PARTIAL PERIPHERAL NEUROPATHY AND DENERVATION INDUCED ADRENOCEPTOR SUPERSENSITIVITY

FUNCTIONAL STUDIES IN AN EXPERIMENTAL MODEL

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Sciatic nerve ligation in rats (chronic constriction injury (CCI)) induces clinical signs and symptoms that mimic human conditions of neuropathic origin, such as reflex sympathetic dystrophy (RSD). Denervation-induced supersensitivity to (circulating) catecholamines has been implicated in sympathetic dysfunction in the CCI model as well as in RSD. In the present paper we studied functional properties of sympathetic innervation in subcutaneous resistance arteries, isolated from the hind paw of rats 3 weeks after ligation. Contractile responses to electric field stimulation of adrenergic nerves and exogenously administered cumulative doses of various adrenergic agonists were studied using a myograph.

As compared to the contralateral side, subcutaneous arteries from the ligated side were less responsive to electrical field stimulation. Besides, as compared to the contralateral side, subcutaneous arteries from the ligated side showed increased sensitivity to α_1-adrenoceptor stimulation.

This study demonstrates that sympathetic dysfunction in an experimental model of neuropathic pain consists of denervation-induced supersensitivity to catecholamines rather than of an afferently-induced increase in efferent sympathetic nerve impulses.

Keywords : reflex sympathetic dystrophy ; catecholamines ; denervation.
Mots-clés : dystrophie réflexe sympathique ; catécholamines ; dénervation.

INTRODUCTION

The chronic constriction-injury (CCI) model has proven to be useful as a model for clinical conditions of neuropathic origin, such as reflex sympathetic dystrophy (RSD) (3, 23). Among others, this type of nerve injury induces autonomic dysfunction, characterized by sympathetically maintained sensory abnormalities (13, 17) and trophic changes (3), as well as abnormalities in skin temperature (4, 24) and skin blood flow (14).

The precise nature of autonomic dysfunction in experimental animal models of neuropathic pain remains obscure. Because surgical or chemical sympathectomy may alleviate sensory abnormalities and because it improves skin blood flow in RSD patients (5, 25) as well as in experimental animal models of neuropathic pain (4, 17), sympathetic dysfunction has been suggested to consist of an increase in efferent sympathetic nerve impulses. Some recent studies however, demonstrated evidence of autonomic denervation in the affected extremity, both in the CCI model (24) and in RSD patients (8). Sympathetic denervation of vasculature increases the catecholamine sensitivity (21). Therefore, denervation-induced supersensitivity to (circulating) catecholamines is a likely candidate to precipitate sympathetic dysfunction in RSD patients as well as in the CCI model.

Functional aspects (19) of sympathetic innervation as well as sensitivity to adrenergic agonists (18) can be investigated adequately in subcutaneous arteries. This prompted us to study these aspects

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in arteries that had been isolated from the hind paws of rats 3 weeks after sciatic nerve ligation. In a myograph, we recorded contractile responses to stimulation of adrenergic nerves (functional density) and exogenously administered agonists (adrenoceptor function).

MATERIALS AND METHODS

Chronic Constriction Injury

The experiments were performed on subcutaneous arteries isolated from 15 male Lewis rats (local inbred, University Maastricht, The Netherlands), weighing 250-350 grams at the start of the experimental protocol. At this point, the animals were anesthetized with 100 mg/kg ketamine hydrochloride intraperitoneally (Nimatek, AUV, Cuijk, The Netherlands) and 0.5 mg diazepam subcutaneously (Centrafarm BV, Etten-Leur, The Netherlands). The right sciatic nerve was exposed under aseptic conditions from high-thigh to mid-thigh level, just proximal to the trifurcation into the tibial, sural, and peroneal nerves. To this end, a dissecting microscope (25 × magnification) was used. In a first group of 10 rats (ligated group) the sciatic nerve was loosely ligated with 4 chromic catgut ligatures (4-0, Ethicon, Norderstedt, Germany), just proximal to the trifurcation. The spacing between these ligatures was about 1 mm (3). On the contralateral (left) side, the sciatic nerve was exposed, but not ligated. In the remaining 5 rats (control group), we performed only a sham-procedure during which the right sciatic nerve was exposed, but not ligated. The wounds were closed with mersilene muscle sutures (5-0, Ethicon) and mersilene skin sutures (2-0, Ethicon). All experimental studies were carried out under a protocol approved by the Institutional Animal Care Committee of the University of Maastricht, The Netherlands.

Myograph studies

Three weeks after sciatic nerve ligation, the animals were killed by a sharp blow on the neck and exsanguinated. Immediately after killing the animals, we isolated from the plantar surface of the hind paw the most laterally located subcutaneous artery. In the ligated group this was done in both hind paws and in the control group in the left hind paw. The isolated artery is innervated by the tibial nerve, which originates from the sciatic nerve (22). Thereupon the following experiments were performed :

In order to examine mechanical properties, the isolated artery segment was mounted on two stainless-steel wires with a diameter of 40 μm and placed horizontally in a myograph (volume 10 ml) between an isometric force transducer (Kistler Morse DSC 6, Seattle, Washington, U.S.A.) and a displacement device (Mitutoyo, Tokyo, Japan) for recording of isometric force development (7). The organ chamber was filled with Krebs-Ringer bicarbonate solution (KRB, composition in mM : NaCl 118.3, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 0.7, H_2O 1.2, $NaHCO_3$ 25.0, and glucose 5.5), which was maintained at 37 °C and gassed with 95% O_2 / 5% CO_2. Before experimentation, all arteries were distended to their individual optimal lumen diameter, i.e. that yielding the maximal force development, by stepwise increases in diameter with intermittent exposure to 5-HT (10 μM). Subsequent experimentation was performed at this optimal diameter.

Next, contractile responses to electrical field stimulation (EFS) were evaluated. Therefore, two platinum electrodes were placed along the longitudinal axis of the isolated vessel preparations. These electrodes were connected to a constant current source (amplitude 85 mA) delivering electrical impulses of constant duration (2 msec) at a variable frequency (0.25 to 32 Hz). These conditions of electrical field stimulation (EFS) have been reported to selectively stimulate intramural nerves in isolated blood vessels (10). In line with this, it was observed in preliminary experiments that EFS induced frequency-dependent contractions that could be abolished by 1 μM prazosin, 1 μM guanethidine or 1 μM tetrodotoxin as well as by pretreatment with 0.3 mg/ml 6-hydroxydopamine. Moreover, EFS applied in the presence of guanethidine (a procedure that is routinely used to evaluate perivascular sensory motor nerve function (12)) failed to reduce contractile responses induced by phenylephrine, endothelin, prostaglandin F_2 or vasopressin. Collectively these observations indicate that vascular responses to EFS result exclusively from stimulation of intramural adrenergic nerves.

In the following experiment, contractile responses to cumulative doses of various adrenergic agonists were assessed. Concentration-response curves were obtained by increasing the concentrations in half-log increments. Sensitivities to the agonists ($pD_2 = -\log EC_{50}$, where the EC_{50} is the agonist concentration needed to produce 50% of the maximal response) were calculated by interpolation on a logistic curve fit of the individual concentration response curves (Graphpad Inplot 3.01, Institute for Scientific Information, San Diego, CA, USA). Concentration response curves (10 μM - 30 μM)

were constructed for the α_1-selective agonist phenylephrine, the nonselective adrenoceptor agonist noradrenaline and the α_2-agonist B-HT 933 (azepexole). The ratio of the maximal response to EFS and the maximal response to noradrenaline was calculated as a functional measure of adrenergic innervation.

Statistics

In the ligated group, all experimental data from the ligated side were compared with those from the contralateral side. In addition, we compared contractile responses (to adrenergic agonists and EFS) of the left hind paw of the ligated group with those from the left hind paw of the control group. This was done in order to exclude the possibility that loose sciatic nerve ligation alters vascular reactivity in the contralateral hind paw. Statistical significance of differences was evaluated by Student's t test for paired or unpaired observations. The Pearson correlation coefficient was determined to assess the strength of linear association between maximum contractile responses to electrical field stimulation and sensitivity to noradrenaline. The value of $p < 0.05$ was taken to denote statistical significance.

RESULTS

The optimal lumen diameter at which maximal contractile responses to 5-HT were obtained did not differ, either between the ligated and nonligated side in the ligated group (269 ± 9 vs 275 ± 10 μm), or between the contralateral nonligated and the control group (275 ± 10 vs 273 ± 25 μm). Maximal contractile responses to 5-HT (10 μM) also did not differ between these groups (ligated 5.43 ± 0.28 ; contralateral nonligated 5.28 ± 0.28 and control 4.59 ± 0.45 mN/mm).

The stimulation parameters used during EFS (range 0.25-32 Hz) elicited distinct contractions during the course of each experiment (Fig. 1). In the ligated group, these responses for the whole frequency range were decreased on the ligated side when compared with the nonligated side (3.70 ± 0.66 vs 5.67 ± 0.32 mN/mm at 32 Hz, $P < 0.05$). No differences were observed in maximal contractile responses to EFS between arteries obtained from contralateral nonligated and control extremities (5.67 ± 0.32 vs 4.85 ± 0.57 mN/mm). In the ligated group, the ratio of maximal responses to

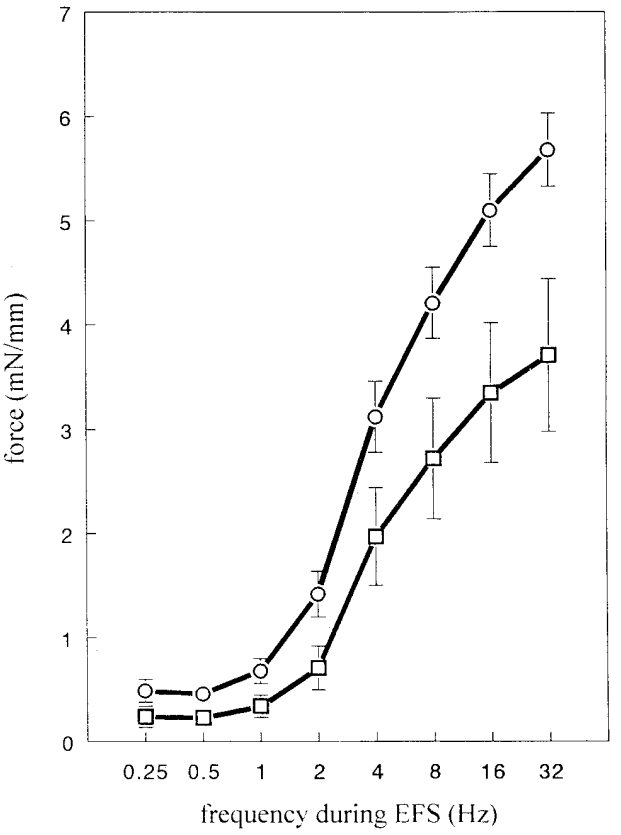

Fig. 1. — Frequency-force relations during electrical field stimulation (EFS) in rat arteries from the ligated side (open square) and nonligated side (open circle). Increases in wall tension are shown as mean values ± SEM (n = 10).

EFS divided by the maximal responses to noradrenaline was also reduced on the ligated side, when compared with the nonligated side (0.95 ± 0.03 vs 0.61 ± 0.21, $p < 0.05$). No differences in this ratio were observed between the contralateral nonligated side and controls (0.61 ± 0.21 vs 0.94 ± 0.05).

All arteries demonstrated concentration-dependent contractions in response to the α_1-adrenergic agonist phenylephrine. Figure 2 shows that in the ligated group, the sensitivity for phenylephrine was increased on the ligated side, when compared with the nonligated side (pD_2 6.24 ± 0.09 vs 6.01 ± 0.08, $p < 0.05$), whereas no differences were observed in the maximal responses induced by phenylephrine (5.68 ± 0.37 vs 5.47 ± 0.23 mN/mm). No differences were observed between ar-

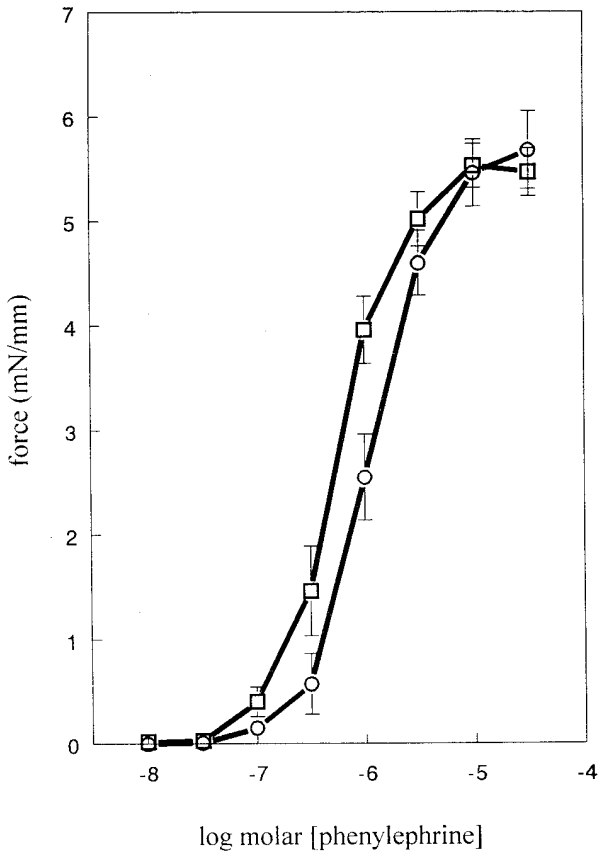

Fig. 2. — Concentration-force relations for phenylephrine in rat arteries from the ligated side (open square) and nonligated side (open circle). Increases in wall tension are shown as mean values ± SEM (n = 10).

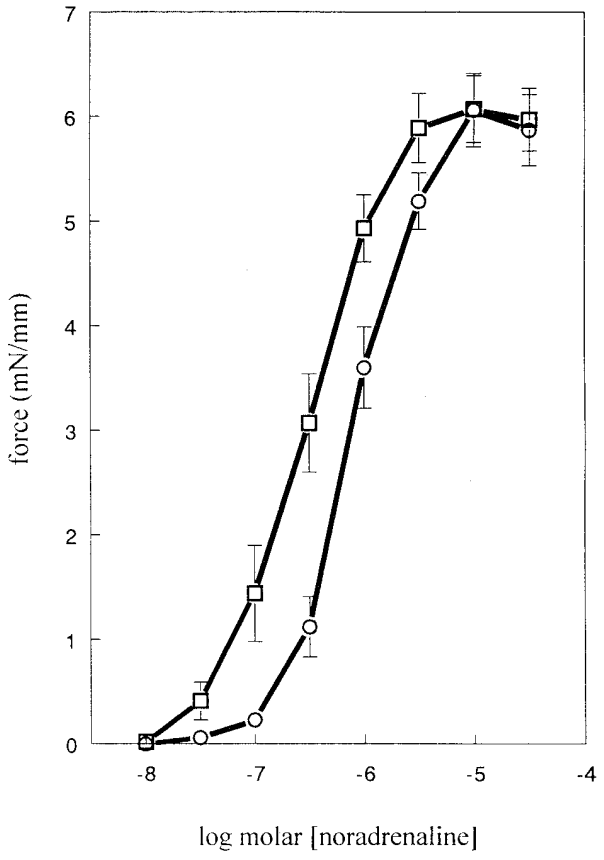

Fig. 3. — Concentration-force relations for noradrenaline in rat arteries from the ligated side (open square) and nonligated side (open circle). Increases in wall tension are shown as mean values ± SEM (n = 10).

teries obtained from contralateral nonligated sides and controls, either in sensitivity for (pD_2 6.01 ± 0.08 vs 5.81 ± 0.09) or in maximal responses elicited by phenylephrine (5.47 ± 0.23 vs 5.03 ± 0.65 mN/mm). The α_2-selective agonist B-HT 933 did not evoke contractile responses in arteries of either the ligated or the control group.

In all arteries, concentration-dependent contractions were evoked by noradrenaline. Since no contractile responses to the α_2-selective agonist B-HT 933 were observed, it is likely that these contractile responses reflect activation of α_1-adrenoceptors. As is shown in fig. 3, in the ligated group the sensitivity for noradrenaline was increased on the ligated side, when compared with the nonligated side (pD_2 6.56 ± 0.14 vs 6.11 ± 0.07, $p < 0.01$), whereas no differences were observed in the maximal forces induced by noradrenaline (5.97 ± 0.30 vs 5.87 ± 0.34 mN/mm). No differences were observed between the contralateral nonligated and control sides, either in sensitivity for (pD_2 6.11 ± 0.07 vs 6.08 ± 0.18) or maximal response induced by noradrenaline (5.87 ± 0.34 vs 5.69 ± 0.49 mN/mm). A significant negative correlation was observed between pD_2 values for noradrenaline and maximal contractile responses to electrical field stimulation on ligated sides (n = 10, $p < 0.01$, R = -0.72), but not on contralateral nonligated sides (n = 10, R = -0.46) or in controls (n = 5, R = -0.01).

DISCUSSION

The present findings are in favor of the concept that sympathetic dysfunction in the chronic constriction-injury (CCI) model involves denervation-induced supersensitivity to catecholamines. Functional sympathetic denervation of arteries on the ligated side is indicated by the reduced contractile responses to electrical field stimulation. This sympathetic denervation may be related to degeneration of small unmyelinated axons in the loosely ligated sciatic nerve, which has been reported to vary from 20 % (16) to as much as 84 % (6).

Since no dissimilarities were observed in contractile responses to adrenergic agonists and electrical field stimulation between arteries from the left side of control rats and those from the nonligated left side of ligated rats, the latter is an adequate control for studies on reactivity of subcutaneous arteries on the ligated side.

Control arteries from the plantar surface of the rat hind paw contracted in response to phenylephrine but not to B-HT 933, which is suggestive for the presence of functional postjunctional α_1-adrenoceptors but not of α_2-adrenoceptors. Besides, postjunctional (adaptive) sensitivity to catecholamines is increased in arteries on the ligated side when compared to the nonligated side, as indicated by contractile responses to the α_1-adrenoceptor agonist phenylephrine.

It has been demonstrated previously that sympathetic denervation of vascular structures may increase sensitivity to catecholamines (2, 21). Hence, the development of supersensitivity to catecholamines may be a consequence of sympathetic denervation as observed on the ligated side in our study. This hypothesis is justified by the observed negative correlation between maximal contractile responses during electrical field stimulation and sensitivity to noradrenaline.

It is likely that, beside the vasculature, other peripheral structures are subjected to autonomic denervation. Hence, one would be able to hypothesize that denervation-induced supersensitivity is present in sensory nerve fibers as well (20). Nociceptors would become more excitable and interact directly with (circulating) noradrenaline, which may explain why nociception in clinical conditions of neuropathic pain (5, 20), as well as in experimental animal models (13, 17), is often increased after sympathetic stimulation. The finding that in humans sympathectomy alone may induce spontaneous pain (15) confirms this hypothesis.

The notion of adrenoceptor supersensitivity to catecholamines leads to the hypothesis that blockades of (supersensitive) adrenoceptors in turn have beneficial effects on sensory and skin blood flow abnormalities. The latter is supported by the finding that administration of phentolamine lessens signs of allodynia in a rat model of neuropathic pain (13). Beneficial effects on sensory abnormalities in humans with neuropathic pain have been reported after administration of adrenoceptor antagonists such as phentolamine (1) or phenoxybenzamine (9, 11). A benefit of adrenoceptor blocking over sympathectomy would be that the effectiveness of the latter may be negatively influenced by a further increase in (super-) sensitivity to noradrenaline. Besides, in cases where (partial) sympathetic denervation is already present as a result of the initiating event (most often peripheral nerve trauma), one may expect few propitious effects of a sympathectomy, whereas blockade of the hypersensitive adrenoceptors may still be expedient.

In conclusion, the present study indicates that loose ligation of a sciatic nerve in rats brings on denervation-induced arterial supersensitivity to catecholamines rather than an afferently-induced increase in efferent sympathetic nerve impulses. As the animal model we have used has become extensively validated in recent years, similar pathophysiological mechanisms may account for human conditions associated with sympathetically maintained sensory dysfunction such as RSD.

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SAMENVATTING

H. KURVERS, M. DAEMEN, D. SLAAF, F. STASSEN, F. VAN DEN WILDENBERG, P. KITSLAAR, J. DE MEY. Reflex sympathische dystrofie en denervatie geïnduceerde adrenoceptor supersensitiviteit.

Ligatie van de nervus ischiadicus middels aanbrengen van vier losse catgut ligaturen (chronic constriction injury - CCI) induceert in de rat symptomen welke overeenkomsten vertonen met klinische neuropathische pijn zoals reflex sympathische dystrofie (RSD). Eerder werd geïmpliceerd dat denervatie geïnduceerde supersensitiviteit voor (circulerende) catecholamines betrokken is bij de sympathische dysfunctie welke prominent aanwezig is in zowel het CCI model als bij RSD.
De huidige studie beoogt enkele functionele eigenschappen van sympathische innervatie in subcutane weerstandsarteriën, geïsoleerd uit de achterpoot 3 weken na

ligatie, te evalueren. Contractiele reponsen tengevolge van stimulatie van adrenerge zenuwen in een elektrisch veld, alsook exogene applicatie van cumulative doses adrenerge agonisten werden bestudeerd met behulp van een myograaf.
Vergeleken met de contralaterale niet-geligeerde achterpoot, vertoonden subcutane arteriën geïsoleerd uit de geligeerde achterpoot een verminderde respons op stimulatie in het elektrisch veld. Bovendien waren deze arteriën toegenomen gevoelig voor α_1-adrenoceptor stimulatie vergeleken met de contralaterale zijde.
Deze resultaten tonen aan dat sympathische dysfunctie in het gebruikte model voor neuropathische pijn veroorzaakt wordt door adrenoceptor supersensitiviteit voor catecholamines in plaats van een vaker verondersteld mechanisme van afferent-geïnduceerde toename in efferente sympathische zenuwactiviteit.

RÉSUMÉ

H. KURVERS, M. DAEMEN, D. SLAAF, F. STASSEN, F. VAN DEN WILDENBERG, P. KITSLAAR, J. DE MEY. Algodystrophie réflexe sympathique et hypersensivité aux catécholamines induite par la dénervation. Modèle expérimental.

La ligature du nerf grand sciatique du rat induit une symptomatologie similaire à celle de la dystrophie réflexe sympathique neurogène. Une hypersensibilité aux cathécholamines circulantes induite par la dénervation a été impliquée dans les phénomènes de dysfonction sympathique observés dans le modèle de ligature du nerf sciatique comme dans l'algodystrophie.
Les auteurs ont étudié les propriétés fonctionnelles d'innervation sympathique des artères sous-cutanées isolées des pattes arrières de rats, 3 semaines après la ligature nerveuse. A l'aide d'un myographe, les auteurs ont étudié les réponses contractiles à la stimulation électrique des nerfs adrénergiques, et l'effet de doses cumulatives de différents médiateurs adrénergiques exogènes.
Les résultats ont été comparés au côté controlatéral sain. Du côté de la ligature, la réponse contractile des artères sous-cutanées était moindre après application d'une stimulation électrique. En outre, les artères sous-cutanées du côté de la ligature présentaient une sensibilité accrue à la stimulation adrénergique α_1.
Cette étude démontre que les anomalies sympathiques présentes dans ce modèle expérimental de douleur neuropathique consistent en une hypersensibilité aux cathécolamines induite par la dénervation, plus qu'en une augmentation des influx d'origine afférente au sein des efférents sympathiques.