalene muscle. The origin of the long thoracic nerve was found by pulling the C6 root to the front.

After the trunks and branches were exposed, we have proceeded to the next stage. It concerned a separation of the pectoral major muscle from the clavicle in the area of 2-3 cm and then a separation of the clavicile from medial to ist lateral third. This stage exposed the clavipectoral fascia, subclavius and pectoral minor muscle, with enabled to observe the inferior trunk and medial fascicle. In the area covered with the pectoral minor muscle were the nerves of the plexus in close contact to each other around the artery. Discission of the minor and pectoral major muscles finally enabled to observe the infraclavicular part of the brachial plexus and all pectoral nerves as well as the terminal nerves in full integrity.

We have exposed the lateral fascicle, which was fixed with fibers of connective tissue to the fascia of the subclavius muscle, using a more proximal preparation. The middle fascicle was present more inwards from the lateral and deeper. A close preparation near the middle fascicle enabled localization of the posterior fascicle. A bifurcation of the posterior fascicle to the radial and axillary nerve is in the level of the origination of the coracobrachial branches from the lateral fascicle. A deeper preparation downwards to the inferior border of the pectoral minor muscle projection enabled an exposure of the median nerve.

Working upwards, the preparation of the proximal part of the median nerve led to the lateral and medial root of bifurcation of the median nerve.

The level of the origination of the axillary nerve from the posterior fascicle was used as an orientation point. Proximally from this level were located fascicles, distally then nerves originating from them.

The formation of fascicles took place usually not above the projection of the lower margin of the clavicle.

When anomalies were detected, we continued the preparation using a magnifying lens.

**RESULTS**

Complicated connections between nerves were observed in the infra- as well as the supraclavicular area. Variations, supra- or infraclavicular, were observed more frequently on the left side.
Simultaneous contribution of the C4 and Th2 roots to the formation of the brachial plexus was not observed. The elasticity and strength of the nerve was significantly decreased in older cadavers. They were more easily damaged during preparation, they were like „gleamless“.

**Variations in the level of the neural plexiform roots:**

The roots C4, C5, C6 and C7 were fixed in the spinal sulcus nerve by transversal radicular ligaments to the transverse processes to the vertebrae, they were missing in C8 and Th1.

Upper cervical nerves C4, C5, C6 and in lesser rate C7 were located due to the physiologic curve of the spine more in the front in relation to the roots C8 and Th1. The roots forming the plexus were usually going through the fissure between the anterior and middle scalene muscle.

In one case the root C5 and in one case both the C5 and C6 ran over the anterior scalene muscle.

The branches to the scalene muscles and to the musculus longus colli exited from the lower parts of the anterior cervical roots as small lateral and medial branches or as branches leaving these proximal spinal nerves near their exit from the intervertebral foramina. The root Th1 did not have proximal branches. The phrenic nerve was connected by one and in two cases by more branches with the root C5.

The most proximal branch of the C5 root is the nervus dorsalis scapulae. It was observed to originate in the lower side of root C6 in 4 cases. The long thoracic nerve was formed from contributions of C5 in all cases. In 16 cases (32%) from C5, C6, C7 and in four cases from contributions of C4, C5 and C6. Mostly from roots C5 and C6 in 30 cases (60%). The biggest trunk was always from the root C6.

We have observed variations of the formation of the neural roots 18 times in 14 cadavers, out of which four were bilateral (Table 1, Fig.1 and 2).

**Variations in the level of the neural plexiform trunks:**

We have detected several variants concerning the branches originating from the neural trunks. The subclavian nerve originated from the superior trunk on various sites following the convergence of C5 and C6. Distally, along the superior trunk, there is the origin of angle wise running suprascapular nerve. It originated from the root C5 in three cases.

Variations in the level of neural trunks formations and their branches were observed in 25 cadavers 32 times, they were bilateral 7 times, see (Table 2).

The superior trunk was not formed in 3 cases. The roots C5 and C6 did not connect in one

| Table 1. Variations of the brachial plexus in the level of neural plexiform roots |
|-----------------------------------|---|---|
| Roots C5 and C6 divided into anterior and posterior branch | 2 | |
| Root C7 is thin and does not form and anterior branch | 1 | 1 |
| Root C7 makes a branch to the root C8 | 1 | 1 |
| Root C8 makes two branches to the posterior branch of the middle trunk | 1 | 1 |
| C8 divides into 2 branches, the anterior goes to the anterior branch of the superior trunk and the posterior connects with the root Th1 | | 1 |
| Two branches of the C8 root connect with the anterior branch of C6 root | 1 | |
| Branches of the C8 root connect with the lateral and posterior fascicle | 1 | |
| Root Th1 is broad as root C8 | 2 | 1 |
| Total | 9 | 1 | 4 |

SIN. – left, DX. – right, Bilat. – bilateral
The roots C5 and C6 divided into an anterior and posterior branch in two cases and then the anterior branches of C5, C6, C7 and the posterior branches of C5, C6, C7 connected. The middle trunk converged in two cases and connected with the superior trunk (Fig. no. 3), in all cases bilaterally and in three cases with the inferior trunk. An impression developed in one case so that all the branches of the middle and inferior trunk originated from this junction and the posterior fascicle did not develop. After a preparation under a magnifying lens that apart from an anterior branch to the anterior branch of the superior trunk there are 4 anterior branches to the inferior trunk. The posterior branch of the middle trunk was thinner than the anterior, it received the posterior branch from the superior and inferior trunk. The greatest number of anatomic variations in the supraclavicular region was observed in the level of inferior trunk formation.

**Fig. 1.** Brachial plexus, left side: root C5 (1), root C6 (2), root C7 (3), root C8 (4) splits into several branches, the root Th1 (5) is thicker than the root C8.

**Fig. 2.** Brachial plexus, left side: root Th1 (1), root C8 is splitted into two branches (2,3), root C7 (4), root C6 (5), root C5 (6). A branch of the root C8 (2) joins the root Th1 (1); another branch of the root 8 (3) joins the root Th1 over the arteria subclavia.

**Fig. 3.** Brachial plexus - convergence of the root C7 (1) with truncus superior (2), root C8 (3), root Th1 (4).
Table 2. Variations of the brachial plexus in the level of plexiform trunks and their branches

<table>
<thead>
<tr>
<th>Type</th>
<th>SIN.</th>
<th>DX.</th>
<th>Bilat.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Truncus superior</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The roots C5 and C6 divided into anterior and posterior branch</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No connection between roots C5 and C6</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>It consisted of short 2 mm connection of C5 and C6 roots</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The roots C5 and C6 connected on the level of the upper margin of the clavicle</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Truncus medius</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Connects with truncus superior</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Connects with truncus inferior</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>After making the anterior branch to the anterior branch of the truncus superior it connects with the posterior branch of the truncus superior and with the truncus inferior</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Truncus inferior</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No connection of the C8 and Th1 roots developed</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>As a small connection of 2 mm with a branch between C8 and Th1 roots</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Connects with two branches with the anterior and posterior branch of the truncus superior</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The root C8 connects with the Th1 root above the subclavian artery</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Does not make a posterior branch</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>It is made up after more than 4 cm parallel course of the C8 and Th1 roots</td>
<td>2</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>11</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

SIN. – left, DX. – right, Bilat – bilateral

The inferior trunk did not develop in one case. The roots C8 and Th1 were parallel, Th1 was even broader. The inferior trunk did not develop immediately in 8 cases, but after more than four centimeters of a parallel course of the roots C8 and Th1.

Each trunk had usually two branches, an anterior and a posterior. The posterior branch of the superior trunk was always longer than the anterior. This was valid for the middle trunk as well, except for 3 cases. The middle trunk (C7) divided to more than two branches in two cases. In such cases was one branch posterior and the rest were anterior branches. The posterior branch of the inferior trunk is significantly smaller than the others and its length varies. It often originates in the eighth cervical root before the formation into trunks. There was no posterior branch of the inferior trunk present in one case.

*Variations in the level of the neural plexiform fascicles:*

We have encountered variations of the neural fascicles 26 times in 22 cadavers. They were unilateral 18 times (Table 3). The connection between the anterior branch of the superior trunk with the anterior branch of the middle trunk did not develop in four cases, but once the later fascicle developed from a branch of the C8 root and once from the inferior trunk. The lateral fascicle was made up from the anterior branch of the superior trunk in two cases. In cases when the middle trunk was divided into more branches was the accessory anterior branch not connecting to the anterior branch of the superior trunk as usually, but it contributed to the formation of the medial root of bifurcation of the median nerve.
Table 3. Variations in the level of the plexiform fascicles

<table>
<thead>
<tr>
<th>Type</th>
<th>SIN.</th>
<th>DX.</th>
<th>Bilat.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fasciculus lateralis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The anterior branches of C5, C6 and C7 connected to make up the lateral fascicle</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The fascicle is made up from the anterior branch of the superior trunk</td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>It is made up from a connection of the anterior branch of the superior trunk and the anterior branch of the C8 root</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>It is made up from a connection of the anterior branch of the superior trunk with a branch from the inferior trunk</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>It is made up from a connection of the anterior branch of the superior trunk with the anterior branch of the roots C8 and C7</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>It releases a branch to the medial bifurcation of the median nerve</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>It is made up of practically the whole volume of the anterior branch of C7 and releases a branch to the ulnar nerve</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Fasciculus posterior</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The posterior branches of C5, C6, C7 and the inferior trunk connected to form the posterior fascicle</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Releases branches to the medial pedicle of the median nerve</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Releases branches to the lateral pedicle of the median nerve</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>The posterior branch of the superior trunk is missing on its formation</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>It develops low under the clavicle projection</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>The posterior fascicle is made up from the whole posterior branch of the superior trunk, which makes up 90% of its volume and a small thin posterior branch of the middle trunk</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Releases connection branches to the ulnar nerve</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Fasciculus medialis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Receives the posterior branch from the superior trunk</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>It is formed from an individual branch from Th1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>11</td>
<td>7</td>
<td>4</td>
</tr>
</tbody>
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SIN. – left, DX. – right, Bilat – bilateral

The anterior branch of the inferior trunk makes up the medial fascicle, which received a branch from the seventh cervical root in two cases.

Variations of the lateral fascicle were frequent, we have observed several combinations and in many cases multiple connections between the lateral and medial fascicle, before the origination of the median and musculocutaneous nerves. Thanks to these connections the medial bifurcation of the root of the median nerve is always containing fibers from the lateral fascicle and in three cases the ulnar nerve is also receiving fibers on this level from the same fascicle.

The lateral fascicle released one or more branches to the pectoral major muscle. The greatest variations in the nerve formation were also detected in the lateral fascicle, which continues as the musculocutaneous nerve (C5, C6, C7) after the origination of the terminal branch to the median nerve. The lateral fascicle divided as late as in the coracobrachial and in the biceps muscles in one case. The musculocutaneous nerve originated there. Then it conne-
cted with the medial bifurcation of the medial fascicle in the lower third of the arm forming the median nerve.

The medial fascicle, in cases when the C7 trunk divides to more than two branches, receives fibers not only from C8, Th1 (Th2) but also from C7. It can receive the same fibers from C7 in cases when the lateral branch of the ulnar nerve is present. The typical medial fascicle did not develop in two cases.

The medial fascicle continued as the ulnar nerve after the release of nervus cutaneus antebrachii medialis, brachii medialis and of the terminal branch to the medial root of the median nerve.

The posterior branches of all three trunks made up the posterior fascicle. The posterior fascicle was usually the thickest of the fascicles in the plexus, sometimes made up from a connection of four branches instead of three. The posterior branch of the superior trunk (C5, C6) is usually the thickest in most cases. The posterior branch of the inferior trunk (C8, Th1) is the thinnest in all cases. The terminal branches of the posterior fascicle of radial nerve and axillary nerve were the most constant.

There were even more variations found. They formed deeply under the clavicle in one case. The posterior branch of C7 was thin in one case and it did not connect with the posterior branch of the superior trunk but only with a thin branch originating from it. It did not lead to formation of the axillary nerve, either to the nervus subscapularis and thoracodorsalis. These branches originated in the posterior branch of the superior trunk, which continued as the axillary nerve into the foramen quadrilaterum.

Clinical, motor and innervation peculiarities in relation to the diagnosis

It is important to understand what neural functions belong to each part of the plexus. It is also important to be aware that due to the presence of variable connections between the trunks of the plexus the innervation of muscles may change independently of the number of the root going out of the plexus. Several anomalous nerve structures are, apart from atypical clinical and electromyography findings, also a source of a diagnostic confusion.

In case of the prefixed type of the nerve trunk it receives more fibers from neurons located more cranially. Root C4 may significantly contribute to the suprascapular nerve, but even to the axillary nerve. Radial nerve receives more fibers from C5. The same is being observed in relation to the musculocutaneous nerve and others. Injuries to superiorly located nerves or nerve trunks, such as truncus superior, are associated with more extensive plegia on periphery, the same as in the postfixed type.

Injury to the C5 root may manifest itself primarily by plegia of the deltoid, biceps and brachialis muscles but also by plegia of the wrist extensors, brachioradialis muscle, supinator muscle and pronator teres. The same injury of the C5 root in case of caudal localization of the plexus may not manifest at all or it can lead only to a mild impairment of the function of the forearm muscles. Brachialis and biceps muscles may be affected only partially.

In case of the postfixed type, the Th1 root may contain several fibers normally carried in the C8 root and a contribution of the Th2 to the plexus may be greater.

The root C7 provides an extensive innervation of the upper limb and variably contributes to the formation of all nerve trunks of the upper limb. In the rare cases of its injuries we can observe rather a diffuse impairment of function, without complete anesthesia or paralysis of some significant muscle group. The eighth cervical nerve contributes to the innervation of the thumb and finger extensors. The first thoracic nerve provides almost the same and it partially contributes to the innervation of the triceps muscle. The inferior trunk contributes to the three main nerves of the shoulder and in case of an injury each may manifest with signs of impairment. Its supply is full on the forearm, therefore the muscles of the forearm and hand innervated with the radial, ulnar and median nerves may be affected.

The posterior fascicle supplies mainly the extensor muscles, the medial and lateral mainly the flexor muscles. The medial fascicle contributes to the inervation of the proper hand muscles
innervated from the median nerve. The lateral fascicle contributes to the innervation of the sensory component of the median nerve. Presence of the Horner syndrome indicates avulsion of the C8 and Th1 spinal roots.

Three branches of the nerve roots seem to be clinically important. Nervus thoracicus longus and nervus dorsalis scapulae – if the function of the muscles supplied by these nerves is impaired, it indicates a proximal injury of the plexiform roots or an avulsion from the spinal cord. On the other hand, if muscles innervated by these nerves work well, even if no other muscle is functional, there is a presumption that the proximal nerve roots are preserved and they can be identified and used for a reconstruction surgery of the brachial plexus.

The radicular supply of the pectoral major muscle (C5-Th1) is a reduced picture of the condition of the whole brachial plexus. The clavicular part is supplied from C5 and C6, the sternal part from C7 and the abdominal part from C8-Th1 by means of the pectoral nerves, which clinically corresponds with the upper, middle and lower type of lesion of the brachial plexus. It can be of some importance in the localization of the lesion on the level of the fascicles.

**Mechanisms and morphological reasons of some types of injuries**

The root is most vulnerable in the site of its origination from the spinal cord. The motor roots contain less radicular fibers and they are thinner than the sensory roots. They are therefore pulled out more frequently. The sheaths of the spinal nerves are firmly connected with the dural bag and the traction forces are therefore transferred on its infundibular concavity into the intervertebral foramina, where the C8 and Th1 roots are freely movable. In comparison with the spinal nerves C5, C6 and C7 that are located in the sulcus spinalis nerve where they are firmly fixed with strong fibrous connective tissue and thereby protected. They are arranged the way to be less vulnerable during traction forces compared with the roots C8 and Th1, which are not fixed. The upper roots are therefore affected more distally during traction – more on the periphery. If an avulsion is to occur, the fixation of these roots has to be broken as well or a transverse vertebral process has to be fractured. A presence of avulsion fractures on this level is strongly suspicious of an avulsion of the nerve roots from the spinal cord.

Even in case of an avulsion of C5, C6, C7 and C8 the injured patient may be able to perform movements in the shoulder. The motility can be preserved thanks to the function of the Xth cranial nerve and the cervical plexus.

The biceps brachii muscle may, even in complete types of plexus injury with avulsion of the roots, in some cases restore its function. The roots C3, C4 and the phrenic nerve contribute to the innervation (8).

**DISCUSSION**

The variations of the formation of the brachial plexus are of a clinical and surgical importance. Knowledge of its anatomic variations may contribute to the explanation of inconceivable clinical pictures. It is supposed that the variations of the formation of the brachial plexus are caused by a deviation of the normal development. Some anatomic peculiarities are important especially for surgeons engaged in the reconstruction of plexus injuries.

It is important to be aware of these variations but also of their relation to the great vessels, because the topographic relations of fascicles and arteries may be various and they can lead to problems during an urgent surgery (1,6). There was not always the same type of variation in case of bilateral variations.

The main supraclavicular variations described in literature concerned the contribution of C4 or Th2 (9,10). We have not encountered any study in literature available to us concerning the variations of the formation of the neural roots, trunks and fascicles.

We have only detected the actual origination of the terminal branches of the plexus in 6 cases after a preparation of the variation of the trunk and fascicle formation.

Attention deserves sparse connective tissue forming sleeves, often multilayer, which was gre-
atest in the level of the trunk branching and fascicle formation, especially medial. Less of this tissue was present in the area of terminal branches formation. Its function is probably to prevent a damage of nerves by each other or by arteries or bone structures in these sites during movements with the upper limb. We have detected fibrotic changes in this tissue in several cases. The greatest fibrotic changes were in individuals heavily physically working, former sportsmen and in cases of postcaval catheters (7).

**Conclusion:** The anatomical arrangement of the brachial plexus is complicated. It is necessary to be aware of the individual variations in the development of the clinical picture, diagnosis and surgical treatment, because it can prevent surprises from some findings during surgery.

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THE ROLE OF NITRIC OXIDE IN BRONCHODILATORY EFFECTS OF PROVINOL - THE RED WINE POLYPHENOLIC COMPOUNDS

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Abstract
Nitric oxide (NO), as a basic mediator of inhibitory nonadrenergic and noncholinergic (i-NANC) neurotransmission in the airways, plays an important role in the pathophysiology of the respiratory diseases. Deficiency of NO is probably one of the important factors in the allergen – induced hyperreactivity of the airways.

Using a guinea pig model of ovalbumin (OVA) induced airway inflammation, the aim of the study was to determine the effect of Provinol (red wine polyphenolic compounds) on the tracheal smooth muscle reactivity in vitro, and to evaluate the role of NO in the bronchodilatory effects of Provinol.

Amplitude of the tracheal smooth muscle contraction as a response to bronchoconstrictor mediators - histamine (10⁻⁸–10⁻³ mol.l⁻¹), acetylcholine (10⁻⁸–10⁻³ mol.l⁻¹) and to allergen (OVA 10⁻⁵–10⁻³ g/ml), was used as a parameter of tracheal smooth muscle reactivity. The isolated tracheal strips were in organ bath pre-treated 30 min with Provinol (0.01 mg/ml), and 30 min with Provinol in combination with L-NAME (10⁻⁶ mol.l⁻¹).

Provinol antagonised OVA-induced contraction of the tracheal smooth muscle strips prepared from guinea pigs after 2 weeks of OVA-sensitization, and this reaction was partially inhibited with L-NAME. 30 minutes incubation of the tracheal smooth muscle with Provinol resulted in a decreased amplitude of contraction to bronchoconstrictor mediators – histamine and acetylcholine and the effect of Provinol was partially diminished by L-NAME (mainly in low doses of bronchoconstrictor).

In conclusion, Provinol inhibited the allergen- and spasmogen-induced contraction of the tracheal smooth muscle in OVA-sensitized guinea pigs, and this mechanism of action is probably partially mediated through the metabolism of NO.

Key words: nitric oxide, red wine polyphenolic compounds, tracheal smooth muscle reactivity, ovalbumin

INTRODUCTION
Nitric oxide (NO) is a highly active small molecule that plays a key role as a vasodilator, neurotransmitter and inflammatory mediator in the airways. NO is derived from amino acid L-arginine, in a stereospecific reaction catalysed by a family of nitric oxide synthases (NOS). NOS isoforms expression and NO production have been demonstrated in vascular endothelial cells, smooth muscle cells, airway epithelial cells, platelets, non-adrenergic and non-cholinergic neurons, macrophages, mast cells, and neutrophils (1). Three NOS isoforms, differing in activity and tissue distribution, have been identified: endothelial NOS (eNOS or type 3) neuronal NOS (nNOS or type 1) and inducible (iNOS or type 2). Endothelial and neuronal NOS are collectively termed constitutive nitric oxide synthase (cNOS). The constitutive NOS (eNOS and nNOS) are localized mainly in endothelial and epithelial cells and in some airway nerves. The cNOS are activated by calcium and calmodulin and produce a small amount of NO (picomole). NO produced this way regulates in airway tone in the respiratory tract, the gas exchange, function of surfactant and mucociliary clearance (2).

Under pathological conditions temporal high levels of NO are produced in the body after induction of the expression of an inducible type of NO synthase (iNOS, NOS-II). iNOS exists mainly in airway epithelial cells and inflammatory cells (3). Inducible way produced NO can exert beneficial effects in the defence of the airways; on the other hand, high levels of NO, if uncontrolled, can be detrimental (4). The detrimental effects are produced because persistent
high amounts of NO can react with concomitantly produced superoxide anions, which thereby generate highly toxic compounds, such as peroxynitrite and hydroxyl radicals (5).

Some pathological conditions, (respiratory disease) lead to damage of the physiological equilibrium of NO: either by decreasing production of NO (mainly constitutive forms), or by increasing formation of NO by iNOS. Fortunately, the body is probably equipped with the ingenious mechanism which carefully regulates the expression of iNOS. The physiological NO levels may probably inhibit the activity of transcription factor NF-κB and thus decrease the expression of the gene encoding iNOS (6).

For that reason, could substances stimulating cNOS by positive way influence the hyperreactivity of the respiratory tract during the inflammatory process. The purpose of the present experiments was to study the effect of Provinol (red wine polyphenolic compounds) on tracheal smooth muscle hyperreactivity in guinea pigs with allergen-induced asthma, and in the following stage to estimate the role of NO in its bronchodilatory effect.

METHODS

Provinol (RWPC- red wine polyphenolic compounds) was provided by D. Ageron (Société Francaise de Distillerie, Vallont Pont d’Arc, France) and the composition of RWPC was (mg/g of dry powder): proanthocyanins 480, total antocyanins 61, free anthocyanins 19, catechin 38, hydroxycinnamic acid 18, flavonols 14. Other chemicals were purchased from Sigma Chemicals Co, Germany.

Sensitization of guinea pigs: Trik guinea pigs (200 g) of both sexes were divided into 2 groups: control and ovalbumin sensitized. The guinea pigs in sensitized group were injected 5 mg of ovalbumin intraperitoneally and 5 mg subcutaneously in 1 ml of saline on day 1. The animals were boosted with 5 mg of ovalbumin injected i.p. on day 4. The guinea pigs were used 2 weeks later (7).

In vitro tracheal muscle contraction experiments: The reactivity of the tracheal smooth muscle was estimated by in vitro method, after 14 days ovalbumin sensitization. The preparation of guinea pig tracheal strips were placed in 20-ml organ chamber containing Krebs-Henseleit buffer of the following composition (µM): NaCl, 110,0; KCl, 4,8; CaCl₂, 2,35; MgSO₄, 1,20; KHPO₄, 1,20; NaHCO₃, 25,0; in glass-distilled water. Organ chambers were maintained at 36,5±0,5 °C and were aerated continuously with the mixture 95% O₂ and 5% CO₂, to maintain pH (7,5±0,1. The tissue strips were initially set to 4 g of tension (30 minutes-loading phase). After this period, the tension in each tissue segment was readjusted to a baseline of 2 g (30 minutes adaptation phase). During these periods the tissue was washed at 15 min. intervals [8,9].

The isolated tracheal strips were in organ bath pre-treated 30 min with Provinol (0,01 mg/ml), and in second case 30 min with Provinol (0,01 mg/ml) in combination with L-NAME (Nω-nitro-L-arginine methyl ester) in concentration 10⁻⁶ mol.l⁻¹ [10].

The amplitude of contraction (mN) of the tracheal smooth muscle to the cumulative doses of histamine (10⁻⁸-10⁻³ mol.l⁻¹), acetylcholine (10⁻⁸-10⁻³ mol.l⁻¹) and ovalbumin (10⁻⁵-10⁻³g/ml) was used as a parameter of tracheal smooth muscle reactivity [11].

For the statistical analysis Student’s t - test for unpaired data was used. Results are expressed as mean ±S.E.M. for 12 tracheal strips in each case. A probability level of p<0,05 was accepted as significant.

RESULTS

Increased reactivity of the smooth muscle of the airway is one of the feature of allergen-induced hyperreactivity of the airways in animal model. The degree of hyperreactivity in in vitro conditions is assessed by increased amplitude of contraction to bronchoconstrictor mediators (12). In our experiments we used the preparation of tracheal strip of guinea pig after 14 days sensi-
tization with ovalbumin. In this interval in all sensitised animals increased amplitude of tracheal smooth muscle contraction to cumulative doses of histamine (10nM-1mM) and acetylcholine (10nM-1mM) was detected (Fig. 1,2).

This animal model of allergen-induced hyperreactivity of the airways was used for evaluation of Provinol bronchodilatory activity. Second step of the experiment was to evaluate the role of NO in the bronchodilatory efficiency of Provinol. For this reason Provinol (0.01 mg/ml) and Provinol (0.01 mg/ml) in combination with the non-selective inhibitor NO-synthase L-NAME (10^-6 mol.l^-1) were directly placed in organ bath 30 minutes before bronchoconstrictor.

The results of experiments, which evaluated the reactivity of tracheal smooth muscle to histamine, showed that after 14 days ovalbumin sensitization, the Provinol pre-treatment caused statistically significant decline in the amplitude of tracheal smooth muscle contraction, and this bronchodilatory efficiency was partially diminished by NO-synthase inhibitor L-NAME (Fig.3).

The similar picture of the changes was observed in contraction induced by acetylcholine,
where Provinol showed significant bronchodilatory effect, which was partially inhibited with L-NAME (Fig. 4).

In the case of contraction induced by allergen (ascended amounts of ovalbumin $10^{-5}$-$10^{-3}$g/ml), the result was the decline of the amplitude of contraction after 30 minutes pre-treatment with Provinol and its significant inhibition with L-NAME, mainly in low doses of allergen (Fig. 5).

**DISCUSSION**

Allergic asthma is characterized by allergen-induced early and late asthmatic reactions, airway inflammation and airway hyperreactivity to bronchoconstrictor mediators (13). Using guinea pig model of allergic asthma, we demonstrated in our experiments, after 14 days ovalbumin sensitization, increased tracheal smooth muscle reactivity to cumulative doses of histamine and acetylcholine.
The precise mechanisms underlying allergen-induced airway hyperreactivity are largely unknown. One of the important factors participating in this pathological process is a dysbalance in NO metabolism. Deficiency of constitutively formed NO, possibly caused by epithelial damage due to inflammation, may contribute to allergen-induced airway hyperreactivity after the early asthmatic reaction (14). The inflammatory mediators cause the decreased function of cNOS, manifested by lower bronchodilatory effect of NO; and stimulation of iNOS, which results in increasing formation of inducible NO and asthmatic complications (15). According to new knowledge about the control of nitric oxide levels, under normal conditions, physiological concentration of NO, maintained by constitutive NO synthases (nNOS, eNOS) can keep the activation of nuclear factor \( \kappa \)B (NF-\( \kappa \)B) suppressed, which thus contributes to limiting processes such as immune response and inflammation (16). When a pathological event occurs rapidly it shifts the equilibrium towards phosphorylated (inactivated) nNOS and eNOS, which thereby results in the abrupt fall in intracellular NO levels. This situation can favour the activation of NF-\( \kappa \)B, by removing the NO-induced inhibitory effect followed by the induction of expression of the gene encoding inducible NO synthase (iNOS) and by the production of high levels of NO. Any compounds that reduce the intracellular concentration of NO, such as inhibitors nNOS or eNOS, might mimic this situation (17). And the other hand the substances able the stimulate cNOS may have positive effect.

Provinol (red wine polyphenolic compounds) in concentration \( 10^{-4} \) - mg/ml is in in vitro conditions able to activate endothelial NO synthase activity in the cardiovascular area. (18). In our experiments Provinol influenced the reactivity of the tracheal smooth muscle and its effect was partially mediated through NO metabolism. Provinol antagonised OVA-induced contraction of the tracheal smooth muscle strips prepared from guinea pigs after 2 weeks of OVA-sensitization, and this reaction was partially inhibited with L-NAME pre-treatment. Incubation of the tracheal smooth muscle with Provinol resulted in a decrease of the amplitude of contraction to bronchoconstrictor mediators – histamine and acetylcholine, and the effect of Provinol was partially diminished with L-NAME (mainly in low doses of bronchoconstrictor).

Flavonoids are a large group of polyphenolic compounds with wide spectrum of biological activities (19). Polyphenols have been shown to be able to positively modulate the process of thrombosis, have cardioprotective, antihypertensive, antiischemic and other positive effects. Our results demonstrate the protective effect of Provinol on the tracheal smooth muscle reactivity to bronchoconstrictor mediator and allergen in the model of ovalbumin induced allergic asthma.
can be presumed that the mechanism of bronchodilatory effect of Provinol is probably partially mediated through the metabolism of NO.

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IN VITRO REACTIVITY OF URINARY BLADDER SMOOTH MUSCLE INFLUENCED BY OXYBUTYNIN, PROPRANOLOL, AND INDOMETHACIN IN GUINEA PIGS

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Abstract

Introduction: The representative of a conservative therapy standard of overactive urinary bladder in humans is oxybutynin, which decreases the hyperactivity of detrusor by blocking muscarinic receptors and by direct relaxation. The aim of our study was to evaluate the effect of oxybutynin on in vitro reactivity of the urinary bladder smooth muscle in guinea pigs and evaluate its effect in a combination with propranolol and indomethacin.

Methods: The reactivity of the urinary bladder smooth muscle was estimated by in vitro method using organ chambers. The smooth muscle strips were prepared from guinea pig urinary bladders and aerated under the tension in Krebs-Henseleit’s solution in the organ bath. Oxybutynin (10⁻⁶, 10⁻⁵, 10⁻⁴ mol.l⁻¹) and oxybutynin in the same concentrations with propranolol (10⁻⁴ mol.l⁻¹) and indomethacin (10⁻⁴ mol.l⁻¹) was added for 15 minutes and after that cumulative concentration-response curves to acetylcholine were plotted.

Results: Oxybutynin in concentrations of 10⁻⁶ and 10⁻⁵ mol.l⁻¹ significantly decreased the contractile responses of the urinary bladder smooth muscle. In the concentration of 10⁻⁴ mol.l⁻¹ a relaxation of smooth muscle without any reaction to the cumulative concentrations of acetylcholine was observed. Although indomethacin caused a non-significant lowering of the contractile responses, propranolol significantly decreased the contractile response to acetylcholine in concentrations of 10⁻⁴ and 10⁻³ mol.l⁻¹. A combination of oxybutynin with indomethacin and propranolol in all tested concentrations significantly decreased the contractile responses and in the concentration of oxybutynin 10⁻⁶ mol.l⁻¹ this decrease was significantly greater comparing to oxybutynin alone.

Conclusion: Our experiments supported the finding that oxybutynin is a very effective inhibitor of the contractile response of urinary bladder smooth muscle in guinea pigs in vitro and in higher concentrations it has relaxation effects. The combination with propranolol and indomethacin was more effective with a predominance of propranolol asset.

Key words: urinary bladder, contraction, oxybutynin, indomethacin, propranolol

INTRODUCTION

Many studies have documented a large prevalence of overactive bladder in the older population (1). Detrusor hyperactivity could be a result of several pathomechanisms, most probably of myogenic and neurological origin (2). Muscarinic receptors mediate normal bladder contraction, but also contractions of overactive bladder, so antimuscarinic drugs can block detrusor contractions in patients with bladder hyperactivity (3). The other way how to influence hyperreactivity is the usage of drugs which have primary effects on membrane ion channels (Na⁺, Ca²⁺, K⁺) and cause direct relaxation of urinary bladder smooth muscle. Prostaglandin synthesis inhibitors may also decrease contractile response by blocking of one part of non-adrenergic, non-cholinergic (NANC) system. To sum up, also α-adrenoceptor antagonists, β-adrenoceptor agonists, vasopresin analogue, antidepressants like imipramine, botulotoxin, and capsaicin should be mentioned.

Conservative therapy standard of overactive urinary bladder in humans is oxybutynin, which is able to decrease hyperactivity of detrusor by summation of effect of muscarinic receptors blockade and local anesthetic effect.

The aim of our study was to study the effects of oxybutynin on in vitro reactivity of urinary bladder smooth muscle in guinea pigs and to evaluate its effects in combination with propranolol and indomethacin. We used indomethacin, cyclooxygenase inhibitor, to eliminate possible

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effects of prostaglandins (4), and propranolol, non-selective β-receptor antagonist, to eliminate possible effects of remaining adrenergic agents in urinary bladder sample, because existence of β₂ and β₃ adrenoreceptors was reported (5,6). This study, evaluating in vitro effects of oxybutynin on reactivity of detrusor, will be the basis of our future studies focusing on modulation of the urinary bladder smooth muscle contraction.

**METHODS**

The reactivity of urinary bladder smooth muscle was estimated by in vitro method (7,8,9). 12 animals weighting 250-300 g were used. The preparations of urinary bladder smooth muscle strips (2 x 2 x 15 mm) from guinea pigs were mounted between two hooks and placed into a 30 ml organ chamber containing Krebs-Henseleit buffer of the following composition: NaCl 110.00 mmol.l⁻¹, KCl 4.80 mmol.l⁻¹, CaCl₂ 2.35 mmol.l⁻¹, MgSO₄ 1.20 mmol.l⁻¹, KH₂PO₄ 1.20 mmol.l⁻¹, NaHCO₃ 25.00 mmol.l⁻¹ and glucose 10.00 mmol.l⁻¹ in glass-distilled water. The organ chambers were maintained at 36.5 ± 0.5 °C and were aerated continuously with a mixture of 95% O₂ and 5% CO₂, to maintain pH 7.5 ± 0.1. One of the hooks was connected to a force transducer (TSR 10G, Vývoj Martin, Slovakia) and an amplifier (M1101 SUPR, Mikrotechna Praha, Czech Republic) and tension recordings were made on a Line Recorder TZ 4620 (Laboratorní přístroje Praha, Czech Republic). The tissue strips were initially set to 4 g of tension (30 minutes loading phase). After this period, the tension in each strip was readjusted to a baseline of 2 g (30 minutes adaptation phase). During both of the periods the tissue strips were washed at 10 minutes intervals. Thereafter, cumulative doses of acetylcholine (10⁻⁸ to 10⁻³ mol.l⁻¹) were added and a continual graphical recording of contractions was made. This recording was named „Control“. After 25 minutes of washing up period, 200 µl of oxybutynin (subst. Sigma-Aldrich) was added into each chamber in order to get the concentrations of 10⁻⁶, 10⁻⁵ or 10⁻⁴ mol.l⁻¹, or 200 µl of indomethacin (subst. Sigma-Aldrich) or propranolol (subst. Sigma-Aldrich) in order to reach the concentration of 10⁻⁴ mol.l⁻¹ as well as a combination of oxybutynin with propranolol and indomethacin together in concentrations mentioned above. After 15 minutes period of incubation the amplitudes of contractions (g / 100g) of urinary bladder smooth muscle strips to the cumulative doses of acetylcholine (10⁻⁸ to 10⁻³ mol.l⁻¹, subst. Sigma-Aldrich) were recorded. These records were used for evaluation of the contractile responses (10).

A non-parametric ANOVA test was used for the statistical analysis. Results are presented as mean ± standard error of the mean (SEM). A probability level of p < 0.05 was accepted as significant. All experiments were conducted in concordance with basic ethic norms and Helsinki Declaration of 1975, revised in 1983.

**RESULTS**

Addition of acetylcholine into the organ bath with urinary bladder smooth muscle strip in cumulative manner resulted in dose-dependent increasing of the contractile responses in controls. In the organ baths with oxybutynin in the concentrations of 10⁻⁶ and 10⁻⁵ mol.l⁻¹ the contractile responses of urinary bladder smooth muscle were significantly decreased (Fig.1). In concentration of 10⁻⁴ mol.l⁻¹ a relaxation of the smooth muscle without any reaction to cumulative concentrations of acetylcholine was observed (Fig.1).

Adding of indomethacin in concentration of 10⁻⁴ mol.l⁻¹ caused a non-significant lowering of the contractile responses to acetylcholine cumulative concentrations of 10⁻⁸ to 10⁻³ mol.l⁻¹ (Fig.2).

Propranolol in concentration of 10⁻⁴ mol.l⁻¹ significantly decreased the contractile responses to cumulative doses of acetylcholine in concentrations of 10⁻⁴ and 10⁻³ mol.l⁻¹ (Fig.3).

A combination of oxybutynin with indomethacin and propranolol together in all tested concentrations (10⁻⁶ to 10⁻⁵ mol.l⁻¹) significantly decreased the contractile responses to cumulative doses of acetylcholine (Fig.4). The response to acetylcholine in the combination of oxybutynin 10⁻⁶ mol.l⁻¹, indomethacin and propranolol was significantly decreased comparing to oxybutynin alone in the same concentration.
Fig. 1. Reactivity of guinea pig urinary bladder smooth muscle after adding of oxybutynin to cumulative doses of acetylcholine. The columns represent mean contraction (g/100 mg) and the range of the standard error of the mean (SEM). One, two and three asterisks represent statistical significance of difference with p < 0.05, 0.01, and 0.001, respectively (ACH = acetylcholine).

Fig. 2. Reactivity of guinea pig urinary bladder smooth muscle after adding of indomethacin to cumulative doses of acetylcholine. The columns represent mean contraction (g/100 mg) and the range of the standard error of the mean (SEM) (ACH = acetylcholine).

Fig. 3. Reactivity of guinea pig urinary bladder smooth muscle after adding of propranolol to cumulative doses of acetylcholine. The columns represent mean contraction (g/100 mg) and the range of the standard error of the mean (SEM). One asterisk represents statistical significance of difference with p < 0.05 (ACH = acetylcholine).

Fig. 4. Reactivity of guinea pig urinary bladder smooth muscle after adding of combination of oxybutynin, indomethacin and propranolol to cumulative doses of acetylcholine. The columns represent mean contraction (g/100 mg) and the range of the standard error of the mean (SEM). One and two asterisks represent statistical significance of difference with p < 0.05, and 0.01, respectively (ACH = acetylcholine).
DISCUSSION

The characteristics of a urinary bladder smooth muscle influence the behavior of not only urinary bladder, but of the whole body, as the ability to accumulate urine and consecutively release it belongs to basic social needs. Only a normal and coordinated bladder function can maintain good social adaptation of an individual. Any changes in this basic need can disturb the integrity and social positioning of individual and so could lead to a significant decrease of the quality of life. Therefore it is very necessary to know all the mechanisms participating in the filling of urinary bladder, voiding, and in case of an impairment to be able to eliminate it.

In clinical practice we meet the problems of hyperresponsiveness or hyperreactivity of smooth muscle in various organ systems, like respiratory system, gastrointestinal tract, skin, as well as urinary system (11,12). From a clinical point of view, the urinary bladder „stability” problems are very often, as Švihra et al. (2001) showed in recent study, that the overactive, „unstable”, bladder incidence in population of Slovakia raises especially with age (1). The typical symptoms of overactive bladder include the frequent voiding and bladder fullness sensations, sensation of not complete bladder emptying after voiding and later also impaired ability to accumulate urine – incontinence. The incontinence can significantly impair the patients’ quality of life.

There are various ways and levels how to influence the urinary bladder reactivity. The micturition reflex represents a classical reflex with its own receptors, afferent nerves, reflex center, efferent nerves and finally effectors. The receptors are located in and under the urothelial layer as well as in the urinary bladder smooth muscle. The sensory nerve endings can be irritated by various stimulants of different origin (chemical substances, temperature, distension, pressure) (13). An over-stimulation (e.g. impairment of urothelium) and increased afferent mediation can lead to increased reactivity of the voiding center and increased frequency of voiding (14,15,16).

The parasympathetic nervous system, similarly to other organ systems, plays the major role in the regulation of the urinary bladder smooth muscle (17). Nowadays five pharmacologically different muscarinic receptors (M₁-M₅) are distinguished. In the human urinary bladder are present especially receptors M₂ a M₃. Although M₂ receptor was found to be the predominant one, the major role in contraction responses plays the M₃ subtype (18,19). The M₂:M₃ ratio in the human smooth muscle is 4:1 (20), in rat 9:1, and in other animals 3:1 (21). The role of M₂ receptors is going to be elucidated, too. The ability to act against beta-adrenergic receptors, whose activation enables the urinary bladder smooth muscle relaxation and urine accumulation, seems to be the major function (22,23). Except of that, M₂ receptor stimulation is associated with an activation of non-specific cation channels and inactivation of potassium channels (20,24). Concluding this, M₂ receptors are responsible for a direct contraction of the smooth muscle during voiding, until M₂ receptors prevent the relaxation of the smooth muscle evoked by sympathetic activation. These two effects are acting synergically in order to expel the urine from the urinary bladder more effectively (21).

Parasympathetic nerves innervating the urinary bladder possess also a huge number of presynaptic inhibitory and excitatory muscarinic receptors (autoreceptors), which can participate in regulation of neurotransmitter release. The inhibitory presynaptic receptors include muscarinic receptor M₂ in the rabbits urinary bladder (25) and rats (26), and receptor M₁ in the urinary bladder of guinea pigs (27) and humans (28). Their irritation leads to a decreased release of acetylcholine into the synaptic cleft (negative feed-back) and to a decreased stimulation of postsynaptic receptors (29).

Muscarinic receptors are located on the external side of the cell membrane and connected to a transmembrane G-protein. There are significant differences in the signal transduction among various subtypes of muscarinic receptors. M₁, M₃ and M₅ receptors are connected to the Gₙ/α protein and phospholipase C with consecutive hydrolysis of phosphatidylinositol biphosphate and mobilization of intracellular calcium. The stimulation of M₂ and M₅ receptors leads to the activation of the G₁ protein and to the inhibition of adenylate cyclase. Furthermore, the muscarinic receptors are able to inhibit ATP dependent potassium channels (K₄ATP), by an activation of protein kinase C (24).
There can be found also adrenoceptors in the smooth muscle of the urinary bladder. The most important subtypes are $\alpha_1$-receptors and $\beta_2$-receptors.

Yamamoto et al. (2001) found that in rats presynaptic $\alpha_1$-receptors are present in neonate as well as in adult animals. A postsynaptic localization was demonstrated only in adult rats (30).

 Postsynaptic $\alpha_1$-receptors have in the urinary bladder relatively small importance. Maggi et al. (1985) found that clonidine caused a concentration dependent inhibition of contractions evoked by electrical stimulation in rat detrusor (31,32). This effect was probably based on the stimulation of $\alpha_2$-adrenoceptors located on postganglionic nerve endings (presynaptically). Their stimulation led to the decreased release of excitatory neurotransmitters (acetylcholine and norepinephrine) (33). Therefore, $\alpha_1$-receptor agonists and antagonists are able to affect the voiding on both levels – central (brain, spinal cord) and peripheral (ganglia, nerve endings, smooth muscle) (3).

The presence of $\beta$-adrenoceptors in urinary bladder smooth muscle is essential for a progressive relaxation of the detrusor during filling phase and increasing of bladder volume. Yono et al., (2000) showed that $\beta_1$-adrenoceptors are present especially in myocardial cells and represent the target of antagonists – beta-blockers, which decrease heart rate (34). The $\beta_2$-adrenoceptors are expressed predominantly in the uterus and the airways and are the targets of beta-mimetics causing tocolysis, and bronchorelaxation, respectively. The $\beta_3$-adrenoceptors could be found in fat tissue cells and regulate the energy metabolism changes and thermogenesis caused by norepinephrin (35,36). The relaxation of urinary bladder smooth muscle is caused by $\beta$-adrenoceptors associated with the transmembrane $G_\alpha$ protein. There is a great variability in the presence of $\beta$-adrenoreceptor subtypes among different species (6). In rabbits are present especially $\beta_2$-adrenoceptors (5), in humans it is $\beta_3$-adrenoceptor subtype (6).

Propranolol, a non-selective $\beta$-blocker, is used in the smooth muscle reactivity tests as an inhibitor of the receptor stimulation by various agents released from resting urothelial cells in the strip. The observed effect was due to relatively high dose of the agent ($10^{-4}$ mol.l$^{-1}$).

The muscarinic receptors are responsible for the contractile properties of the urinary bladder smooth muscle not only in normal, but also in diseased state (overactive bladder). Therefore, the anticholinergic therapy (trospium, oxybutynin, tolterodin) represents one of the most effective treatments for this disease (37).

Atropin is not a standard therapy due to its adverse effects and low specificity. In some resistant cases it is used in intravesical administration for its local action. It leads to an increase of the capacity and decrease of the intravesical pressure (38).

Oxybutynin possesses several pharmacological effects. Depression of detrusor hyperactivity is reached by the blockade of muscarinic receptors, direct relaxation of detrusor and by a local anesthetic effect. The local anesthetic effect is present only by intravesical administration of oxybutynin. The agent has higher affinity to muscarinic receptors M1 and M3 than to M2 subtype. The clinical importance of this affinity is still unclear, as oxybutynin acts through its active metabolites. Many clinical studies showed that the drug significantly influences the detrusor hyperactivity with 70 % efficacy. The dryness in mouth during a standard dosage regimen was reported in a relatively high number of patients – 80 %. Oxybutynin solidly inhibits the urinary bladder hyperreactivity and is therefore recommended as first line therapy of the overactive bladder (37).

There are also other agents used in the therapy of the overactive bladder. The mechanism of action of propiverine is the anticholinergic action and the blocking of calcium channels. This agent is changed to metabolites, which affect the detrusor and inhibit spontaneous contractions (37).

A human bladder mucosa has the ability to synthesize eicosanoids. These agents can be liberated from bladder muscle and mucosa in response to different types of trauma. Although prostaglandins can cause contraction of the urinary bladder smooth muscle, more important than direct effects on the bladder muscle may be sensitization of sensory afferent nerves, increasing the afferent input produced by the given degree of bladder filling. Therefore, treatment of ove-
reactive bladder with prostaglandin synthesis inhibitors (such as indomethacin or flurbiprofen) could be expected to be effective (39).

Other drugs with potential effect on the urinary bladder smooth muscle are calcium channel blockers verapamil and nifedipine (40), highly selective antagonist of M₃ receptor subtype daf
fenacine (41), or imipramine with anticholinergic action and 5-hydroxytryptamine blocking effect (37). Local effect and desensitization of the sensory receptors in urinary bladder participates in the effect of intravesically-administered capsaicin (37).

In conclusion, according to our results we can confirm that oxybutynin is a very effective inhibitor of the contractile responses of the urinary bladder smooth muscle in guinea pigs in vitro to acetylcholine and in higher concentrations has relaxation effects. In combination with propranolol and indomethacin, the inhibition was more potent, especially due to the effect of propranolol. These findings are still objects of further research.

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SMOKING IN PREGNANT WOMEN IN SOME REGIONS OF SLOVAKIA IN THE LIGHT OF EDUCATIONAL, NATIONAL AND SOCIAL BACKGROUND

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Abstract

Introduction: Smoking of women, especially during pregnancy, poses a significant public health problem. In its epidemiology regional and cultural individualities play an important role. The goal of the cross-sectional study was to find out differences in smoking habit in pregnant women between Central Slovakia (Martin) and Southern Slovakia (Nové Zámky and Komárno), where a high proportion of Hungarians live.

Material and methods: Questionnaire data were collected from hospitalized women after a physiologic delivery at Martin Faculty Hospital (April 1999 - January 2000) and hospitals in Komárno and Nové Zámky (February 2001 - August 2001). Data on smoking before and during pregnancy, attitudes towards healthy life-style and awareness of risk factors (represented by a number of correctly identified risk factors) were compared.

Results: Of the total number of 832 women (227 in Martin, 253 in Komárno, 352 in Nové Zámky), 31.5 % reported smoking before pregnancy, while strong negative association with educational level has been found and smoking of Hungarian women prevailed. 18.3 % of the smokers continued smoking also during pregnancy. Similarly, strong negative association with educational level has been found. 68.3 % of respondents reported feeling of healthy life-style. Higher educated subgroups and Slovak women have shown better knowledge on risk factors.

Discussion: Relatively high prevalence of smoking before pregnancy, compared to similar studies carried out in Slovakia, may reflect an increasing trend of women’ smoking. A high need for effective measures is apparent in the lowest educated groups. A relatively worse situation in Hungarian minority can be partially explained by different cultural background as well as a language barrier, preventing access to information.

Key words: smoking, pregnancy, education, cultural background

INTRODUCTION

The onset of smoking habit and its further development is significantly influenced by social background in a given community. On the other hand, differences in life-style, including smoking, determined by specific regional and cultural features, can to some extend explain a relatively high level of heterogeneity in cardiovascular mortality in Slovakia (1, 2). The study of differences in smoking habit and related factors in particular communities help us to estimate the role of regional and cultural features, which could be important in tobacco control measures.

In our survey, we studied smoking habit and related life-style factors before and during pregnancy in three samples of women from two different regions of Slovakia: Central Slovakia region represented by the town of Martin, and Southern Slovakia region represented by the towns of Komárno and Nové Zámky. The population of Central Slovakia is almost entirely of Slovak nationality. Social environment in Southern Slovakia is significantly influenced by Hungarian minority living here, which can be reflected also in specific features of life-style in this region.

METHODS

Questionnaire data on smoking before and during pregnancy, attitudes towards healthy life-style and awareness of risk factors (represented by a number of correctly identified risk factors) were collected. Samples included women hospitalized after a physiologic delivery at the Obste-
The Obstetric Clinic in Martin Faculty Hospital (from April 1999 to January 2000) and the Obstetric Departments of hospitals in Komárno and Nové Zámky (from February 2001 to August 2001). All women with physiological delivery within a given time period, reporting either Hungarian or Slovak nationality and willing to cooperate, were included. Data were analyzed by logistic regression and associations were mutually controlled for region (Martin, Nové Zámky, Komárno), nationality (Slovak, Hungarian) and self-reported educational level (elementary, apprentice, secondary and college/university).

RESULTS

From the total number of 832 respondents, 352 were from Nové Zámky, 253 from Komárno and 227 from Martin. Hungarian national minority in Nové Zámky represented 35.2% and in Komárno 71.1% of the respondents. In Hungarian women a higher proportion of women with elementary education has been found (Tab. 1). The average age of respondents was 26.0±4.9 years and no significant difference in age distribution has been found among samples.

Table 1. Educational structure of the sample (only respondents reporting their educational level were included)

<table>
<thead>
<tr>
<th>Educational level</th>
<th>Elementary</th>
<th>Apprentice</th>
<th>Secondary</th>
<th>University</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nationality</td>
<td>abs.</td>
<td>%</td>
<td>abs.</td>
<td>%</td>
<td>abs.</td>
</tr>
<tr>
<td>Slovak</td>
<td>41</td>
<td>7.9</td>
<td>145</td>
<td>27.9</td>
<td>272</td>
</tr>
<tr>
<td>Hungarian</td>
<td>50</td>
<td>16.4</td>
<td>99</td>
<td>32.6</td>
<td>125</td>
</tr>
</tbody>
</table>

From all the respondents, 31.5% reported regular smoking before pregnancy, with clear predominance of lower educated women (p<0.001). Also Hungarian women have shown higher prevalence of smoking before pregnancy, however after controlling for education level, the difference between nationalities was not found significant (Fig. 1).

5.8% of all the respondents reported smoking during pregnancy and also here a strong association with education level has been found. Among lowest educated respondents 19.4% smoked, while among university educated none of the respondents reported smoking during pregnancy (Fig. 2).

Considering the knowledge about healthy life-style, 35.5% of the respondents reported very good knowledge, 55.9% moderate level of knowledge and 3.0% poor knowledge. There is a clear

Fig. 1. Proportion of women regularly smoking before pregnancy.
association with education level (p<0.001) and higher educated respondents had also better subjective feeling of good knowledge. Actual knowledge, represented by the number of correctly identified risk factors, also depended on education level (p<0.001) Among the women with elementary education as much as 76.3% could not name any risk factor, while among all the respondents it was only 47.5%. Evaluating the impact of education, no significant association with nationality and region has been found (Fig. 3). 35.8% of all respondents identified smoking as a risk factor.

**DISCUSSION**

The prevalence 31.5% of smoking women before pregnancy in our presented study exceeds the rates found within the CINDI project in 1993 (3), where in the age group 15-34 years
smoked 20.7% of women. Our results exceed also smoking prevalence of women found in a survey carried out by the Statistical Office of the Slovak Republic, i.e. 26.0% (4). These differences indicate gradually increased proportion of smoking women in Slovakia, which deserves our attention.

High proportion of smoking among lower educated women indicates that one of the priorities of tobacco control in our population should be preventive measures focused on this population group. Proportion of the women reporting continuation smoking during pregnancy is lower than in the studies carried out in western countries (5, 6, 7, 8, 9, 10), however, considering the relatively low validity of subjectively given information found in several studies (11, 12, 13), we have to expect higher actual data in this field. Strong association of smoking with low education emphasizes the need for more efficient tobacco control activities in this target groups.

It is not surprising that higher educated women showed clearly better knowledge on risk factors. This finding again emphasizes the need for more intensive preventive measures addressed to lower educated population groups. On the other hand, relatively low level of subjective feeling of good knowledge among these women speaks for the need of increasing the motivation of target group to look for information.

Taking into consideration that harmful effect of smoking are only rarely doubted even among lay public, and its significance is frequently mentioned in mass media and educational materials, less than 36% of respondents reporting smoking as a risk factor should be considered as a small proportion. This finding indicates that despite a large quantity of information, the target audience is not appropriately addressed, and another more effective ways of how to educate people should be sought for.

Considering differences in life-style in the compared regions, the nationality, after assessing other possible confounding factors (region and education) showed only limited influence, which had reflected in a slightly higher smoking prevalence among Hungarian women. So, existing differences in life-style and mortality rate among regions and nationalities, at least partially, can be explained by different educational structure of compared communities.

Considering validity of our results, our samples include all the population groups of Slovak and Hungarian women living in studied regions, however, samples reflect proportionally not only actual numbers in the population, but also birthrate in the population groups and their willingness to cooperate in health related issues. If this study is considered as an outcome for primary prevention and health promotion among pregnant women (e.g. as a part of a psychoprophylaxis), such potential bias does not influence an appropriate interpretation of obtained data and their implementation in practice. Romany minority were not included into the study as a separate population group, since their identification is problematic and it may by easily considered as a discrimination issue. Beside this, population groups not included in the study due to either not willingness to cooperate or insufficient intellectual level, even illiteracy (particularly among Romany minority) require special approach beyond the scope of our study and methods effective among other population groups cannot be implemented here. On the other hand, it should be assumed that a part of Romany women reported themselves as Hungarians.

Eventually, despite of some above-mentioned weak points, we hope that our study brings some valuable data that can be implemented in health promotion and prevention, particularly if its limitations and possibilities are appropriately respected.

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THE IMPORTANCE OF AMBULATORY BLOOD PRESSURE MONITORING IN THE DIAGNOSIS OF WHITE COAT HYPERTENSION IN CHILDREN

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Abstract
The article points out the importance of the ambulatory blood pressure monitoring (ABPM) in the diagnosis of white coat hypertension in children. In pediatric cardiology we often meet patients with high blood pressure. Most of them are children whose blood pressures are higher only in the presence of a doctor.

Objective: To point out the importance of the 24-hour blood pressure monitoring in different diagnoses in children with repeatedly measured higher blood pressure.

Methods: Our study carried out in 2001-2003 included 77 patients (60 boys, 17 girls) in which a high blood pressure was repeatedly found (in average 139/86 mmHg).

In all patients included in our study the 24-hour blood pressure monitoring was made to confirm the white coat hypertension. We used Meditech ABPM-04. The size of the inflatable cuff depended on the age of children. In all our patients the family history was taken and the complete cardiologic examination was carried out.

Results: By using ABPM we found the white coat hypertension in 30 children (39%). The blood pressure measured in outpatients was in average 137/82 mmHg and during ABPM 116/63 mmHg. From this group, 19 children (63 %) had positive family histories.

Conclusion: ABPM seems to be very important in the diagnosis of white coat hypertension in children.

Key words: white coat hypertension, children, ABPM, repeatedly measured higher blood pressure

INTRODUCTION
White coat hypertension is a common finding in children. The recorded incidence varied between 10% and 50%, depending on the defined criteria (1). White coat hypertension is a phenomenon in which a patient’s blood pressure rises in the presence of a physician (white coat) and, presumably, returns to normal at home (2). Another definition of white coat hypertension: an elevation of clinical pressure with a normal daytime ambulatory profile (3). The condition compared with established hypertension is associated with a relatively benign cardiovascular risk (4). The etiopathogenesis may reflect an abnormally vigorous sympathetic response to the environment of the measurement, especially the presence of the measuring nurse or physician (5).

Ambulatory blood pressure monitoring (ABPM) is the most frequent mechanism used in measuring the presence of the white coat effect (3). It is a 24-hour non-invasive, continuous measuring of blood pressure. The ABPM device needs connection to PC and the software for ABPM. The size of the inflatable cuff for blood pressure measurement must be adjusted to the age of the children patient and the size of the arm. The Figure 1 shows the device for ABPM that we have used.

The objective of our work was to point out the importance of using ABPM in differential diagnosis in children patients in which blood pressure was repeatedly higher than normal values.

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Fig. 1. ABPM device
METHODS

Our series consisted of 77 patients, 60 boys and 17 girls, their age ranging from 12 to 19 years (the average age was 17.3). These children were usually sent to the cardiologic outpatient department because of the repeatedly (3 and more times) higher blood pressure for their age. The measurements were carried out by classical auscultatory method. As normal values of blood pressure we have taken Soergel graph and table of normal values (Soergel at al., The Journal of Pediatrics, 1997).

In all patients of the group:
1. detailed family history was taken
2. the patient’s blood pressure was taken by using the classical auscultatory method after 5 minutes of relaxing in sitting position
3. ECG with the use of a standard 12 lead placement was taken
4. ECHO was made
5. ABPM was made

Ambulatory blood pressure monitoring was performed with the Meditech ABPM-04. The monitor was applied to the nondominant arm and the patient and his/her parents were instructed to perform normal activities between measurements but to rest the arm at heart level during measurements (6). Monitors were programmed to measure blood pressure at 20-minute intervals during a day and 40-minute intervals at night. Daytime was defined as hours between 6:00 and 22:00 and nighttime as the hours between 22:00 and 6:00 (7). The monitor was removed the next day and the data were transferred to a personal computer and loaded into specialized software. Recordings were not included in our study if there were < 28 valid readings during the day or < 7 valid readings during the night. The phenomenon of white coat hypertension in our study was defined as:
1. mean 24-hour blood pressure less than Soergel-defined 95th percentile
2. less than 25 % of blood pressure during 24-hour period exceeding 95th percentile (8).

Our study was in the accordance with the ethical norms and the Helsinki’s Declaration (1983).

RESULTS

Records of 73 children patients were available for the study. All the patients had repeatedly elevated physician referral blood pressures (exceeded 95th percentile). The mean blood pressure was 139/86 mmHg.

ABPM has given us 3 kinds of results depending on the mean 24-hour blood pressure, mean daytime blood pressure and mean nighttime blood pressure, if these blood pressures have or have not exceeded 95th percentile of normal values for a specific age.
1. **Juvenile (essential) hypertension**

Most of the blood pressure measurements (more than 25 %) were higher than 95th percentile of normal values during the whole 24-hour period. But very important was that we have found the decrease of blood pressure during the nighttime. The Figure 2 presents ABPM record of a patient with the diagnosis of juvenile hypertension.

2. **Secondary hypertension**

The blood pressure in these patients was usually above 95th percentile during whole 24-hour period too but the nocturnal fall was not found. It is the basic diagnostic difference between juvenile and secondary hypertension, which we can find in ABPM record.

3. **White coat hypertension**

The measured values of blood pressure during 24-hour period we divided into two parts. The first one was at the beginning of the ABPM, when the blood pressure was still higher than 95th percentile of normal values. It was the period when the patient was in the presence of a physician. The second one was when the values of blood pressure were normal during the whole day-and nighttime (with the nocturnal fall). Sometimes we found 1-2 higher values of blood pressure during the second part, but it was usually then when the child was examined at school or had some other stress.

White coat hypertension (group A) was confirmed in 30 children (39%) 17 boys and 13 girls with the average age of 16.6 years. The ambulatory measured values of blood pressure were from 130 to 150 mmHg of systole and from 60 to 100 mmHg of diastole. But the values of blood pressure during ABPM were lower, from 100 to 130 mmHg of systole and from 50 to 70 mmHg of dia-

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**Figure 3.** ABPM record in a patient with the secondary renal hypertension in dialysis. The types of lines are the same as in Fig.2

**Figure 4.** ABPM record in white coat hypertension. The types of lines are the same as in previous figures.
stole. The group B consisted of the patients with systemic hypertension (juvenile or secondary hypertension).

<table>
<thead>
<tr>
<th>Table 1. Comparison of the values in the group A (white coat hypertension) to group B (juvenile or secondary hypertension)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group A</strong></td>
</tr>
<tr>
<td>Number of patients</td>
</tr>
<tr>
<td>Positive family history</td>
</tr>
<tr>
<td>BP in the outpatient dpt. (mmHg)</td>
</tr>
<tr>
<td>ABPM (mmHg)</td>
</tr>
</tbody>
</table>

**DISCUSSION**

White coat hypertension is a relatively common finding in patients with elevated clinical blood pressure. The specific numerical definitions vary, but the essential component of the diagnosis is an elevated clinical blood pressure above accepted normal levels, in association with a normal ABPM profile (9). Why is the diagnosis of white coat hypertension so important? It is connected with a pathological risk. However the risk associated with white coat hypertension is substantially lower than the risk associated with sustained hypertension, but higher than the risk of true normotensive patients (9).

ABPM has become more widely used in the assessment of elevated blood pressure in children. The accurate diagnosis of white coat hypertension is particularly important in children because the detection of elevated blood pressure often requires expensive and often also invasive diagnostic procedures to detect underlying disease, which could be sometimes very dangerous (kidney diseases and so) for a child (10).

39 % of all the children patients in our study had white coat hypertension. It is between 10% and 50%, which is the incidence of this phenomenon (1). But very interesting is that white coat hypertension has been more common in girls (13 of 17 girls with repeatedly measured higher blood pressure had in our study white coat hypertension and only 4 girls had systemic hypertension). The positive family history in group A was much the same as in group B, which is, in our opinion, a quite interesting information. We know that the family predisposition is very important etiologic factor in essential (juvenile) hypertension. So it seems that white coat hypertension could probably be the first stage of the development of essential hypertension. But we should test (take blood pressure or ABPM) the patients of our study for several years (5, 10, 15) whether they still will have white coat hypertension or they will pass to the group B (systemic hypertension).

Very important is the question of the treatment of children patients with white coat hypertension. It is very important especially in childhood, because if we mark that a child as ill, it could have bad effect. Its parents protect that child more than other, can forbid them to do several activities, his friends can reject them (because he is weaker, ill) and it is also bad for his/her mental maturing. So the doctor must be very careful what to say. Usually we recommend control examinations once a year. An additional method is the management of stress; it seems to be very good way in treatment of white coat hypertension.

In conclusion, we have shown that ABPM can completely, simply and sufficiently diagnose the white coat hypertension. Therefore, we suggest the non-invasive 24-hour measuring of blood pressure as the essential diagnostic method.
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3. Introduction should introduce into the problem and state the purpose of the article.

4. Methods should be complete to allow other workers to reproduce the results. Describe statistical methods. Indicate whether the procedure followed was in accordance with the ethical standards and with the Helsinki Declaration from 1975 as revised on 1983.

5. Results: Present your results in logical sequence in the text, tables and illustrations.

6. Discussion: Emphasize the new and important aspects of the study, link the conclusions with the presented goals of the study, relate the results to other relevant studies with a short summary of results at the end.

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