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FUNCTIONAL STUDIES ON SCIATIC NERVE BLOOD FLOW IN RESPECT TO ITS VASCULAR SUPPLY AND TONIC NEURAL ACTIVITY

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An introduction of laser flow meters for a continuous measurements of a tissue blood flow has opened new avenues for an accurate assessment blood flow in peripheral nerves. The aim of our study was: 1) to carry out a functional verification of anatomical sources of a sciatic nerve blood supply in the rat; 2) develop a measurement technique to facilitate standardisation of results; 3) to determine the role of nerve fibres tonic activity in the maintenance of a resting blood flow in the sciatic nerve. Based on results of the present study the following conclusions have been drawn out: 1) in order to obtain a real values of the blood flow through the sciatic nerve it is necessary to remove its muscular fascia; 2) an uninjured epineurium plays a crucial role in maintaining the resting blood flow; 3) major blood supply of sciatic nerve comes from inferior gluteal and popliteal arteries; 4) the tonic neural activity plays a role in the maintenance of the resting sciatic nerve blood flow in anaesthetised rats.

Key words: *sciatic nerve, blood flow, rat*

INTRODUCTION

Mechanisms regulating blood flow through the blood vessels supplying peripheral nerves remain largely unclear. A gap between anatomical and functional studies has been due to, among other things, a lack of measurement techniques which would allow continuous recording of blood flow through the peripheral nerves. The assessment of blood flow using 'microspheres' or hydrogen ion clearance did not permit continuous measurements of tissue blood flow, therefore were of limited usefulness in unsteady state conditions (1, 2). Implementation of laser flowmeters opened a new era in microvascular research (3, 4). The major advantage of this technique is possibility to get continuous read out of the magnitude blood flow in the tissue adjacent to the tip of the flow probe.

Because of an easy access and relatively big size, the sciatic nerve is frequently used in both morphological and functional studies. The majority of experimental studies on the pathogenesis of different kinds of neuropathies were performed on the sciatic nerve (5). A review of publications on the studies of the sciatic nerve blood flow (SNBF) in rats show that authors employed different procedures to dissect the sciatic nerve and also selected different sites of placement of laser flow probes (4, 6, 7). Therefore, it seems necessary to develop a standard technique which would allow to compare results from different laboratories. To approach this problem in the present study the following issues were addressed:

- the role of the epineurium in maintaining blood flow in rat's sciatic nerve,
- functional identification of the main vascular sources providing blood supply to the sciatic nerve,
- the role of tonic neural activity in maintaining the sciatic nerve blood flow in the anaesthetised rat.

MATERIALS AND METHODS

The study was carried out on 20 male Wistar rats with the body weight ranging from 300g to 350g., obtained from the animal house of the Medical University of Warsaw. Animals were anaesthetised with an intraperitoneal injection of chloral hydrate (36mg/kg body weight). The rats were artificially ventilated with a Harvard Rodent Ventilator (Model 638). Tidal volume and frequency of respirator were set under control acid base and gaseous composition of arterial blood obtained from catheter introduced into common carotid artery (AVL-Compact 2). The same catheter was used for continuous recording of arterial blood pressure (Temed MCK-4011S).

The rats were placed in the right lateral position. The skin incision was performed in the line between the greater trochanter and the knee. After exposure of the intramuscular space between the two heads of the biceps femoris muscle a blunt dissection was done with care to preserve the vascular network of the knee. Therefore neither vascular ligation nor coagulation was used at this step of surgical procedure. In order to provide less strain on the sciatic nerve, the thigh was abducted by 40 degrees. The muscles were divided and retracted and a segment of the sciatic trunk was exposed on the length of approximately 18 mm, from its tibial and fibular division up to the rim of the piriform muscle. The dissected nerve was covered by a thin layer of muscular fascia clearly visible only under the operating microscope. The measurement of the blood flow was done using a laser Doppler flowmeter (Alf 21 Transonic, USA). The flow probe of 0.8mm in diameter was placed under microscopic control on the sciatic nerve approximately 8 mm from the subpiriform opening. The flow probe was mounted in holder (KANETEC MP-BP) which provided its constant position thorough the observation period. The tissue was rinsed with 37°C Ringer's solution during the measurement period.

Four experimental procedures were performed on ten rats in the following order:

1. measurement of SNBF after the fascia covering the nerve was removed,
2. measurement of SNBF after removal of the epineurium,
3. measurement of SNBF after the proximal nerve dissection at the subpiriform opening,
4. measurement of SNBF after the distal nerve dissection, peripherally to the fibular and tibial divisions.

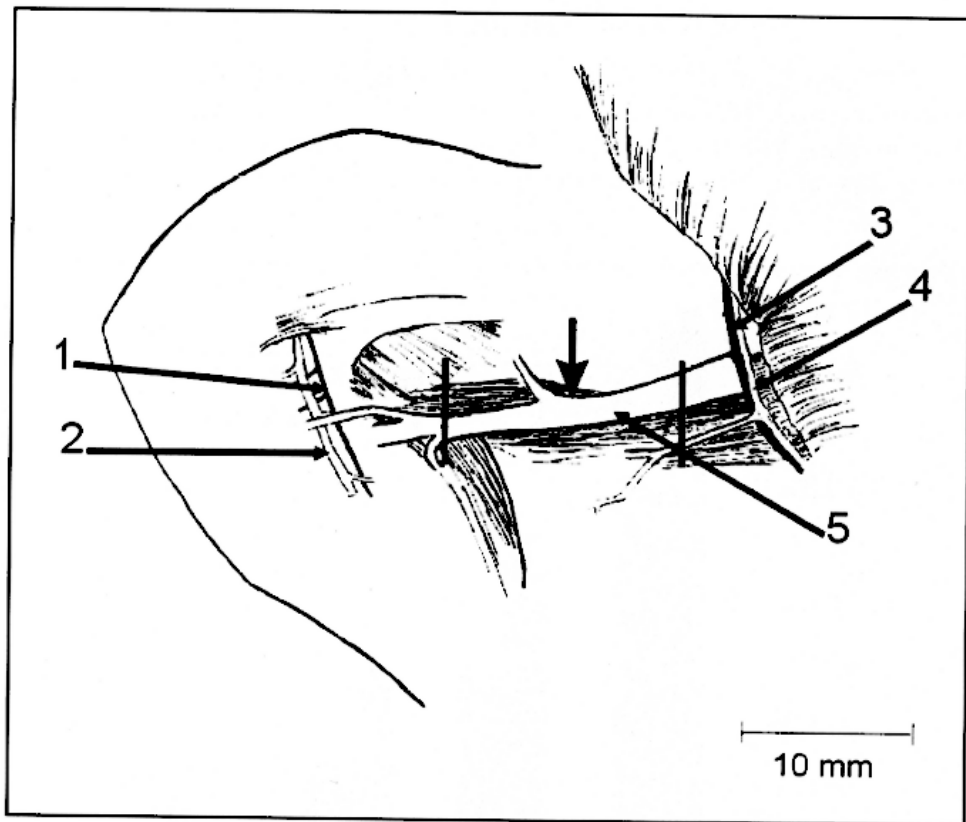


Fig. 1. Sketch presenting topography of the sciatic nerve in rat. 1. popliteal artery, 2. popliteal vein, 3. inferior gluteal artery, 4. inferior gluteal vein, 5. sciatic nerve, arrow — indicates the flow probe placement. Solid lines indicate the places of nerve dissection. in the order to keep clarity the network of vessels supplying sciatic nerve is not included.

The same procedures were applied to the other ten rats after local application of 2% lidocaine (Lignocain, Astra). Tiny swabs soaked with lidocaine were placed over the exposed sciatic nerve for 2 minutes. A complete blockade of nerve conduction was evidenced by absence of evoked electrical response in tibial muscle in response to electrical stimulus of the magnitude three times threshold at 2 Hz applied to the proximal end of sciatic nerve. The value of threshold voltage was established for each rat before placing the flow probe (Neuralog System USA). All parameters were recorded and stored in the computer acquisition system (AcqKnowledge System USA).

In the statistical analysis we used student-t test for the standard distribution to compare both rat groups, whereas, the ANOVA test was used for paired parameters to compare the results of subsequent experimental series. All data are presented as a mean \pm standard error of mean (SE).

RESULTS

The removal of the muscular fascia resulted in a significantly increased blood flow from 16.8 ml/min/ 100g body weight \pm 1.75 to 28.1 ml/min/ 100g body weight \pm 5.46 ($p < 0.01$). For the subsequent calculation the value of the blood flow after removing sciatic nerve fascia were treated as 100%.

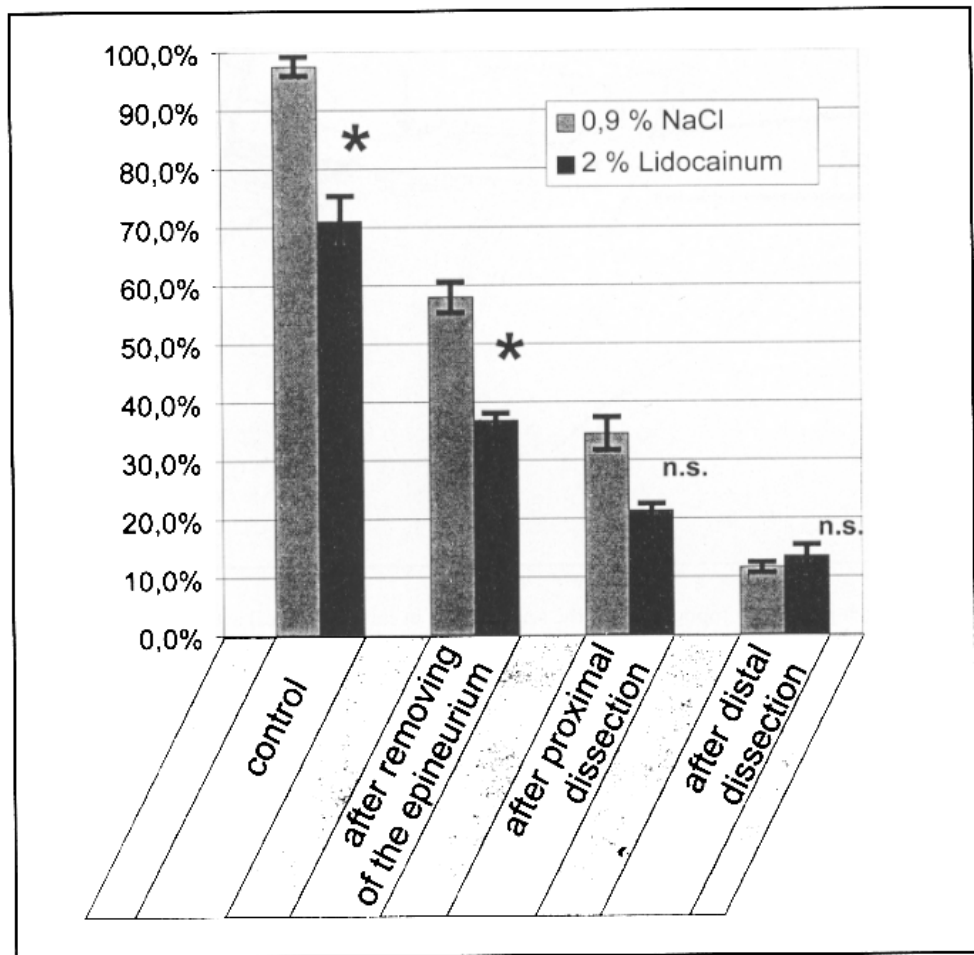


Fig. 2. The values of SNBF before and after lidokaine application in different experimental procedures. Asterix indicates statistical at $p < 0.01$.

The removal of epineurium caused a significant decrease of SNBF down to $58.0\% \pm 5.0\%$. The subsequent application of lidocaine caused further drop in SNBF to $36.8\% \pm 2.5$. The proximal and distal sciatic nerve dissection lead to

a drop of SNBF to the values of $34.5\% \pm 5.14$ and $11.6\% \pm 1.53$ respectively. The topical application of lidocaine did not produce significant changes in SNBF neither after proximal nor distal dissection of the sciatic nerve. Results are presented in *Fig. 2*.

DISCUSSION

Removing the thin muscular fascia which is clearly visible only under the operating microscope is a prerequisite for adequate measurement of SNBF with a laser flowmeter. We can assume that blood flow through sciatic nerve fascia is low as compared to that in nerve tissue. Therefore an increased blood flow after the removal of the above structure is the result of the elimination of a low flow zone. Since the laser flowmeter applied in the present study calculates blood flow from only 1 mm^3 of tissue, then even a tiny (low or high flow) compartment between the flow probe and the tissue of interest may be a source of a substantial error.

The reduction in the SNBF by 58% after the removal of the epineurium points that the majority of vessels supplying the sciatic nerve are penetrating from its surface (8, 9, 10). This finding does not exclude that in the sciatic nerve there are arteries running parallel to nerve fibres in deeper layers of the nerve trunk.

A decreased blood flow following the proximal dissection of the nerve in the subpiriform opening in order to eliminate the source of nerve blood supply stemming from the inferior gluteal artery and the distal dissection to eliminate blood supply from the popliteal artery, provides functional confirmation of the role of these blood vessels in the maintenance of an adequate blood supply of the rat sciatic nerve (10, 11). A reduced blood supply after a similar procedure was also demonstrated by Myers and Heckman (12). Anatomical studies have shown that blood supply of the sciatic nerve comes from numerous vessels arising from both inferior gluteal and popliteal arteries. These vessels are interconnected by abundant anastomoses. In addition, neither gluteal arteries nor popliteal ones are anatomically terminal. Therefore, from the technical point of view, vascular isolation of sciatic nerves by cutting main arteries or its branches is not feasible. For the purpose of the present study we applied simple technique to confirm anatomical data by assessing changes in SNBF after interrupting the continuity of the sciatic nerve at the described above locations.

The most conspicuous result of the present study was a decrease in SNBF after topical application of lidocaine. As lidocaine causes vasodilatation the above finding could not be due to its vascular action (12). The most likely

mechanism responsible for the decreased SNBF blood flow after topical application of lidocaine is the abolition of the tonic activity of the sciatic nerve fibres. Taking into account vascular action of local anaesthetics the observed decrease in SNBF was underestimated. The decrease in SNBF after lidocaine application points that in the sciatic nerve, similarly to another tissues, blood flow is closely related to their metabolic activity. It is reasonable to assume that an increased activity of sciatic nerve fibres would result in augmented SNBF. Our preliminary data indeed substantiated this assumption. The application of a nociceptive stimulus into palmar surface of the foot was immediately followed by an increase in ipsilateral SNBF (13). The mechanism responsible for coupling SNBF with the activity of a given population of fibres is totally unknown. We may say that the regulation of blood flow in nerves still awaits its appreciation.

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