

PERIPHERAL NERVE LESION-INDUCED UPTAKE AND TRANSPORT OF CHOLERAGENOID BY CAPSAICIN-SENSITIVE C-FIBRE SPINAL GANGLION NEURONS*

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In the present experiments the effect of systemic capsaicin treatment on the retrograde labelling of sensory ganglion cells was studied following the injection of cholera toxin B subunit-horseradish peroxidase conjugate (CTX-HRP) into intact and chronically transected peripheral nerves. In the control rats CTX-HRP injected into intact sciatic nerves labelled medium and large neurons with a mean cross-sectional area of $1041 \pm 39 \mu\text{m}^2$. However, after injection of the conjugate into chronically transected sciatic nerves of the control rats, many small cells were also labelled, shifting the mean cross-sectional area of the labelled cells to $632 \pm 118 \mu\text{m}^2$. Capsaicin pretreatment *per se* induced a moderate but significant decrease in the mean cross-sectional area of the labelled neurons ($879 \pm 79 \mu\text{m}^2$). More importantly, systemic pretreatment with capsaicin prevented the peripheral nerve lesion-induced labelling of small cells. Thus, the mean cross-sectional areas of labelled neurons relating to the intact and transected sciatic nerves, respectively, did not differ significantly. These findings provide direct evidence for a phenotypic switch of capsaicin-sensitive nociceptive neurons after peripheral nerve injury, and suggest that lesion-induced morphological changes in the spinal cord may be related to specific alterations in the chemistry of C-fibre afferent neurons rather than to a sprouting response of A-fibre afferents.

Keywords: Sensory ganglion – peripheral nerve section – cholera toxin – neuroplasticity – capsaicin

INTRODUCTION

Lesions of peripheral nerves induce complex changes in the structural, functional and chemical traits of sensory ganglion neurons [1, 8, 9, 13]. These involve alterations in the expression of sensory neuropeptides, neurotrophins and their receptors and other sensory neuron-specific macromolecules [8, 20, 24]. Alterations in the chemistry of the affected nerve elements apparently play a crucial role in the mechanism of functional and pathological changes associated with nerve lesions, including the development of chronic/neuropathic pain states [6, 24]. Changes in the electrophysiological properties of lesioned sensory ganglion cells may also be related to the altered expression of specific ion channel proteins [21]. Although structural changes associated with nerve lesions have been revealed already in early morphological studies,

*Dedicated to Professor József Hámori on the occasion of his 70th birthday.

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comparatively little attention has been paid to the significance of structural alterations in relation to the development of pathophysiological changes which commence in the domain of injured neurons. Previous studies dealing with the effects of peripheral nerve lesions involving specific chemodenervation of capsaicin-sensitive afferent nerves disclosed a delayed degeneration of spinal primary afferent terminals which may be attributed, at least in part, to sensory ganglion cell loss [9, 13]. It has been suggested that this may provide favourable conditions for the development of structural neuroplastic changes in the spinal dorsal horn [2]. Indeed, recent findings have demonstrated that injection of a cholera toxin B subunit-horseradish peroxidase conjugate (CTX-HRP) into damaged but not intact peripheral nerves resulted in strong labelling of primary afferents not only in the deeper (Rexed's laminae III–VI) layers of the spinal cord but also in the superficial dorsal horn, the substantia gelatinosa (Rexed's lamina II) [14, 22, 23]. Since cholera toxin B subunit and its conjugates have been reported to be specifically bound to and transported by medium and large dorsal root ganglion neurons giving rise to myelinated afferent fibres, which terminate normally in the deep dorsal horn [18], these findings have been interpreted in terms of a vigorous sprouting response of A primary afferent fibres in response to peripheral nerve injury [14, 22]. The present experiments were initiated in an attempt to study the role of C-fibre nociceptive dorsal root ganglion neurons in this phenomenon by making use of the neurotoxic effect of capsaicin on this particular population of afferent fibres [10–12]. The results indicate that capsaicin-sensitive sensory ganglion neurons may bear of fundamental significance in the mechanism of this neuroplastic response which may not necessarily involve sprouting of A-fibre afferents.

MATERIALS AND METHODS

Adult male Wistar rats weighing 250–270 g at the start of the experiments were used in this study. This study was approved by the Ethical Committee on Animal Experiments of the University of Szeged.

Capsaicin pretreatment

Rats were injected with increasing doses of capsaicin (10, 30, and 100 mg/kg b.w.) on three consecutive days under ether anaesthesia. Animals injected with similar amounts of the solvent for capsaicin (8% ethanol, 6% Tween 80 in saline) served as controls.

Peripheral nerve transection and retrograde labelling with CTX-HRP

Five to seven days later the animals were anaesthetized and the right sciatic nerve was exposed in the mid thigh and transected distal to a ligature. The wound was

closed and the rats were returned to the animal house. In control experiments the same procedure was followed except that the nerve was left intact. Two weeks later the sciatic nerves were exposed and 1 μ l of a 1% solution of a CTX-HRP conjugate (Sigma) was injected into the nerve using a Hamilton microsyringe under chloral hydrate (400 mg/kg, i.p.) anaesthesia.

Histological procedures

Two days after the injection of CTX-HRP the animals were deeply anaesthetized and perfused transcardially with an aldehyde fixative containing 1% glutaraldehyde and 1.25% paraformaldehyde in 0.1 M phosphate buffer (pH = 7.4) followed by 400 ml of cold phosphate buffer containing 10% sucrose. The L4–L6 dorsal root ganglia were removed and stored in the sucrose-buffer solution. Serial frozen sections of dorsal root ganglia 20 μ m in thickness were cut on a cryostat, mounted on chromalum-gelatin coated slides and they were reacted for the demonstration of peroxidase activity according to Mesulam [15] using 3,3',5,5'-tetramethylbenzidine as chromogen. After the completion of the enzyme reaction, most slides were dried overnight and then dehydrated briefly in ethanol, cleared in xylene and mounted in Permount. Other sections were counterstained with neutral red.

Size-frequency distribution histograms of CTX-HRP-labelled neurons were generated by measuring the sizes of 300 neurons with clear cut nuclei in the L4 and L5 dorsal root ganglia of each animal by means of a light microscope equipped with a camera lucida and a digitising tablet connected to a computerized system.

RESULTS

In dorsal root ganglia of control (vehicle-treated) rats mostly medium to large sized neurons showed intense perikaryal peroxidase activity resulting from the retrograde intraaxonal transport of CTX-HRP injected into the intact sciatic nerve (Fig. 1a). In capsaicin-pretreated rats the size frequency distribution of CTX-HRP labelled neurons relating to the intact sciatic nerves were similar to that seen in control (vehicle-treated) animals (Figs 1c, 2). However, statistical analysis of the mean cross-sectional areas of ganglion cells relating to the intact sciatic nerves of control and capsaicin-pretreated rats revealed a moderate but significant increase in the number of labelled small neurons (Fig. 3). Following the intraneural injection of CTX-HRP into the transected sciatic nerve of control rats, the number of small labelled cells increased markedly (Fig. 1b). This was evident from comparisons of both the size frequency distribution and the mean cross-sectional areas of labelled neurons (Figs 2 and 3). In contrast, in capsaicin-pretreated rats section of the sciatic nerve failed to induce a similar increase in the number of labelled small neurons (Fig. 1d). The size

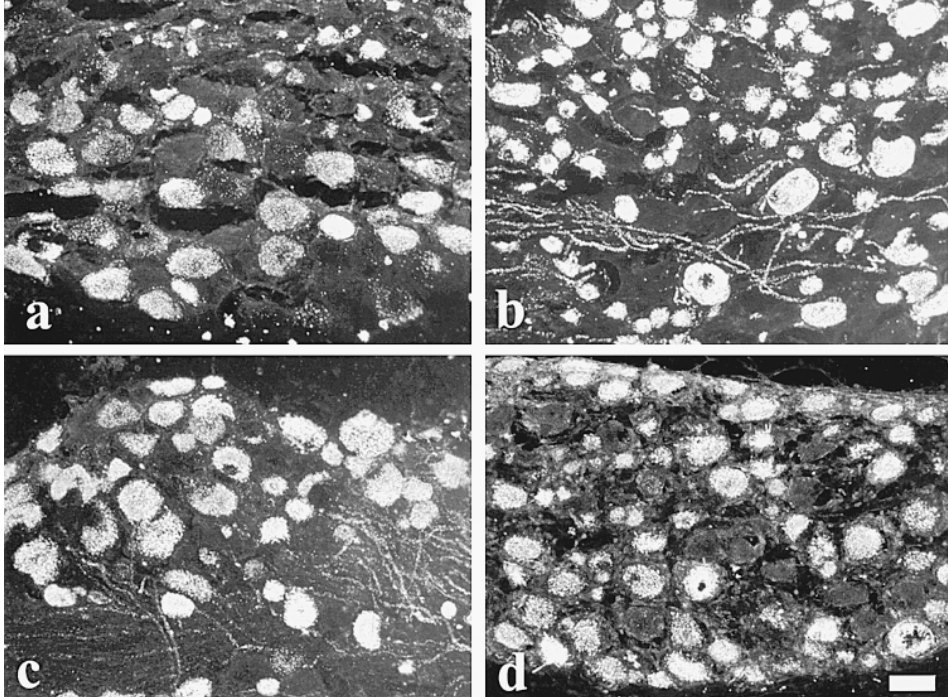


Fig. 1. Microphotographs of labelled neurons in L5 dorsal root ganglia following the injection of CTX-HRP into intact (a, c) and transected (b, d) sciatic nerves of control (vehicle-treated, a, b) and capsaicin-pretreated (c, d) rats. Scale bar in (d) indicates 50 μm and applies to all microphotographs

frequency distribution and the mean cross-sectional areas of labelled dorsal root ganglion neurons relating to the transected and intact sciatic nerves, respectively, did not differ significantly (Figs 2 and 3).

DISCUSSION

The present findings corroborate earlier reports showing that intraneural injection of CTX-HRP into intact peripheral nerves results in the labelling of parent dorsal root ganglion neurons mainly of medium and large sizes [23]. In agreement with recent observations, a significant population of small neurons was also labelled following intraneural injections of CTX-HRP into chronically transected sciatic nerves of control animals [19]. Systemic capsaicin pretreatment per se produced a slight but statistically significant increase in the number of labelled small sensory ganglion cells after the injection of CTX-HRP into an intact sciatic nerve. However, the main finding of the present study was that prior systemic capsaicin treatment largely prevented the nerve lesion-induced increased labelling of small neurons.

These results strongly indicate that capsaicin-sensitive primary sensory neurons are intimately involved in the mechanisms of structural and biochemical changes which follow peripheral nerve injury. Previous studies disclosed that injection of

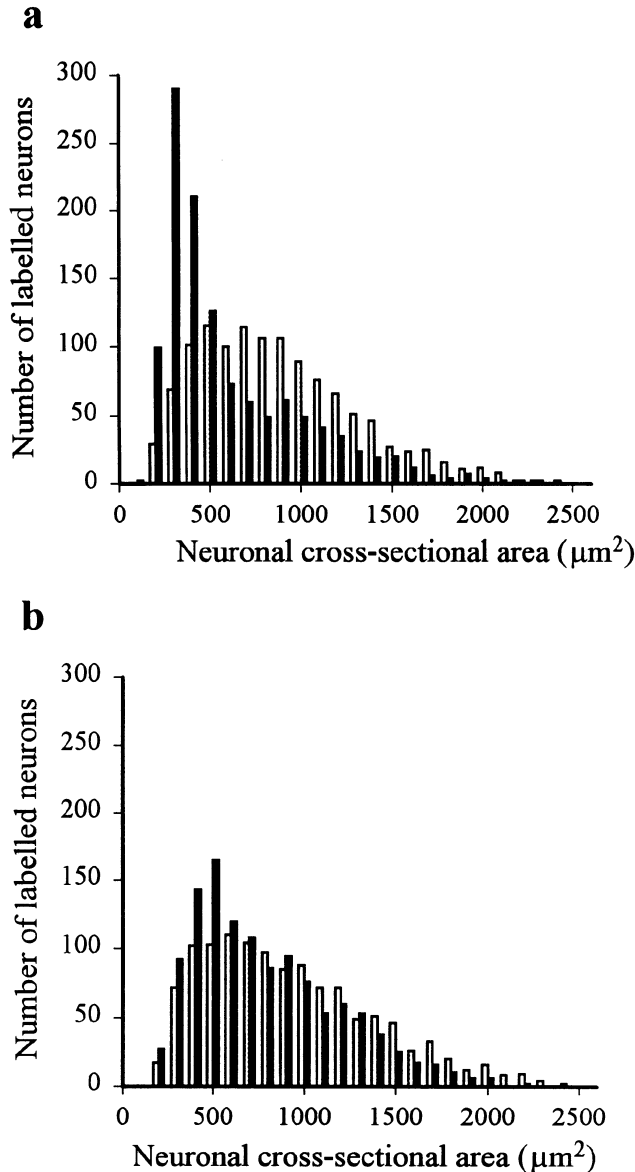


Fig. 2. Size-frequency distribution histograms of L4-L5 dorsal root ganglion neurons labelled with CTX-HRP injected into intact (open bars) or transected (filled bars) sciatic nerves of control (vehicle-treated (a)) and capsaicin-pretreated (b) rats

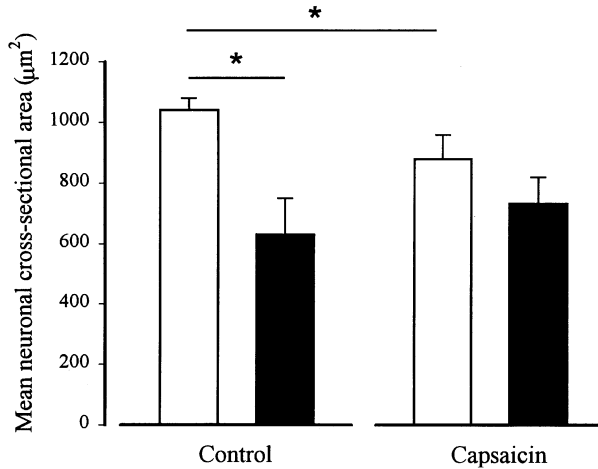


Fig. 3. Mean cross-sectional areas of L4–L5 dorsal root ganglion neurons labelled with CTX-HRP injected into intact (open columns) or transected (filled columns) sciatic nerves of control (vehicle-treated, $n = 4$) and capsaicin-pretreated ($n = 4$) rats. Each column represents the data obtained from 4 animals. *indicates significant differences ($p < 0.05$) between the respective data

CTX-HRP into a previously transected but not intact peripheral nerve resulted in the marked labelling of the substantia gelatinosa of the spinal dorsal horn. This phenomenon was attributed to a sprouting response of injured A-fibre afferents which normally do not terminate in this superficial layer of the spinal dorsal horn [14, 22, 23]. The present observations indicate a significant involvement of capsaicin-sensitive sensory ganglion neurons in the mechanism of this supposed sprouting response. On the one hand, the induction of vacant synaptic sites by producing an extensive but selective C-fibre deafferentation of the substantia gelatinosa due to the degeneration of capsaicin-sensitive primary afferent terminals may promote a sprouting response of A-fibre afferents [9, 11]. On the other hand, however, the possibility arises that the lesion-induced labelling of the substantia gelatinosa may be related to a phenotypic switch of capsaicin-sensitive small sensory ganglion neurons which permits an increased uptake and transport of CTX-HRP by C-fibre afferents normally terminating in that region of the spinal dorsal horn [10, 11, 13, 17]. The present findings support this assumption by showing that systemic capsaicin treatment per se resulted in a moderate but significant increase in the labelling of small sensory ganglion cells after the injection of CTX-HRP into an intact nerve. Previous observations disclosed that systemic injection of capsaicin produced degenerative alterations in a particular subpopulation of primary afferent neurons [11, 17], in particular degeneration of peripheral C-fibre axons [5] and nerve endings [4]. In the affected neurons, these degenerative phenomena, by mimicking a partial nerve section, may initiate chemical changes associated with nerve lesions.

Several factors may contribute to the observed lack of a substantial increase in the labelling by CTX-HRP of injured small ganglion cells after capsaicin treatment.

First, systemic injection of capsaicin in adult rats induces profound degenerative alterations in the domain of C-fibre primary sensory neurons [11, 13, 17]. Although the extent and nature of ganglion cell death is still unclear, the extensive degeneration of peripheral C-fibres [5, 11], nerve endings [4], and spinal primary afferent terminals [11, 13, 17] is well established. It is conceivable that damage to the peripheral terminals of sensory axons may interfere with trophic influences of the target tissues resulting in disturbances affecting the uptake and/or transport of CTX-HRP. Indeed, addition of external nerve growth factor has been shown to prevent neuroplastic changes in the spinal dorsal horn following peripheral nerve transection [3]. Second, capsaicin has been shown to selectively inhibit intraneuronal transport processes in C-fibre afferent fibres [7, 16]. Therefore, both loss of sensory C-fibres and blockade of retrograde axonal transport in C- but not A-fibre afferents may explain the failure of peripheral nerve section to produce a marked increase in the proportion of CTX-HRP labelled small neurons after systemic capsaicin treatment.

In conclusion the present findings revealed a fundamental role of capsaicin-sensitive primary afferent neurons in the mechanisms of peripheral nerve lesion-induced structural neuroplastic changes in spinal sensory ganglia. The results also point to the possibility that the marked transganglionic labelling by CTX-HRP of the substantia gelatinosa observed after peripheral nerve lesions may be attributed mainly to a phenotypic switch of capsaicin-sensitive sensory ganglion neurons rather than an extensive sprouting of A-fibre afferents [14, 22, 23].

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