

MOTOR DYSFUNCTION AND REFLEX SYMPATHETIC DYSTROPHY

BILATERAL MOTOR DENERVATION IN AN EXPERIMENTAL MODEL

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Reflex sympathetic dystrophy (RSD) is a neuropathic pain condition most often occurring in relation to trauma to, or surgery on, an extremity. It is characterized among other things by motor disturbances such as joint stiffness and tremor. Signs and symptoms can be induced in a rat model through chronic constriction of a sciatic nerve (CCI-model). In this study the CCI-model was used to evaluate the extent of bilateral peripheral motor nerve-fiber involvement in relation to ligature localization. In 12 Lewis rats, the common sciatic nerve was loosely ligated with four chromic catgut ligatures at the midhigh level just proximal to the right sciatic trifurcation. Acetylcholinesterase (CE) histochemistry of sciatic (distal and proximal to ligation) and corresponding contralateral nerve biopsy specimens was performed at 21 days after ligation. An additional 12 rats were sham-operated and served as controls. As compared to sham-operated controls or contralateral nonligated sciatic nerves, CE histochemistry after 21 days revealed a marked decrease of CE-positive fibers in cross-sections taken from distal and proximal sciatic nerve biopsies ipsilateral to the ligatures. In addition, as compared to sham-operated controls, there was a decrease of CE-positive fibers in cross-sections taken from contralateral nonligated sciatic nerves. The present findings indicate profound motor denervation, distal as well as proximal to the ligatures. Motor denervation also affected the contralateral nonligated sciatic nerve. The evident usefulness of the CCI-model for the study of RSD places the present results in line with the concept of central nervous system involvement in the pathophysiology of RSD.

Keywords: reflex sympathetic dystrophy; algodystrophy; acetylcholinesterase; sciatic nerve; motor denervation.

Mots-clés: dystrophie réflexe sympathique; algodystrophie; acétylcholinestérase; nerf sciatique.

INTRODUCTION

Abnormal motor function (i.e., joint stiffness, tremor) and its relation to neuropathic pain is a widely discussed topic in reports concerning the clinical characteristics of the patient suffering from reflex sympathetic dystrophy (RSD) (28). After the onset of RSD (most often because of trauma or surgery to an extremity), signs and symptoms can become manifest not only in the injured extremity but also in the noninjured contralateral extremity (21, 24-26). The latter has been reported with respect to roentgenologic manifestations of distinct patchy osteoporosis and histological findings (15, 16). Our group recently demonstrated bilateral involvement with reference to autonomic disturbances in RSD patients (20).

Animal models for the study of neuropathic pain conditions such as RSD have gained much research interest and have been extensively val-

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idated in the last few years (4, 9, 14). In 1988, Bennett and Xie (3) described a rat model of partial peripheral nerve injury (chronic constriction injury (CCI)) in which abnormalities reminiscent of those observed in human conditions such as RSD (hyperalgesia, allodynia, dystonic posture, abnormal gait and atrophic changes of muscles in the affected extremity) could be provoked by loosely ligating the common sciatic nerve. Substantial morphological changes in the sciatic nerve induced by such ligature placement have been observed (2, 6, 10). These studies indicate that the ligation procedure causes severe axonopathy with simultaneous degeneration and regeneration mainly distal, but also proximal, to the lesion. Clearly, denervation associated with this axonopathy plays an important role in at least the development of autonomic dysfunction in the CCI-model. It is also evident that in the CCI-model a bilateral distribution of sensory abnormalities exists (1), in line with many observations in RSD-patients (21, 24).

The present study was undertaken in the CCI-model to determine the effect of ligature placement on axonal cholinesterase (CE) content as a measure of motor innervation by employing histochemical methods according to Karnovsky (8, 12). The extent of peripheral motor-nerve fiber involvement in relation to ligature localization in the sciatic nerve as well as in the nonligated contralateral sciatic nerve was determined to evaluate the bilateral distribution of motor abnormalities, if any indicative of central nervous system involvement.

MATERIALS AND METHODS

Twenty-four male Lewis rats, weighing approximately 250 g at the time of operation, were anesthetized with sodium pentobarbital (50 mg/kg I.P.). In an experimental group (n = 12), under aseptic conditions the right common sciatic nerve was exposed and four loose 4/0 chromic gut ligatures were placed just proximal to the sciatic trifurcation. In a sham group (n = 12) the right common sciatic nerve was exposed but not ligated. Treatment of animals conforms to International Association for the Study of Pain Ethical Guidelines (29). The animals used showed behavioral signs associated with the CCI-model such as eversion, foot

drop and licking of the affected hindpaw. No such behavioral changes were observed in sham-operated rats. In both groups animals were sacrificed and tissue samples were taken 21 days after ligation. At the time of sacrifice, sodium pentobarbital anesthesia (70 mg/kg I.P.) was induced, and sciatic nerve segments were taken just distal and proximal to the site of ligature placement. An additional corresponding nerve biopsy was taken from the contralateral nonligated sciatic nerve (fig. 1).

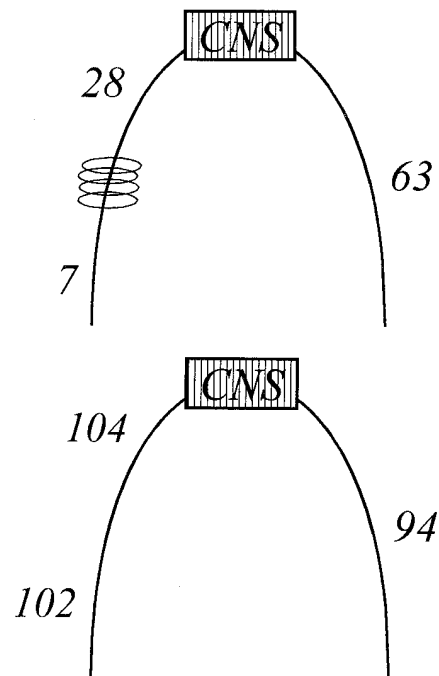


Fig. 1. — Schematic representation of nerve biopsy locations in sciatic nerves relative to the central nervous system (CNS). The numbers indicate the amount of CE-positive axons found at each location. The upper drawing represents the ligation group. The lower drawing represents the sham-operated group.

All tissue samples were frozen immediately in isopentane cooled in liquid nitrogen at -160°C . The nerve segments were cross-sectioned ($9\ \mu\text{m}$) with a cryostat microtome, and CE activity, primarily specific to myelinated motor axons, was demonstrated by the method of Karnovsky (12). The incubation medium, containing 3.0 mM acetylthiocholine and 0.6 mM iso-OMPA for nonspecific cholinesterase inhibition, was adjusted to pH 5.5 and sections were incubated for 1 h at 37°C .

Quantification of total number of CE-positive fibers per cross-sectioned nerve was performed with the aid of a semiautomated (Leica Q570C) image analyzer by

an independent investigator. Data were expressed as medians with interquartile ranges. All experimental data were compared with data obtained from sham-operated controls or contralateral side using the Mann-Whitney-U test or the Wilcoxon signed-rank test respectively for nonparametric statistics. Values of $p < 0.05$; $p < 0.01$ and $p < 0.001$ were taken to denote statistical significance.

RESULTS

Results are shown in fig. 1 and fig. 2. The total number of CE-positive fibers in sciatic nerve segments taken distally to the ligatures was decreased at 21 days after ligation when compared to sham-operated controls (7 vs. 102; $p < 0.001$) or to the contralateral nonligated side (7 vs. 63; $p < 0.01$). Sciatic nerve cross-sections from nerve segments taken proximally to the ligatures when compared to sham-operated controls also showed decreased numbers of CE-positive fibers (28 vs. 104, $p < 0.001$) after ligation. The total number of CE-positive fibers in nerve segments taken from ligated animals from the contralateral nonligated sciatic nerve was decreased (63 vs. 94; $p < 0.01$) when compared to sham-operated controls.

DISCUSSION

Histochemical staining for CE demonstrated a loss of motor fibers in the distal as well as in the proximal segment of the experimentally-injured sciatic nerve at 21 days after ligature placement. These findings are consistent with previous reports demonstrating extensive degeneration of large-diameter fibers distal as well as proximal to the ligatures (2, 10). Besides, in the contralateral nonligated sciatic nerve we found a significant reduction in the number of motor fibers. Since functional and morphological status of skeletal muscle both depend on motor innervation (13, 27), motor denervation, besides sensory abnormalities, may contribute to the observed functional impairment of muscles innervated by the ligated sciatic nerve. In addition, denervation pathology in general can underlie other peripheral manifestations associated with ligature placement such as the microcirculatory dysfunction responsible for temperature dysregulation of the affected extremity (17, 18).

In previous communications we reported upon the involvement of the injured peripheral nerves

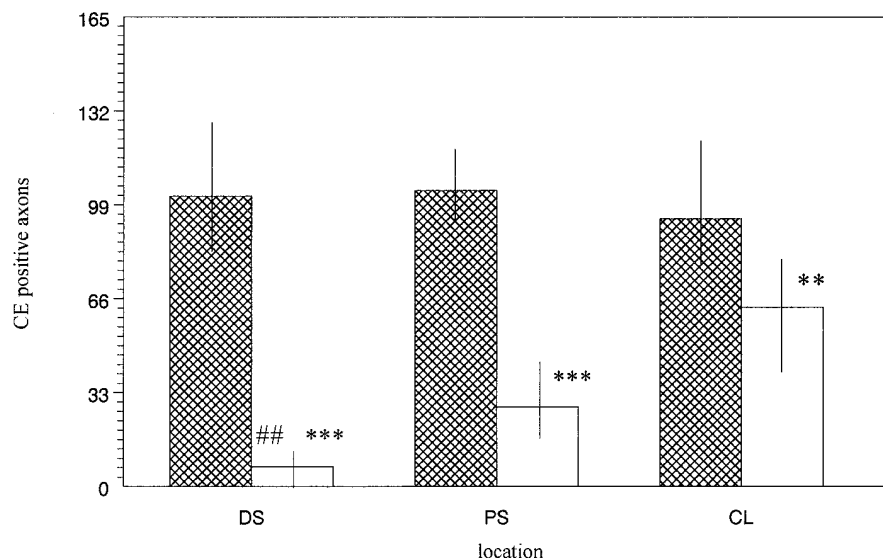


Fig. 2. — Graphic representation of the distribution of CE-positive axons in ipsilateral distal sciatic (DS), proximal sciatic (PS) and contralateral (CL) nerve segments as medians with interquartile ranges. Double-hatched bars represent the amount of CE-positive axons determined in specimens ob-

tained from the sham-operated control group. Open bars represent the ligation group. Symbols are as follows: ** $p < 0.01$ as compared to controls; *** $p < 0.001$ as compared to controls; ### $p < 0.01$ as compared to the contralateral side.

as well as the opposing peripheral nerves in modulating autonomic dysfunction in RSD and in the CCI-model (19, 20, 24). The present study raises suspicion of a similar process with reference to motor dysfunction suggesting the involvement of the central nervous system. Behavioural change of an intact nerve after injury to the contralateral homologous nerve has been reported repeatedly, and several mechanisms have been proposed such as neurophysiologic changes induced by the contralateral injury by transsynaptic signalling (7, 22, 23) and alterations in quality and quantity of trophic substances and exogenous materials carried to the contralateral side by means of axoplasmatic transport (5, 22, 23). Indeed, in the CCI-model the N-methyl-D-aspartate (NMDA) receptor is down-regulated in the dorsal and ventral regions of the spinal cord inducing enhanced susceptibility to excitotoxic injury mediated by barrages of excitatory amino acids released by the injured peripheral nerve (11).

It has been demonstrated by Attal *et al.* (1) that the injured as well as the contralateral noninjured sciatic nerve both modulate sensory dysfunction. The present study indicates a similar process with reference to motor dysfunction suggesting involvement of the central nervous system. Besides, using this well-accepted animal model of neuropathic pain, these data make it tempting to hypothesize that motor denervation is an important pathophysiological process involved in the bilateral distribution pattern of signs and symptoms often seen in patients suffering from neuropathic pain conditions such as RSD.

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SAMENVATTING

P. BULLENS, M. DAEMEN, G. FRELING, P. KITSLAAR, F. VAN DEN WILDENBERG, H. KURVERS. Motordysfunctie en RSD. Bilaterale motorische denervatie in een experimenteel model.

Reflex sympathische dystrofie (RSD) is een neuropathisch pijnsyndroom dat frequent trauma van, of operatieve ingrepen aan extremiteiten compliceert. Het syndroom wordt onder andere gekenmerkt door motorische klachten waaronder gewrichtsstijfheid en tremor. De verschijnselen van RSD kunnen experimenteel worden geïnduceerd in een diermodel door vier losse ligaturen aan te brengen rond de n. ischiadicus van de rat (CCI-model). Deze studie beoogt in het CCI model te bestuderen in welke mate er sprake is van bilaterale betrokkenheid van perifere motorneuronen in relatie tot de locatie van de aangebrachte ligaturen. CCI werd geïnduceerd in 12 Lewis ratten middels aanbrengen van 4 losse catgut ligaturen rond de n. ischiadicus ter hoogte van het midden van het dijbeen. Acetylcholinesterase (CE) histologie werd 21 dagen na liggeren verricht op sciatale axonale dwarsdoorsneden (distaal en proximaal van de ligaturen) evenals op corresponderende contralaterale zenuwbipten. Een tweede groep van 12 ratten onderging een sham operatie en diende als controlegroep. Op dag 21 na liggeren nam het aantal CE-positieve axonen af in bipten genomen distaal, alsook proximaal van de ligaturen, zowel vergeleken met sham-geopereerde dieren als vergeleken met de niet-geligeerde zijde. Bovendien was het aantal CE-positieve axonen in sciatale bipten afgenomen contralateraal van de geligeerde n. ischiadicus gereduceerd, vergeleken met het aantal CE-positieve axonen in bipten afkomstig uit sham-geopereerde dieren. De huidige bevindingen wijzen op de aanwezigheid van motorische denervatie zowel distaal als proximaal van de ligaturen. Van motorische denervatie was eveneens sprake in de contralaterale niet-geligeerde n. ischiadicus. Omdat het CCI een gevalideerd model is, ondersteunen deze resultaten de hypothese dat het centrale zenuwstelsel een belangrijke rol speelt bij de pathologie van RSD.

RÉSUMÉ

P. BULLENS, M. DAEMEN, G. FRELING, P. KITSLAAR, F. VAN DEN WILDENBERG, H. KURVERS. Dystrophie réflexe sympathique et troubles

moteurs. Modèle expérimental de dénervation motrice bilatérale.

La dystrophie réflexe sympathique est une affection clinique douloureuse neuropathique, survenant le plus souvent après un traumatisme ou une intervention chirurgicale au niveau d'un membre. L'affection est caractérisée notamment par des troubles moteurs, comme un enraidissement articulaire ou un tremblement. Une symptomatologie similaire peut être induite chez le rat après ligature chronique du nerf sciatique. Dans la présente étude, le modèle de ligature du sciatique a été utilisé pour évaluer l'importance de l'atteinte bilatérale motrice périphérique en fonction du niveau de ligature. Le nerf sciatique a été ligaturé lâchement à mi-cuisse chez 12 rats Lewis par 4 fils de catgut chromé, juste en amont de la trifurcation sciatique droite. Une histochimie à l'acétylcholinestérase du nerf sciatique, proximale et distale par rapport

à la ligature, et une biopsie nerveuse controlatérale de contrôle ont été réalisées 21 jours après la ligature. Douze rats ayant bénéficié d'un abord chirurgical sans ligature du sciatique ont été utilisés comme contrôles. L'examen histochimique a démontré une augmentation importante des fibres positives pour l'acétylcholinestérase au niveau des biopsies nerveuses réalisées proximale et distale par rapport à la ligature. De plus, par rapport aux contrôles, une diminution des fibres positives pour l'acétylcholinestérase a été retrouvée au niveau du nerf sciatique controlatéral, n'ayant pas subi de ligature. Ces observations suggèrent l'existence d'une dénervation motrice profonde, distale aussi bien que proximale aux ligatures, et affectant également le côté controlatéral non lié. Ces résultats vont dans le sens du concept d'une atteinte du système nerveux central dans la physiopathologie de la dystrophie réflexe sympathique.