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Reversible Blockade of Retrograde Axonal Transport in the Rat Sciatic Nerve by Vincristine

B. V. MACFARLANE, A. WRIGHT* AND H. A. E. BENSON

*Departments of Pharmacy and *Physiotherapy, The University of Queensland, Brisbane, Queensland, Australia 4072*

Abstract

Altered axoplasmic transport has been implicated as a contributing factor in neuropathic pain states. Treatments which produce transient axoplasmic transport blockade have shown initial promise as therapeutic procedures in the management of chronic neuropathic pain. The present study evaluated the capacity of the vinca alkaloid, vincristine to produce reversible blockade of retrograde axoplasic transport.

Results indicated that intraneural administration of 10^{-5} M vincristine resulted in blockade of retrograde axoplasmic transport of [^{3}H]leucine 24 h following administration. Importantly, the effect reversed over the next four days and axoplasmic transport was re-instated fully at day five.

The implications of these results are discussed in relation to the potential use of axoplasmic transport blocking agents in the management of chronic neuropathic pain.

Axoplasmic transport involves specialised intracellular transport mechanisms whereby nerve cell components and extracellular materials can be transported between the cell body and the peripheral limits of the axon. A clear function of anterograde axoplasmic flow (ie. from the cell body to the periphery) is to transport cellular components such as organelles, receptors, structural proteins etc, from their site of production in the cell body to their functional location which may be in the periphery of the cell. The role of retrograde axoplasmic transport (i.e. from the periphery to the cell body) is less clear, however it is suggested that it performs at least two important functions. Firstly, in a cyclical process cellular components may be transported back to the cell body where they can be reprocessed, thereby conserving energy within the system. Secondly, it appears that retrograde transport is important as a means of conveying trophic signals, thus providing the nucleus with information about the state of the axon, synapses, target tissues and the general environment of the neuron (Wall & Melzack 1989).

Nerve growth factors, present in target tissues and transported via axoplasmic transport have been identified (Varon & Adler 1980) and their importance for nerve growth and the continued maintenance of normal nerve function is now well recognised. Any modification of the pattern of signals being received by the cell body will result in morphological and metabolic changes which ultimately lead to changes in neuronal function (Kristensson & Olsson 1976). Indeed, because of the complex interconnections between neurons and the transfer of trophic factors across synapses, altered retrograde axoplasmic transport in one group of neurons is likely to result in changes in morphology and function in second order neurons with which they make synaptic connections. This process has been highlighted as an important component of the pathophysiological processes involved in chronic neurogenic pain (Woolf 1993). One suggested outcome of this process is a reduction in inhibitory influences and an increase in neuronal excitability which may provide a basis for ongoing pain and allodynia (Breuer & Atkinson 1988). The potential importance of altered axoplasmic transport in the maintenance of chronic neuropathic pain has been highlighted by Wall (Wall & Melzack 1989). Altered axoplasmic transport has also been proposed as a pathophysiological mechanism underlying some more common musculoskeletal disorders (Butler 1991).

What is not clear at present is the question of whether alterations in neuronal function occur as a result of the absence of some factor in a damaged neuron, or as the result of uptake and retrograde transport of a factor which is present in damaged tissue, but which is not present in the neuronal environment under normal circumstances. Clearly, if the second situation prevails then it might be expected that temporary blockade of retrograde axoplasmic transport would result in some reduction in morphological and functional changes with consequent improvement in symptomatology.

In a clinical trial using topically applied vinca alkaloids (vinblastine and vincristine) as inhibitors of retrograde axoplasmic flow, Knyihar-Csillik et al (1982) demonstrated that transdermal iontophoretic delivery of these agents produced significant clinical improvements in 51 patients with a variety of chronic neurogenic pain syndromes including post-herpetic neuralgia, trigeminal neuralgia, causalgia, painful metabolic neuropathies and discogenic pain. Iontophoretic treatment was applied for one hour per day over a period of 8–24 days and, in those cases which responded to treatment, pain relief was apparent within 5–7 days of vinca alkaloid administration. Consequently, if modification of retrograde axoplasmic transport can produce relief of chronic neurogenic pain, which is generally unresponsive to most other therapeutic interventions, then further research to explore this phenomenon is warranted.

The present study involved an investigation of the effect of intra-neural administration of the vinca alkaloid vincristine on retrograde axonal transport of [^{3}H]leucine in the sciatic nerve of the rat. It was hypothesised that, vincristine would inhibit retrograde axoplasmic transport of [^{3}H]leucine in the sciatic nerve and that this inhibitory effect would be reversible.

Correspondence: H. A. E. Benson, Department of Pharmacy, The University of Queensland, Steele Building, Brisbane, Queensland, Australia 4072.

Materials and Methods

This research was approved by the Animal Experimentation Ethics Committee of the University of Queensland.

Materials

Vincristine sulphate injection (1 mg mL^{-1}) was obtained from Delta West (Australia) and diluted to 0·1 mg mL^{-1} with sterile isotonic saline, using appropriate cytotoxic handling procedures by the oncology pharmacist, Royal Children's Hospital (Brisbane). [^{3}H]Leucine (specific activity 140 Ci $mmol^{-1}$ in sterile aqueous solution: total activity 5 mCi mL^{-1} [185 MBq]) and Solvable Tissue and Gel Solubiliser were obtained from DuPont NEN Research Products Ltd. (Australia). Optiphase 'HiSafe' 3 was obtained from Fisons Chemicals, UK. All other chemicals used were of reagent grade.

Neural injection procedure

Male Wistar rats (average weight 400 g) were anaesthetised with pentobarbitone sodium (60 mg kg^{-1} i.p.). The rat was placed in the prone position, with the left leg stabilised and rotated slightly to ensure the tibial nerve lay parallel to the operating plane. The operating area was shaved with clippers and the tibial division of the sciatic nerve exposed by making an incision through the skin and subcutaneous connective tissue. A metal microspatula was placed under the nerve to facilitate microinjection. A pulled glass micropipette (external tip diameter 50 μm) was placed in a stereotaxic micromanipulator. The micropipette was attached to a 2 μ-L syringe (Hamilton 7000 series with Chaney adaptor) using polythene tubing. This was preloaded with 1 μL of injectate. The micropipette was inserted into the tibial nerve with the aid of a dissecting microscope (× 40; Olympus SZSTB1). The injectate was introduced into the nerve over a period of 60 s. After a further 60 s the micropipette was removed and the incision closed using Michel clips. The neural injection procedure is shown in Fig. 1A. The animal was allowed to recover and was housed in a climate controlled environment (20°C, 12-h simulated day and night) until further experimentation.

Dissection procedure

The rat was killed by cervical dislocation under anaesthesia. The hair was removed from the back and an incision made lateral to the spinal cord, from the thoracic region to the sacral region. The tibial and sciatic nerves were removed from just below the tibial nerve injection point to the thoracic trifurcation (Fig. 1B).

Tissue preparation and radiolabel assay

The nerve was dissected into 7·5-mm lengths from the end furthermost from the injection site. Each segment was placed in a scintillation vial, 1 mL Solvable tissue solubiliser added and incubated at 65°C for 45 min. Following incubation the samples were vortex mixed and 4 mL of scintillation liquid was added. The samples were shaken and the radioactivity was measured by scintillation counting.

Experimental protocol

The study involved a comparison of three experimental conditions to evaluate the effects of vincristine over a series of time periods from 24–120 h. The time periods tested were 24 h (n = 7), 42 h (n = 4), 72 h (n = 6), 96 h (n = 5) and 120 h (n = 7). This permitted a measure of the extent of vincristine-mediated blockade of axonal transport and the time period of the effect.

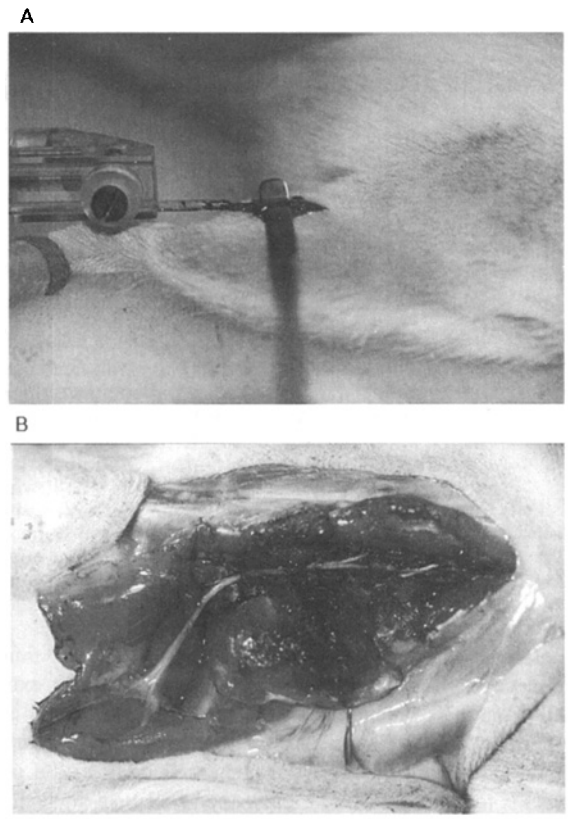

FIG. 1. A. Intraneural injection procedure showing the insertion of a pulled glass micro-pipette under the perineurium of the rat tibial nerve prior to delivery of injectate. B. Dissection of the rat tibial and sciatic nerves and the lumbar plexus used to trace the passage of the radiolabel following intra-neural injection.

The treatment involved injection of vincristine into the nerve with the subsequent administration of [^{3}H]leucine at a site 1 cm distal to the original injection site following a defined time period as indicated above. After a further 24 h the animals were killed and the radioactivity of the nerve analysed as described above.

A saline control was utilised in order to monitor the effects of the injection vehicle. This involved the administration of saline at various time periods prior to an injection of [^{3}H]leucine as described above.

A procedural radiolabelled control was also used. This involved the injection of [^{3}H]leucine only, with subsequent killing of the animal after 24 h and measurement of radioactivity as described above.

Data analysis

Results for each nerve segment were expressed as percentage of total disintegrations per min (d min^{-1}) across the entire nerve. Data were plotted as log % d min^{-1} vs distance from injection site for each time interval and the AUC of % d min^{-1} vs distance from injection site curve was calculated for each

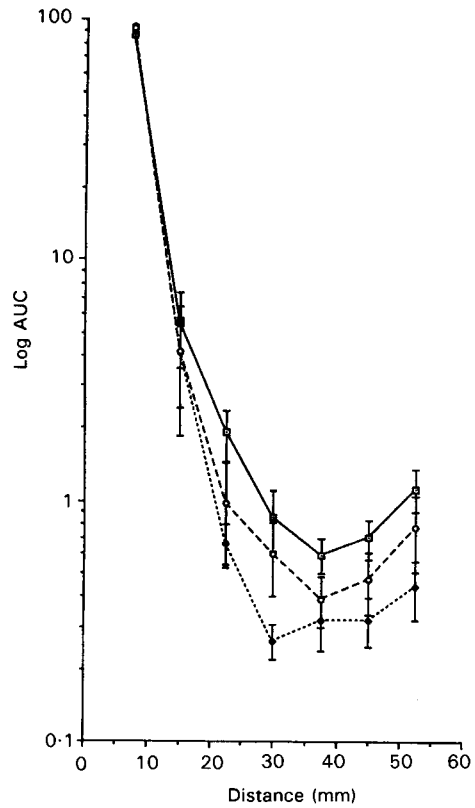

FIG. 2. Effect of intraneural injection of vincristine on retrograde axoplasmic transport of [^{3}H]leucine when applied 24 h before [^{3}H]leucine. AUC is from a plot of % d min^{-1} [mean ± s.e.] vs distance from the injection site in the tibial nerve of Wistar rats. ◆, vincristine; □, radiolabel control; ○, saline control.

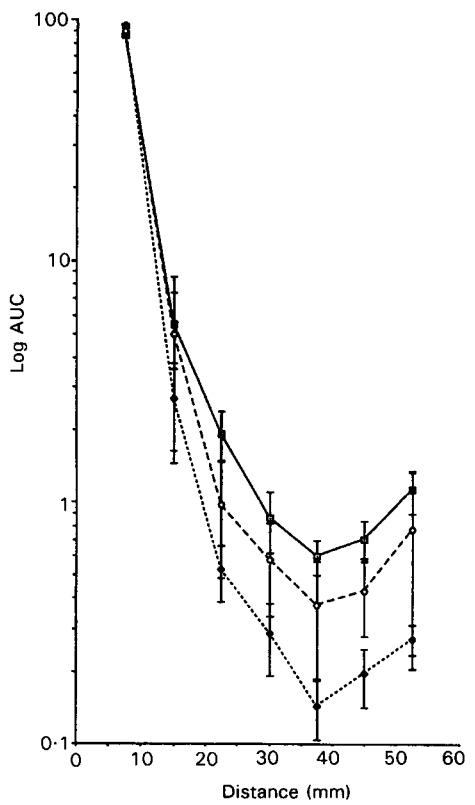

FIG. 3. Effect of intraneural injection of vincristine on retrograde axoplasmic transport of [^{3}H]leucine when applied 42 h before [^{3}H]leucine. AUC is from a plot of % d min^{-1} [mean ± s.e.] vs distance from the injection site in the tibial nerve of Wistar rats. ◆, Vincristine; □, radiolabel control; ○, saline control.

data set (Figs 2–6). Tests for statistical significance (one tailed, independent t-test: $P < 0.05$) were conducted on the area under the % d min^{-1} vs distance from injection site curve (AUC: Fig. 7), excluding the immediate injection site sections (0–15 mm).

Results

All procedures were well tolerated. The rats did not show any signs of altered gait, feeding or grooming habits or autotomy following any of the injection procedures.

P values for the difference between vincristine treatment, saline control and radiolabel control (based on AUC data) are presented in Table 1.

Fig. 2 describes the situation occurring in the nerve 24 h after vincristine treatment or saline control. The trend indicates that the percentage of the radiolabel existing in the nerves treated with both vincristine and saline is less than that in the nerve that was injected with [^{3}H]leucine alone. The difference between the AUC of vincristine and radiolabel control is significant as is the difference between vincristine and saline control (Table 1), indicating that vincristine inhibited the transport of the radiolabel. Area under the curve was reduced from 27·68 ± 5·11, in the case of the radiolabel control, to 10·51 ± 2·51 in the case of the vincristine-treated nerve.

This trend continued in the 42-h (Fig. 3) and 72-h (Fig. 4) treatment groups where the difference between AUC of vincristine is significant compared to AUC of the radiolabel control. At these time periods there was no significant difference between vincristine and the saline control (Table 1).

Table 1. P-values for comparison of vinctristine treatment with saline control and radiolabel control conditions.

Time interval (h)	P-values for vincristine treatment vs control conditions	
	Saline control	Radiolabel control
24	0·022*	0·006*
42	0·157	0·003*
72	0·463	0·038*
96	0·081	0·300
120	0·470	0·281

AUC data: one tailed independent t-test: *significant difference ($P < 0.05$).

As the time period increased to 96 h (Fig. 5) and 120 h (Fig. 6) there was no longer a significant difference between vincristine treatment and the radiolabel control, indicating that axoplasmic transport had been re-instated within the nerve. AUC for vincristine at 120 h (34·28 ± 9·84) is similar to that of the radiolabel control (27·68 ± 5·11).

Fig. 7 summarises the changes in AUC with time following treatment with vincristine, the saline control and the AUC of

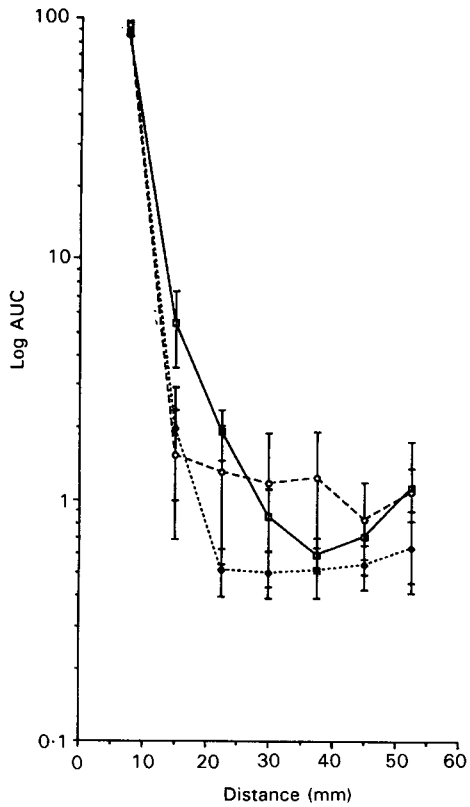

FIG. 4. Effect of intraneural injection of vincristine on retrograde axoplasmic transport of [^{3}H]leucine when applied 72 h before [^{3}H]leucine. AUC is from plot of % d min^{-1} (mean ± s.e.) vs distance from the injection site in the tibial nerve of Wistar rats. ◆, vincristine; □, radiolabel control, ○, saline control.

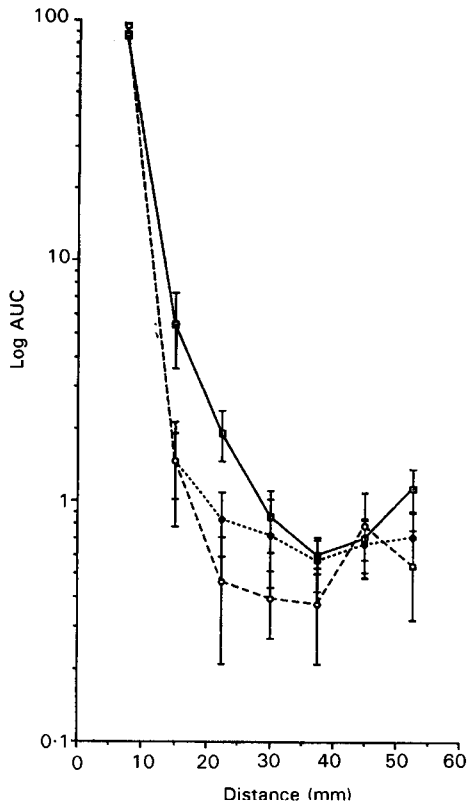

FIG. 5. Effect of intraneural injection of vincristine on retrograde axoplasmic transport of [^{3}H]leucine when applied 96 h before [^{3}H]leucine. AUC is from plot of % d min^{-1} (mean ± s.e.) vs distance from the injection site in the tibial nerve of Wistar rats. ◆, vincristine; □, radiolabel control; ○, saline control.

the radiolabel control. In the case of vincristine, from time zero the AUC decreases to a minimum at 24 h, indicating maximum effect, and then steadily increases over the following four days until axoplasmic transport normalises on the fifth day. In the case of the saline control, the decrease in AUC follows a similar pattern to vincristine, although less pronounced and more variable. Indeed at 24 h the AUC for radiolabel control and saline are very similar whereas the AUC for vincristine is significantly different.

The data indicate that saline also has an inhibitory influence on axoplasmic transport. The effect is variable over time and there seems to be no correlation between the effect of saline and the period of treatment as is the case for vincristine. Nevertheless, the effect was sufficiently strong to ensure that a significant difference between vincristine treatment and the saline control could only be demonstrated following the 24-h treatment period. This could be attributed to the large standard errors witnessed with the saline treatments.

In summary, vincristine significantly blocked retrograde axonal transport of [^{3}H]leucine from 24 h and up to 72 h post injection into the nerve. After 72 h, blockade of retrograde axonal transport was reversed until it was normalised at or around 120 h. Saline control did not induce a predictable reduction in axonal transport of [^{3}H]leucine however it was apparent that the injection vehicle may have contributed in some degree to the inhibitory effect of the vincristine treatment procedure.

Discussion

The vinca alkaloids are recognised as specific inhibitors of retrograde axoplasmic transport. Their suggested mechanism of blockade is via inhibition of microtubules due to their spindle poison properties. The present study demonstrates that intraneural microinjection of 10^{-5} M vincristine produces a relative blockade of retrograde axoplasmic transport. The effect is apparent within 24 h post injection and persists for up to 72 h. Importantly, the results of the study show that the blockade is completely reversible within 5 days. This period of time equates well with the 5–7-day time period required to produce a treatment response following iontophoretic delivery of vincristine in patients with chronic neuropathic pain (Knyihar-Csillik et al 1982).

The results of the study cast some doubt on the suitability of using saline as an injection vehicle in studies evaluating inhibitors of axoplasmic transport. While it is known that sodium ions can have a considerable effect on axoplasmic transport, researchers have previously used saline as a vehicle without reporting a significant influence on axonal transport (England et al 1988; Harry et al 1992). It appears that the inhibitory effect of saline is non-specific directionally. Although vincristine has been shown to be a specific retrograde transport inhibitor, sodium ions affect both retrograde and anterograde movement while also affecting other vital cellular processes. Indeed extended exposure of nerve preparations to saline has resulted in death of the neuron (Ochs 1982). While

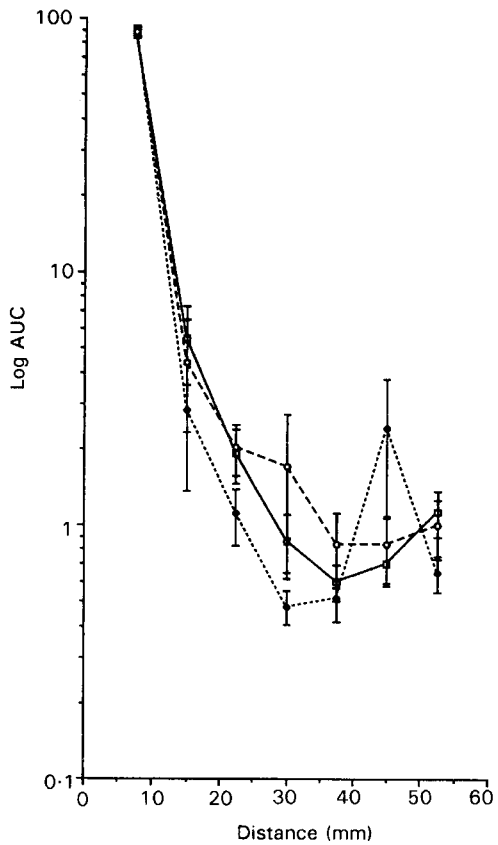

FIG. 6. Effect of intraneural injection of vincristine on retrograde axoplasmic transport of [^{3}H]leucine when applied 120 h before [^{3}H]leucine. AUC is from plot of % d min^{-1} (mean ± s.e.) vs distance from the injection site in the tibial nerve of Wistar rats. ◆, vincristine; □, radiolabel control; ○, saline control.

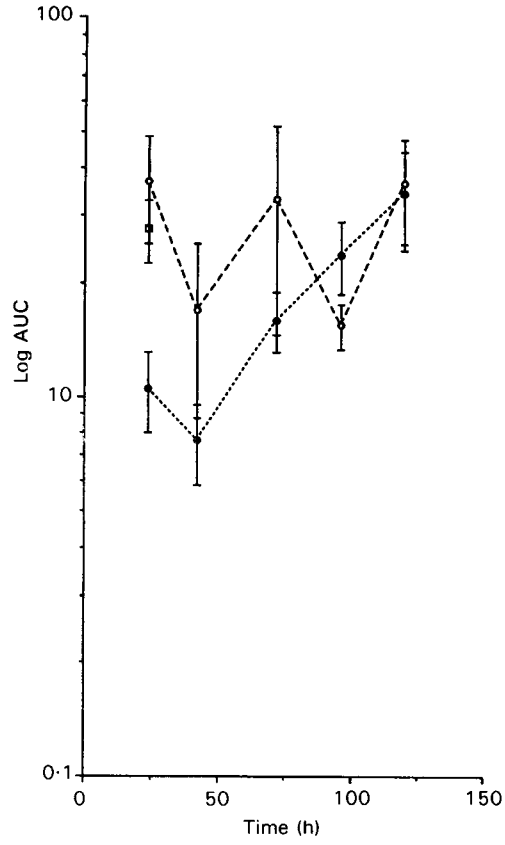

FIG. 7. AUC (from plot of % d min^{-1} (mean ± s.e.) vs distance from the injection site) vs time, showing the radiolabel control and the inhibition of axonal transport 24 h after treatment with vincristine followed by the steady resumption of transport at longer time intervals after treatment. ●, vincristine; □, radiolabel control; ○, saline control.

inhibition of retrograde axoplasmic transport would be desirable in the treatment of neuropathic pain, inhibition of anterograde axoplasmic transport would not since this would deprive the cell of vital gene products produced in the nucleus. Further research is required to determine the most appropriate vehicle for intraneural injections of vinca alkaloids and other axoplasmic transport inhibitors. The need to employ control procedures to evaluate the effects of injection vehicles in studies of this nature has also been clearly demonstrated.

Research is required to evaluate the functional status of the axoplasmic transport system in animal models of neuropathic pain such as that developed by Bennett & Xie (1988) in which loose ligatures are used to produce a chronic constriction injury of the sciatic nerve. In such a model, it would be hypothesised that axoplasmic transport may be reduced across the region of the nerve constriction. Future studies could focus on evaluating the influence of vincristine blockade on pain-related nociceptive measures in this neuropathic pain model with the aim of providing a potential therapeutic intervention in patients with chronic neuropathic pain.

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