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Adrenal Medullary Transplants Attenuate Sensorimotor Dysfunction in Rats With Peripheral Neuropathy

JULIE B. SIEGAN AND JACQUELINE SAGEN

Department of Anatomy and Cell Biology, University of Illinois at Chicago, Chicago, IL 60612

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SIEGAN, J. B. AND J. SAGEN. *Adrenal medullary transplants attenuate sensorimotor dysfunction in rats with peripheral neuropathy.* PHARMACOL BIOCHEM BEHAV **59**(1) 97–104, 1998.—Previous work in our laboratory has demonstrated that adrenal medullary transplants into the spinal subarachnoid space can alleviate neuropathic pain behaviors. The purpose of this study was to test the possibility that motor, as well as, sensory dysfunction is reduced by adrenal medullary transplants. Peripheral neuropathy was induced by a chronic constriction injury (CCI) of the sciatic nerve of rats. In addition to exaggerated responses to noxious and innocuous stimuli characteristic of peripheral nerve injury, severe impairment of hindpaw placing and grasping reflexes following CCI was observed. Two weeks following CCI, either adrenal medullary or control striated muscle tissue was implanted into the spinal subarachnoid space. Adrenal medullary, but not control transplants, produced significant restoration of hindlimb reflex function in animals with peripheral nerve injury. This was reversed by pretreatment with the α-adrenergic antagonist phentolamine, but not the opiate antagonist naloxone, suggesting a role for catecholamines secreted by the implanted cells in reflex recovery. Adrenal medullary transplants also attenuated hyperalgesia and allodynia resulting from nerve injury. These results indicate that adrenal medullary transplants can alleviate sensorimotor dysfunction consequent to peripheral nerve injury. © 1998 Elsevier Science Inc.

Spinal cord Chromaffin cells Neural transplants Motor reflexes Pain Catecholamines Peripheral neuropathy Adrenal medulla

PERIPHERAL nerve injuries produce a constellation of sensorimotor pathologies in animal models and in humans. In recent years, several animal models have emerged that demonstrate some parallels with peripheral neuropathies encountered in the clinic (2,20,30). Behavioral analyses of these models have focused primarily on abnormal sensory function, notably exaggerated responses to innocuous and noxious stimuli characteristic of neuropathic pain. For example, in the chronic constriction injury model (CCI), characteristic symptoms following sciatic nerve ligation include hyperalgesia to noxious thermal and mechanical stimuli and allodynia to innocuous tactile and cold stimuli (1,2). However, in addition to alterations in sensory function, motor dysfunction is likely to be a component of the pathologic symptoms following peripheral nerve injury. In support for this, CCI of the sciatic nerve results in atrophy of the tibialis anterior and gastrocnemius/soleus muscles on the side ipsilateral to the nerve injury (2). This atrophy has been reported to be accompanied by weakness of the dorsiflexors and abnormal gait and posture, possibly due to the observed degeneration of Aα axons of the α motor neurons that innervate muscles below the knee (23). In addition, in tests of sciatic nerve function following CCI, including grasping and ankle dorsiflexion, a transient loss in hindpaw motor function with subsequent recovery was reported to temporally correlate with the sensory hyperalgesia and its functional recovery (21).

Previous studies in our laboratory have demonstrated that the transplantation of adrenal medullary chromaffin cells into the spinal subarachnoid space can attenuate chronic pain behaviors, including thermal and mechanical hyperalgesia and cold and tactile allodynia following CCI (11–15,18,31). Chromaffin cells were initially selected as donor cells due to their secretion of pain reducing neuroactive substances, particularly opioid peptides and catecholamines. However, recent studies have also demonstrated that attenuation of chronic pain by adrenal medullary implants is accompanied by reduction in spinal cord neuropathology consequent to peripheral

Requests for reprints should be addressed to J. Sagen, CytoTherapeutics, Inc., Two Richmond Square, Providence, RI 02906.

nerve injury. These include reductions in nitric oxide synthase (14), cGMP levels (31), and transsynaptic degeneration of inhibitory interneurons (11,18), which have been induced by CCI. Although these alterations are particularly marked in the spinal dorsal horn, morphological changes in the ventral horn have also been noted. In particular, reduced densities of NMDA receptors associated with motor neurons, which may accompany excitotoxic damage following CCI, are restored by adrenal medullary implants (15). Several studies in other laboratories have suggested that increased catecholamine levels in the spinal cord, either by injection or cellular implantation, can improve motor function following spinal cord damage (3,5,7,19,24,42). In addtion, adrenal medullary implants have been used in other injury models to provide an endogenous source of catecholamines, notably in Parkinson's disease and traumatic brain injury models (6,8,35). In particular, an enduring restoration of postural reflexes was reported following adrenal medullary grafting delayed as long as 3 weeks after cortical ablation injuries (6). The goal of the present study was to determine whether adrenal medullary implants in the spinal subarachonoid space could attenuate motor as well as sensory deficits following peripheral nerve injury. Preliminary results of this work have been reported previously (33).

METHOD

Experimental Protocol

All experimental procedures were reviewed and approved by the institutional animal care committee. Male Sprague–Dawley rats (Sasco, WI), weighing 250–275 g, were used as both graft donors and recipients. Animals underwent a battery of behavioral assessments for sensory and motor function (described below) at three time points: prior to peripheral nerve ligation to establish baseline responses, 2 weeks following peripheral nerve ligation, and 1 week following transplantation (3 weeks following peripheral nerve ligation). These time points were chosen based on previous experience with the CCI model, to coincide with the peak effects of the peripheral nerve injury. Animals were divided into five groups (n = 12 per group): CCI + adrenal medullary implants, CCI + control striated muscle implants, CCI only (no implant), adrenal medullary implant only (no CCI, to determine whether the implant itself produces any changes in motor or sensory behaviors), and normal intact animals (no surgical procedures). Following this evaluation, to assess the potential role of catecholamines or opioid peptides released from the transplants in producing behavioral effects, animals were pretreated with either phentolamine (HCl, 10.0 mg/kg, SC) or naloxone (HCl, 2.0 mg/kg, SC) to block host spinal α-adrenergic or opioid receptors, respectively, or saline vehicle prior to behavioral testing. These doses were based on previous studies using these agents to demonstrate the contribution of catecholamines and opioid peptides to analgesic effects (27–29). For this antagonist study, four animals from each group were pretreated with either phentolamine, naloxone, or saline on a given test day. This was rotated on subsequent test days, such that each animal eventually received each of the pretreatments, with at least 48 h elapsed between assessments.

Surgical Procedures

Unilateral chronic constriction nerve injury was induced as described in detail previously (2,12,13). All surgical procedures were done aseptically. Animals were anesthetized with sodium pentobarbital (40 mg/kg, IP, supplemented as necessary), and the right common sciatic nerve was exposed at the midthigh level using blunt dissection. Four 4-0 chromic gut ligatures spaced about 1 mm apart were tied around the sciatic nerve proximal to the trifurcation, loosely constricting the nerve such that circulation through epineural vasculature was not completely blocked. The musculature was closed with silk sutures and the skin closed with wound clips. Two weeks after nerve surgery, some animals were implanted with either adrenal medullary tissue or control striated muscle tissue (see above Experimental Protocol), as described in other studies (11,12,15). Both adrenal medullary tissue and striated muscle tissue were obtained from adult male Sprague–Dawley donor animals. Adrenal medullary tissue was dissected from adrenal cortex with the aid of a dissecting microscope, and cut into small pieces (<0.5 mm^3) in ice-cold Hanks' buffer. Tissue from two adrenal glands was implanted in each recipient animal, as this amount has been shown in previous studies to produce reliable analgesia in several models. Equal volumes of striated muscle tissue obtained from the external abdominal muscle of donor animals was implanted as graft controls. Graft recipients underwent a laminectomy at the level of the lumbar enlargement, and graft tissue was implanted in the spinal subarachnoid space via a slit in the dura at approximately L2. Following implantation, the musculature was sutured and the skin closed with wound clips. Animals were housed three to a cage and food and water were available ad lib.

Motor Dysfunction Assessment

Motor function was assessed using simple reflex tests following a protocol described by others (4). These included reflexes for grasping and placing, which have been reported to be impaired by constrictive nerve injuries in temporal correlation with hyperalgesia (21). To assess grasping reflexes, rats were placed on a wire grid, and observed for grasping of the hindpaws (positive response) to the contacted wire. To assess placing reflexes, rats were elevated slightly above a table and the ventral surface of the hindlimbs were gently brought into contact with the surface. A positive reflex occurs when the hindpaw is extended and placed on the table surface. In addition to these tests of hindlimb function, righting reflexes were also assessed by placing the rat on its back on a flat surface and noted whether the animal immediately assumes a normal upright position. Five trials of each reflex test were given, and animals were scored based on the number of normal reflex responses obtained.

Sensory Assessment

Hyperalgesia and allodynia were assessed as described in detail in previous studies (12,13,18,32). To determine responses to noxious thermal stimuli, a modification of the Hargreave's test (16) was used. Animals were placed beneath an inverted plastic cage on an elevated glass floor and a radiant heat source beneath the glass was aimed at the plantar hindpaw. Testing was alternated between hindpaws for three trials each with at least 30-s intertrial intervals and withdrawal latencies (determined electronically) were averaged for each hindpaw. Difference scores were calculated by subtracting the withdrawal latencies of the left hindpaw (intact side) from that of the right hindpaw (ligated side), such that negative difference scores indicate thermal hyperalgesia on the nerve-injured side. A cutoff time of 15 s was used to avoid tissue damage in the absence of a response. Responses to noxious mechanical stimuli were assessed using the Randall-Selitto test. Pressure was applied to the plantar surface of each hind-

paw sequentially at a constant rate of 64 g/s using a commercially available apparatus (Ugo-Basile), until the animal reacted with a withdrawal response. The apparatus automatically terminates at a scale reading of 25 (=1000 g). Difference scores were calculated as described for thermal responses. Tactile allodynia was assessed using a calibrated series of von Frey hairs ranging from 0.69 to 75.86 g using procedures similar to that described by others (36). Animals were placed beneath an inverted clear plastic cage on an elevated mesh floor and von Frey hairs were indented on the hindpaw midplantar skin five times in rapid succession. Testing was alternated between both hindpaws using increasing von Frey hair forces until the animal responded with a paw withdrawal. The lowest hair in the series that evoked at least one withdrawal response was recorded as threshold.

Morphological and Statistical Analysis

Following completion of behavioral assessments, some animals were perfused and their spinal cords removed and processed for tyrosine hydroxylase (TH) immunocytochemistry to confirm general adrenal medullary graft viability. These procedures have been routinely employed in our laboratory and described in detail elsewhere (39). As numerous previous studies have indicated that there is little variability in chromaffin cell viability in grafted tissue pieces, only a randomly selected sample of the transplants were evaluated ($n = 4$), and none of the animals were excluded from behavioral analyses. In addition, cell survival was not quantified, as loosely adherent graft tissue pieces are often lost during dissection or processing, making an accurate number difficult to obtain. For TH immunocytochemistry, primary antisera was obtained from Incstar (diluted 1:500); secondary antisera from Cappel (diluted 1:100, rhodamine linked goat-antimouse). Behavioral data were analyzed using ANOVA (repeated measures) and the Newman–Keuls test for multiple posthoc comparisons (SigmaStat, Jandel Scientific).

RESULTS

Motor Reflexes

In all cases, the righting reflex was not impaired, either by CCI or tissue implantation into the spinal subarachnoid space. However, both the placing reflex and the grasping reflex were significantly impaired following CCI [Fig. 1; overall, $F(4, 2) = 2013.0$ and 233.4, for placing and grasping reflex respectively, $p < 0.001$]. Responses of the right (ligated) side only are shown for clarity, as reflex responses on the intact side were unaltered from baseline. Normal intact animals (no CCI or implant) showed positive reflex responses (5 of 5) for both tests at all three time points tested. Peripheral nerve ligation resulted in severe impairments in both reflexes on the injured side two and three weeks following CCI ($p < 0.05$ compared to both baseline and normal intact animals). Similar severe impairments were sustained following implantation of control striated muscle tissue ($p < 0.05$ compared to baseline and normal intact animals). In contrast, following adrenal medullary implantation, the severe impairments resulting from CCI were attenuated, and some positive reflex responses were restored ($p < 0.05$ compared to post-CCI preimplant responses). This group was markedly improved compared to the muscle implanted group ($p < 0.05$). However, recovery was not completely restored to baseline levels for either test, as some residual impairment remained ($p < 0.05$ compared to baseline and normal intact animals). The implantation of adrenal medullary tissue into the spinal subarachnoid space did not alter reflex responses in animals without CCI ($p > 0.05$ compared to baseline and normal intact animals).

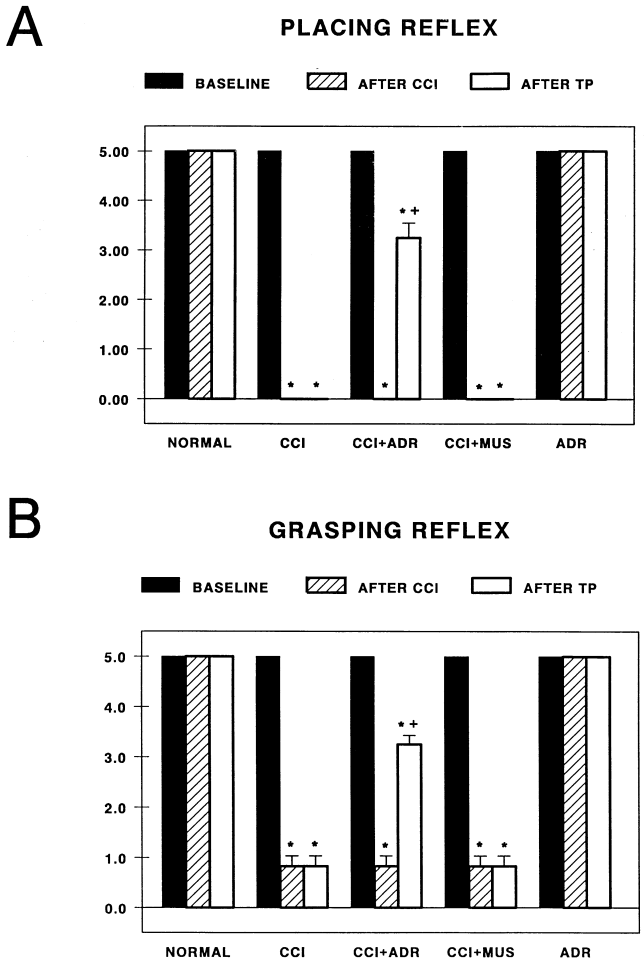

FIG. 1. Alterations in hindpaw reflexes following chronic constriction injury (CCI) of the sciatic nerve and transplantation of adrenal medullary (ADR) or control striated muscle (MUS) tissue in the spinal subarachnoid space. A shows the number (mean ± SEM) of normal placing reflexes (out of five trials), and B shows the number (mean ± SEM) of normal grasping reflexes (out of five trials). Five groups of animals were evaluated ($n = 12$/group): NORMAL = intact animals with no CCI or transplant; CCI = animals with sciatic nerve injury only (no transplant); CCI + ADR = animals with CCI followed by adrenal medullary transplantation; CCI + MUS = animals with CCI followed by striated muscle implantation; ADR = animals with adrenal medullary transplants only (no CCI). BASELINE indicates responses prior to any surgical manipulations; AFTER CCI indicates responses 2 weeks following sciatic nerve ligation in animals with CCI or at the same time point in animals without CCI; AFTER TP indicates responses 1 week following transplantation of either adrenal medullary or muscle tissue (corresponding to 3 weeks following CCI) in animals receiving transplants or at the same point in animals without transplants. $^{*}p < 0.05$ compared to baseline; $^{+}p < 0.05$ compared to after CCI.

Figure 2 shows the effects of antagonist pretreatment on placing (Fig. 2A) and grasping (Fig. 2B) reflexes in the five groups of animals. In normal intact animals, α-adrenergic antagonist phentolamine produced a slight, but statistically in-

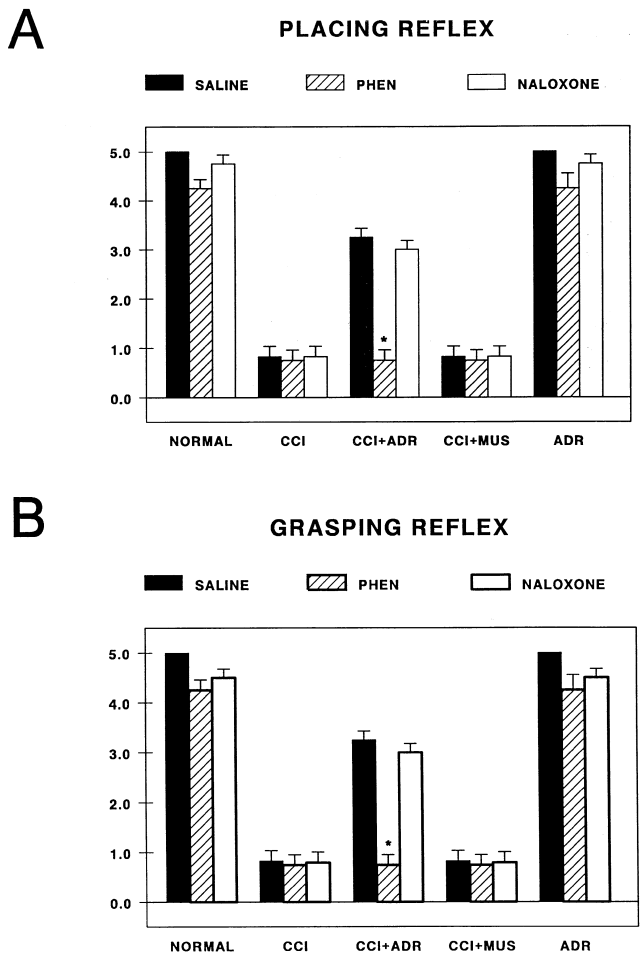

FIG. 2. Effects of antagonist pretreatment on alterations in hindpaw reflexes. A shows the number (mean ± SEM) of normal placing reflexes (out of five trials) and (B) shows the number of normal grasping reflexes (out of five trials). Five groups of animals were evaluated (n = 12/group): NORMAL = intact animals with no CCI or transplant; CCI = animals with sciatic nerve injury only (no transplant); CCI + ADR = animals with CCI followed by adrenal medullary transplantation; CCI + MUS = animals with CCI followed by striated muscle implantation; ADR = animals with adrenal medullary transplants only (no CCI). Animals were pretreated either with saline vehicle, α-adrenergic antagonist phentolamine (PHEN; HCl, 10.0 mg/kg, SC), or opiate antagonist naloxone (HCl, 2.0 mg/kg, SC) 15 min prior to testing. *$p < 0.05$ compared to saline.

significant reduction in positive motor reflex responses ($p > 0.05$ compared to saline vehicle). In the CCI only group, none of the pretreatments altered the impaired reflex responses resulting from peripheral nerve injury. Similarly, these antagonists had no effect on motor impairments in animals with control striated muscle implants. However, pretreatment with phentolamine in adrenal medullary-implanted animals reversed the restorative effects of these implants on motor reflex function, both for hindlimb placing and grasping ($p < 0.05$ compared to saline pretreatment). In these animals, reflex responses were reduced to levels of impairment similar to that observed in nonimplanted or control-implanted animals with peripheral nerve injury ($p > 0.05$ compared to both). In contrast to phentolamine, the opiate antagonist naloxone did not alter the beneficial effects of adrenal medullary implants on hindlimb reflexes ($p > 0.05$ compared to saline). Neither of the antagonists altered responses in adrenal medullary implanted animals without peripheral nerve injury.

Hyperalgesia and Allodynia

Responses to thermal and mechanical stimuli are shown in Fig. 3. Difference scores obtained in normal intact animals in response to a noxious thermal stimulus were near zero at all three test times, due to similar response latencies of both hindpaws (Fig. 3A). However, thermal hyperalgesia was induced following CCI [overall, $F(4, 2) = 87.3$, $p < 0.001$]. This was apparent as negative difference scores obtained at 2 weeks following peripheral nerve injury in all groups receiving CCI ($p < 0.05$ compared to baselines and normal intact animals). The thermal hyperalgesia was maintained at 3 weeks following peripheral nerve injury in both nonimplanted and control-implanted animals ($p < 0.05$ compared to baseline and normal intact animals). However, thermal hyperalgesia was completely reversed in animals with adrenal medullary implants ($p < 0.05$ compared to post-CCI; $p > 0.05$ compared to both baseline and normal intact animals). Responses following adrenal medullary implantation were also significantly different than those following control striated muscle implantation ($p < 0.05$). These findings were similar to those described in previous studies (11–13).

Similar findings were observed for responses to noxious mechanical stimuli (Fig. 3B). In normal intact animals, difference scores approached zero at all three determinations. Following CCI, negative difference scores indicative of mechanical hyperalgesia on the nerve-injured side was apparent [overall, $F(4, 2) = 328.4$, $p < 0.001$]. This was reversed in animals with adrenal medullary implants ($p < 0.05$ compared to post-CCI; $p > 0.05$ compared to baseline and normal control animals), but not in control-implanted or unimplanted animals with peripheral nerve injury ($p > 0.05$ compared to 2 weeks post-CCI; $p < 0.05$ compared to baselines and normal intact animals).

Responses to innocuous tactile stimuli are shown in Fig. 3C. These are shown as cumulative response curves (% of animals reaching threshold at each bending force) 3 weeks following CCI. In normal intact animals, none of the animals responded to von Frey hairs with forces lower than 8.51 g. In contrast, in animals with peripheral nerve injury, the cumulative response curve is shifted leftward such that all the animals reach threshold below 8.51 g, some responding to very light hairs indicative of tactile allodynia [overall, $F(4, 59) = 28.7$, $p < 0.001$]. Similar tactile allodynia was observed in animals with control striated muscle implants. In contrast, tactile allodynia was attenuated in animals with adrenal medullary implants ($p < 0.05$ compared to nonimplanted and control-implanted animals). Von Frey responses in these animals were similar to normal intact animals without peripheral nerve injury ($p > 0.05$). In all three sensory tests, adrenal medullary implants alone (no CCI) did not alter responses compared to normal nonimplanted animals ($p > 0.05$).

Figure 4 shows the effects of antagonist pretreatment on sensory responses. Similar to findings described above for nonpretreated animals, adrenal medullary implants in animals with CCI resulted in attenuated hyperalgesia and allodynia when these animals were pretreated with saline. Pretreatment with either the α-adrenergic antagonist phentolamine or the opiate antagonist naloxone did not alter difference scores for either noxious thermal (Fig. 4A) or noxious mechanical (Fig.

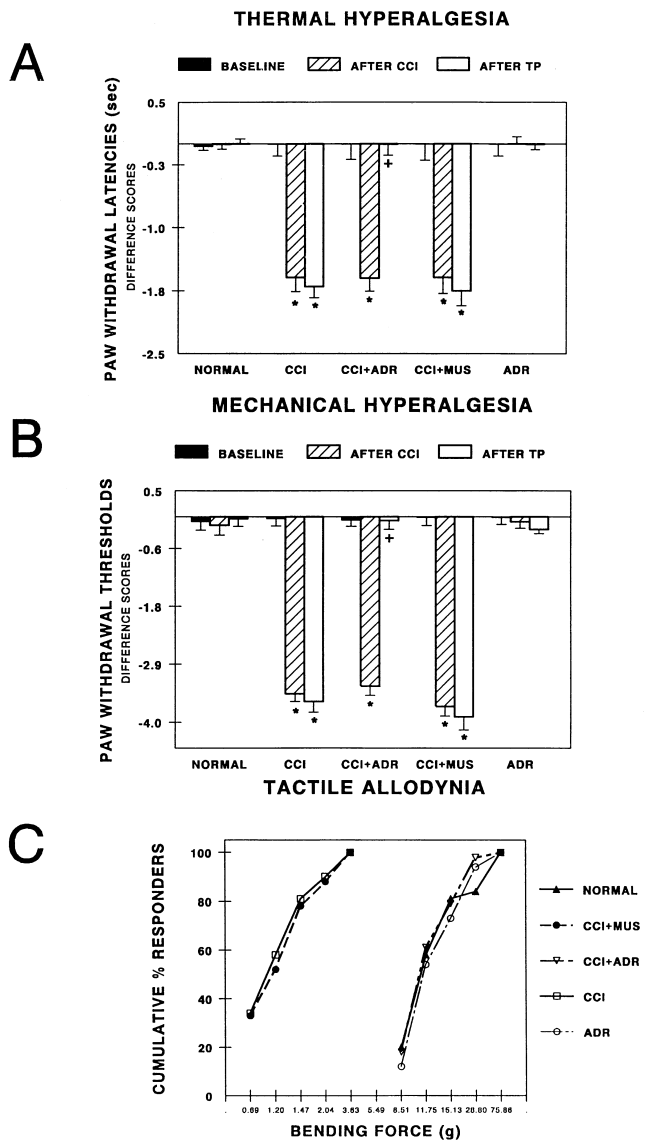

FIG. 3. Responses to noxious thermal (A), noxious mechanical (B), or innocuous tactile (C) stimuli following chronic constriction injury (CCI) of the sciatic nerve and transplantation of adrenal medullary (ADR) or control striated muscle (MUS) tissue in the spinal subarachnoid space. Five groups of animals were evaluated (n = 12/group): NORMAL = intact animals with no CCI or transplant; CCI = animals with sciatic nerve injury only (no transplant); CCI + ADR = animals with CCI followed by adrenal medullary transplantation; CCI + MUS = animals with CCI followed by striated muscle implantation; ADR = animals with adrenal medullary transplants only (no CCI). BASELINE indicates responses prior to any surgical manipulations; AFTER CCI indicates responses 2 weeks following sciatic nerve ligation in animals with CCI or at the same time point in animals without CCI; AFTER TP indicates responses 1 week following transplantation of either adrenal medullary or muscle tissue (corresponding to 3 weeks following CCI) in animals receiving transplants or at the same point in animals without transplants. Thermal and mechanical hyperalgesia are shown as difference scores (mean ± SEM) obtained by subtracting responses on the left (intact) side from responses on the right (ligated) side; $^{*}p < 0.05$ compared to baseline; $^{+}p < 0.05$ compared to after CCI. Tactile allodynia is shown as a leftward shift in the cumulative percentage responders to von Frey hairs in the lighter ranges.

4B) stimuli ($p > 0.05$ compared to saline pretreatment). It should be noted that phentolamine pretreatment did result in slightly reduced paw withdrawal thresholds even in normal control animals; however, differences between responses of both hindpaws were not altered in any of the groups. Response thresholds to innocuous tactile stimuli were not changed by antagonist pretreatment (Fig. 4C; $p > 0.05$ compared to saline pretreatment for all groups). For clarity of presentation, only responses in the CCI-transplant groups are shown; however, responses in the normal intact animals and adrenal implant only animals were similar to those of the CCI-adrenal implant group ($p > 0.05$) and responses of the CCI only group were similar to responses of the CCI-muscle implant group ($p > 0.05$).

Figure 5 shows the appearance of an adrenal medullary implant approximately 5 weeks following transplantation. Clusters of viable chromaffin cells stained with a tyrosine hydroxylase antibody are found in the spinal subarachnoid space between the spinal cord and the dura mater. The cells retain their in situ cuboidal morphology, and dense clusters are highly vascular. The graft pieces are usually loosely adherent to the meningeal surfaces, and do not appear to migrate into the host parenchymal tissue. Because only a small sampling of the transplants were evaluated, detailed analysis of chromaffin cell viability and relationship to behavioral changes was not done. However, the general appearance of all graft tissue pieces evaluated appeared similar in appearance, i.e., containing clusters of TH-positive chromaffin cells, as found consistently for adrenal medullary transplants described in previous studies (12,13,32,34,39).

DISCUSSION

Results of this study demonstrate that adrenal medullary implants into the spinal subarachnoid space can attenuate motor as well as sensory dysfunction in rats with peripheral nerve injury. Emphasis on dysfunction resulting from peripheral nerve injuries, such as that induced in the CCI model, has been focused primarily on sensory abnormalities, particularly with respect to exaggerated responses to both noxious and innocuous stimuli. However, in animals with constriction injuries of the sciatic nerve, abnormal posture, weight-bearing, and locomotion are characteristic features of the resultant syndrome (2), and transient loss in hindpaw motor function, including grasping and ankle dorsiflexion has been reported (21). These disabilities may be due to degeneration of Aα axons of the α motor neurons that innervate muscles below the knee (23). In the present study, sciatic nerve constriction resulted in severe deficits in hindlimb placing and grasping reflexes. Reflexes that involve muscles above the knee, such as hindlimb withdrawal from a noxious stimulus, appear to be intact. To some extent, the deficits in hindpaw reflexes may result from reluctance to use the injured paw due to pain, although neither of the reflexes require substantial weight bearing. In addition, results from the present study suggest that deficits in motor and sensory responses are pharmacologically distinct. Nevertheless, as the effects of peripheral nerve injury on motor and sensory function are not completely separable, dysfunction following CCI should be considered as a constellation of sensorimotor deficits.

Adrenal medullary implants significantly attenuated or reversed all of the sensorimotor deficits quantified in this study. Previous findings in our laboratory have demonstrated that these transplants can alleviate both acute and chronic pain behaviors using several animal models (12,27,28,32,34,39). Ad-

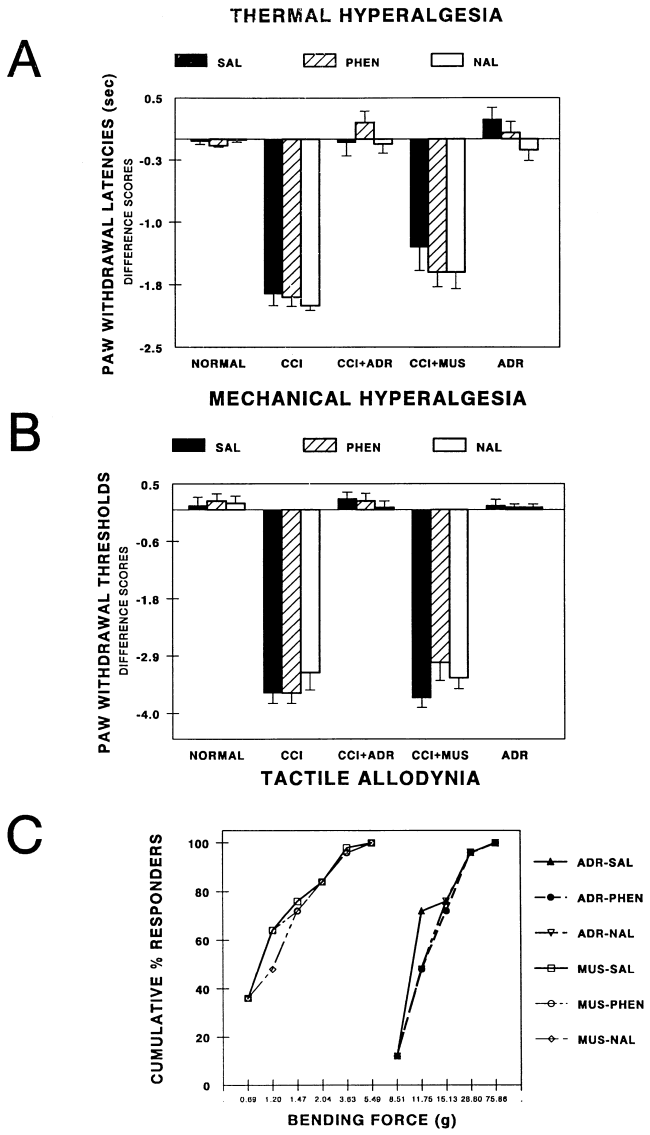

FIG. 4. Effects of antagonist pretreatment on responses to noxious thermal (A), noxious mechanical (B), or innocuous tactile (C) stimuli following chronic constriction injury (CCI) of the sciatic nerve and transplantation of adrenal medullary (ADR) or control striated muscle (MUS) tissue in the spinal subarachnoid space. Five groups of animals were evaluated (n = 12/group): NORMAL = intact animals with no CCI or transplant; CCI = animals with sciatic nerve injury only (no transplant); CCI + ADR = animals with CCI followed by adrenal medullary transplantation; CCI + MUS = animals with CCI followed by striated muscle implantation; ADR = animals with adrenal medullary transplants only (no CCI). Animals were pretreated either with saline vehicle (SAL), α-adrenergic antagonist phentolamine (PHEN; HCl, 10.0 mg/kg, SC), or opiate antagonist naloxone (NAL; HCl, 2.0 mg/kg, SC) 15 min prior to testing. Thermal and mechanical hyperalgesia are shown as difference scores (mean ± SEM) obtained by subtracting responses on the left (intact) side from responses on the right (ligated) side. Responses to tactile stimuli are shown as the cumulative percentage responders through a range of von Frey hairs.

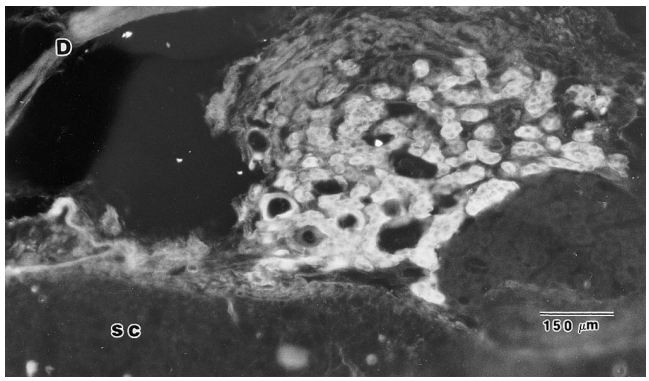

FIG. 5. Appearance of an adrenal medullary transplant in the spinal subarachnoid space 5 weeks following implantation. Chromaffin cells in the transplants were identified using a tyrosine hydroxylase primary antibody and rhodamine-linked secondary antibody. SC = spinal cord; D = dura mater.

renal medullary chromaffin cells were initially selected as donor material for these implants as they secrete several pain-reducing neuroactive substances, notably catecholamines and opioid peptides, agents that can act at host spinal α-adrenergic and opioid receptors to produce analgesia (22,40,41). This notion has been supported in studies utilizing acute pain models, as analgesia following adrenal medullary implantation in the spinal subarachnoid space is blocked by either α-adrenergic or opioid antagonists (27–29). In addition, increased levels of both catecholamines and opioid peptides are found in the spinal CSF of animals with adrenal medullary implants (25,26). However, recent studies in our laboratory have suggested that mechanisms of tonic pain reduction may be pharmacologically distinct (32,34). In particular, using the formalin model that produces an early acute pain response and a delayed tonic pain phase, α-adrenergic or opioid antagonists, which prevented attenuation of the first phase, had no effect on attenuation of the tonic phase by adrenal medullary implants. The possibility that adrenal medullary implants reduce chronic pain by alternate mechanisms is supported by results of the present study, as neither the α-adrenergic antagonist phentolamine nor the opioid antagonist naloxone reversed the beneficial effects of the implants on abnormal pain behaviors.

In contrast to pain behaviors, catecholamines do appear to play a role in the restorative effects of the adrenal medullary implants on motor reflexes, as this was reversed by phentolamine. In intact animals, motor facilitation is thought to involve descending brainstem catecholaminergic projections, notably from the dorsolateral pontine coeruleospinal system [for review, see (9,10,17). Activation of this system potentiates the lumbar monosynaptic reflex and depolarizes hindlimb flexor and extensor motoneurons, partially via an α_1 receptor mechanism (10). Other colocalized neurotransmitters, including enkephalin and glutamate, have also been implicated in these processes (9). These findings have led to studies utilizing fetal locus coeruleus intraspinal implants to restore motor function following spinal cord transection (3,5,42). This resulted in some improvements in locomotion (42) and increased force of the hindlimb flexor reflex that was partially blocked by α-adrenergic antagonist phenoxybenzamine (3). A similar recovery in force associated with the hindlimb withdrawal reflex in spinally transected animals was reported following chromaffin cell implants into ventral regions of the spinal cord (24).

In the present study, adrenal medullary implants, when placed in the spinal CSF, can also partially restore severe motor reflex deficits consequent to peripheral nerve injury. This may be mediated by catecholamine release from the implanted chromaffin cells as it is reversed by α-adrenergic blockade, and is supported by previous studies in our laboratory demonstrating increased spinal CSF catecholamine levels in animals with adrenal medullary transplants (26). Previous findings in our laboratory have additionally suggested that adrenal medullary implants can promote recovery in spinal cord pathology resulting from peripheral nerve injury, including restoration of spinal inhibitory interneurons (11,18) and reduction in components of the NMDA-NO cascade (14,31). In addition to changes in the dorsal horn, motor neuron function may also be altered, as reduced NMDA receptor immunoreactivity associated with motor neurons following CCI is restored by adrenal medullary implants (15). It is possible that neurotrophic effects of adrenal medullary implants contribute to these restorative effects as chromaffin cells produce a "cocktail" of neurotrophic factors, including basic fibroblast growth factor (β-FGF), transforming growth factors β (TGF-β), interleukin-1 (IL-1), and neurotrophin (NT)-4/5 (37,38).

In summary, the results of this study indicate that adrenal medullary implants in the spinal subarachnoid space can improve sensorimotor function following peripheral nerve injury. In particular, severe deficits in hindlimb reflexes resulting from sciatic nerve constriction are markedly restored by adrenal medullary implants, possibly mediated, at least in part, by catecholamine release from the implanted cells.

ACKNOWLEDGEMENTS

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