Acta Neurochir (Wien) (1990) 105: 39–43

Acta Neurochirurgica

Study of Nerve Regeneration in Centrocentral Anastomosis

J. L. Gil-Salú and **J. M. González-Darder**

Department of Neurosurgery, Faculty of Medicine, University of Cádiz, Cádiz, Spain

Summary

Nerve regeneration was studied in a model of centrocentral anastomosis (CCA) performed on the sciatic nerve of the rat. Experimental CCA was made by suturing the proximal end of the peroneal branch on the proximal end of the sural branch, placing between them a peroneal nerve graft (Group I, 20 rats) or a silicone chamber (Group II, 12 rats). Nerve grafts had a length of 5 mm and silicone chambers 7 mm. In six silicone chambers an 1 mm nerve graft was placed in the centre of the tube. In group I animals anterograde degeneration was studied by cutting the graft 60 days after surgery. In group II, nerve regeneration was studied 2, 4 and 8 weeks after surgery. Results indicate that in CCA: 1) regenerated axons coming from one nerve end grow into the graft but do not cross the contralateral suture line; 2) regeneration is poorer in silicone chambers than in nerve grafts; and, 3) in silicone chambers regeneration is related to time. The reduction in the regenerative capability in CCA seems to be related to the alteration of nerve sprouts aiming for the peripheral targets.

Keywords: Peripheral nerve regeneration; centrocentral anastomosis; silicone chambres technique; nerve growth factors; sciatic nerve; rat.

Introduction

The centrocentral anastomosis (CCA) may be defined as the terminoterminal suture among the fascicles of the proximal stump of a sectioned peripheral nerve, with the interposition of a graft between each pair of fascicles.

The CCA was introduced by Slooff[10] and Samii[8], and some clinical and experimental studies have shown this to be effective in reducing the size of the terminal neuroma an neuroma related pain[1–3,8,10]. The first point of our experimental work was directed towards study of nerve regeneration in a CCA model to determine how the CCA could modify the factors involved in the pathogenesis of neuroma-related pain. To achieve this, an experimental model of a single CCA was developed in the sciatic nerve of the rat. On the other hand, some authors have proposed that the reduction in the regenerative capability of the axon sprouts observed in the CCA is due to the isolation of those from the nerve growth factors (NGF). To clarify this point, a second experiment was designed studying nerve regeneration in a CCA created in the sciatic nerve of the rat with the interposition of a silicone chamber instead of the nerve graft.

Materials and Methods

Adult Sprague-Dawley male rats were used for the study, weighing 200–220 g at the beginning of the experiments. For surgical procedures animals were intraperitoneally anaesthetized with ketamine, diazepam, and atropine sulphate. The sciatic nerve was approached in the mid-thigh and the tibial, peroneal, and sural branches were dissected using microsurgical techniques (Fig. 1 a). Both peroneal and sural branches were cleanly severed about three mm distal to their origin. In a first group of twenty animals (Group I), a nerve graft of 5 mm lenght was taken from the peroneal branch and end-to-end sutured to the terminal stumps of the peroneal and sural nerves (Fig. 1 b). In a second group of twelve rats (Group II) peroneal and sural nerves were severed and each stump was insersted into the opposite ends of a silicone tube[5,6]. The silicone tube had an internal diametre of 1 mm, an outer diametre of 1.2 mm and a length of 7 mm. The silicone tube was left empty in six rats and in the others a small piece of 1 mm of peroneal nerve was placed in the centre of the tube before the insertion of the nerve stumps (Fig. 1 c). A single 10/0 Ethilon stich was used to suture the nerve grafts in group I animals and to secure the nerve stumps into the silicone tube in group II rats. In all animals the surgical wounds were closed in layers and no antibiotic agents or topical or parenteral drugs were administered. The animals were housed under standard colony conditions. At the end of the experiments the animals were sacrificed by means of a lethal dose of sodium thiopental.

Sixty days after CCA was performed the animals belonging to group I were operated on for the second time. By approaching the CCA and using a gentle microsurgical technique the mid-point of the nerve grafts were identified and cleanly severed, fixing each stump

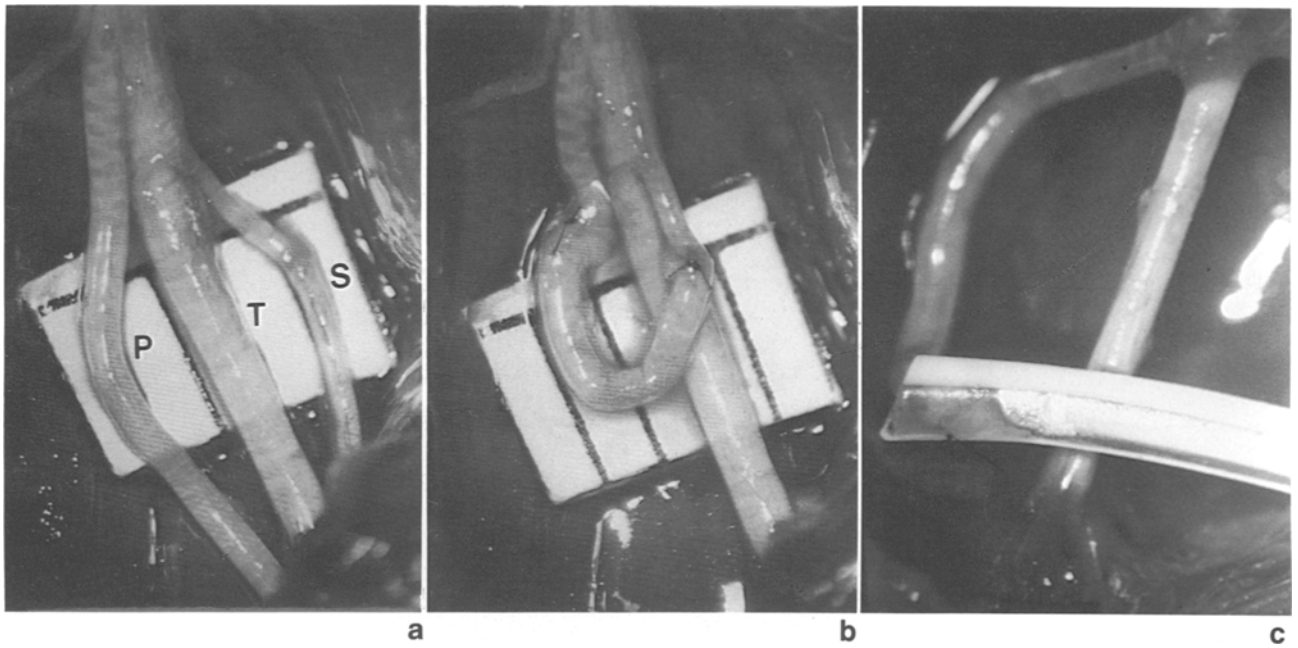

Fig. 1. Surgical technique of centrocentral anastomosis. a) Microsurgical exposure of the sciatic nerve in the rat, showing the tibial (*T*), peroneal (*P*), and sural (*S*) branches. b) Operative photograph of the completed centrocentral anastomosis between the sural and peroneal branches in group I. A nerve graft is interposed between both nerves. c) Operative photograph of the centrocentral anastomosis in group II. The central nerve stump of the peroneal branch is inserted in the end of a silicone tube and secured by means of a single stich. The central stump of the sural nerve was placed in the same way in the other end of the chamber. In this case no isolated nerve graft was left into the chamber. In photographs a and b measure is in millimeters.

to the neighbouring muscles with a perineural 8/0 stich. Ten days later, the CCA was microsurgically approached again and the stumps of the previously severed nerve graft and the parent peroneal and sural nerves were removed, identified, orientated, and stored for histological study. Animals of group II with silicone tubes were sacrified 2, 4, and 8 weeks after surgery and the tubes with the adjacent peroneal and sural nerves were removed for macroscopic and histological examination.

The specimens were prepared for pathological study in the following manner: double fixation in glutaraldehyde and osmium tetroxide, subsequent dehydratation through graded alcohols, and final inclusion in araldyte. Transverse sections of 2 μm thick were cut on a Ultracut Reichert-Jung Ultramicrotome, stained with the metachromatic toluidine blue technique and examined on a Leitz Ortopan Optic Microscope. Sections were taken in sequence at different levels of the specimens for the purpose of covering the entire length of grafts or silicone tubes and parent nerves.

Results

The surgical technique used was uneventful and there was no mortality nor complication during the period of observation.

In group I animals three areas were specially considered in the pathological study: the distal portion of the cut nerve graft, corresponding to the centre of the graft; the proximal portion of the nerve graft, just distal to the suture line; and the distal portion of the parent peroneal and sural nerves above the suture lines. The former area consisted in a large number of fascicles containing fibres in a recent wallerian degeneration stage and fibres in different stages of regeneration (Fig. 2 a). Some occasional fascicles were seen containing simultaneously regenerative and degenerative nerve fibres (Fig. 2 b). This mixed regenerative-degenerative pattern was also observed along the nerve graft. However, near the suture line there was a predominance of regenerative sprouts. The regenerated axons growing into the nerve graft showed medium to large diameters. The parent nerves above the suture line enlargement showed essentially histological pattern of a normal peripheral nerve, without evidence of wallerian degeneration or regeneration.

Silicone tubes of group II animals sacrified 2 weeks after surgery were seen filled with a yellowish or xanthochromic fluid on macroscopic examination. However, silicone tubes belonging to 4 and 8 week animals showed a vascularized structure bridging the gap between both nerve stumps (Fig. 3 a). The tubes showed a relatively thick thread regardless of the regeneration time or the presence of the nerve piece left free in the

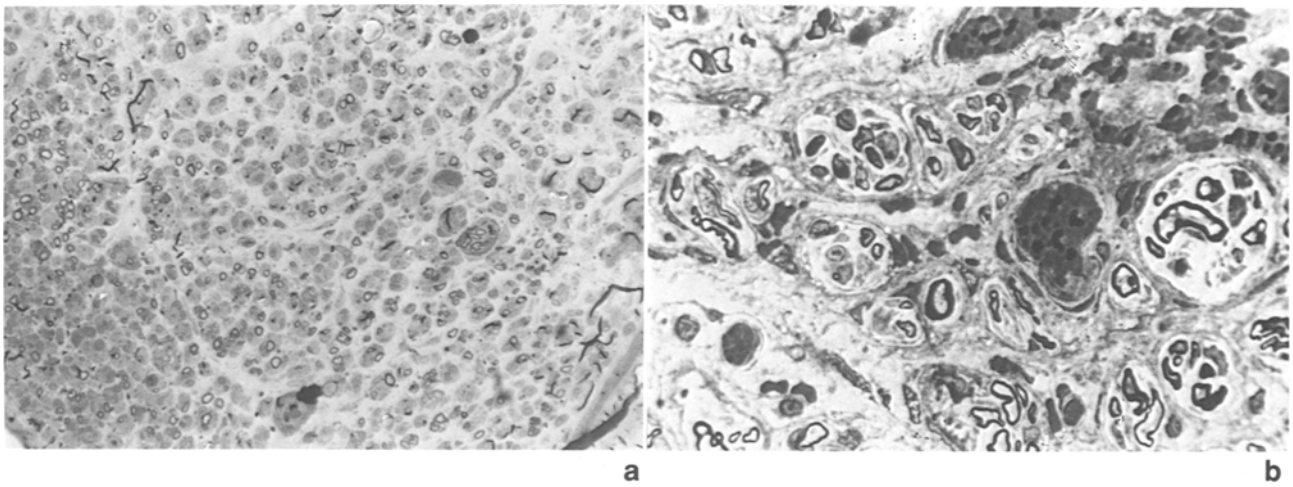

Fig. 2. Photograph of transverse sections of centrocentral anastomosis (CCA) in group I. a) Section from the end of the cut nerve graft, corresponding to the central area of the CCA, showing a regenerative fascicular pattern. Fascicles contain regenerative and degenerative fibers (×40). b) Magnification of the same area showing into some singles fascicles both regenerated and degenerated fibers (× 100)

chamber. Microscopic examination demonstrated an amorphous material without vessels, nerve fibres or connective tissue in the 2 week specimens. Both nerve ends showed a regenerative pattern extending only 0.5 to 1 mm into the tube (Fig. 3 b). In the 4 week chambers the regenerated axons extended almost through the entire length of the chamber, although their number, density and thickness decreased in the centre of the chamber (Fig. 3 c). When the regeneration time was 8 weeks the axonal pattern was similar near the nerve stumps and in the centre of the tube (Fig. 3 d). There were no significant differences between tubes with or without isolated nerve pieces.

Discussion

The centrocentral anastomosis is an ingenious attempt in the prevention and treatment of terminal or postamputation neuromas. CCA reduces the size of experimental neuroma[1–3, 8]; reduces the autotomy behaviour in the rat if the procedure is performed immediately after nerve section as well as following resection of a terminal neuroma[1]; and, clinical studies have shown a remarkable pain reduction when it is used for the treatment of painful neuromas[3, 8, 10]. In addition to that clinical interest, CCA is an interesting experiment itself to study peripheral nerve regeneration since the model involves the growing within a nerve graft by axons coming from both graft ends.

In our experiments we have studied the regeneration pattern using an anterograde wallerian degeneration technique. Following CCA would have a nerve growth sprout across both suture lines and along the interposed nerve, reaching and crossing eventually the contralateral suture lines. Sixty days were left allowing this regeneration process. Cutting the nerve graft an anterograde wallerian degeneration in the axons regenerated in the contralateral portion of the nerve graft is promoted. These degenerative changes were histologically studied ten days later and let us know the point reached by the nerve sprouts. The results of our experiments show that the axonal sprouts are growing within the interposed nerve graft. They pass in both directions from one side to the other of the graft developing clusters into the graft of crossing inside a single cluster. However, the sprouts are not able to cross over the contralateral suture lines. Most of these regenerative fibres are fully myelinated and the resemblance of the graft is quite similar to a normal nerve.

These findings lead to some neurophysiological and neuropathological considerations. However, the main issue is to explain why the regenerating fibres cannot cross the contralateral suture line. Gorkisch *et al.*[2] suggested an alteration in the axonal flow due to the increase of the intraperineurial pressure. Also it would be related to the mechanical barrier developed in the suture line by the scar. An additional factor would be the difficulty or impossibility of finding free Hanke-Bugner's bands on the other side of the suture line. Seckel[9] postulated the isolation from target derived neurotrophic factors and the confinement of the nerve sprouts into a non-target environment. The CCA model with silicone tube was designed to explore the last hypothesis. Recent basic research studies have demonstrated that macrophages coming from the neigh-

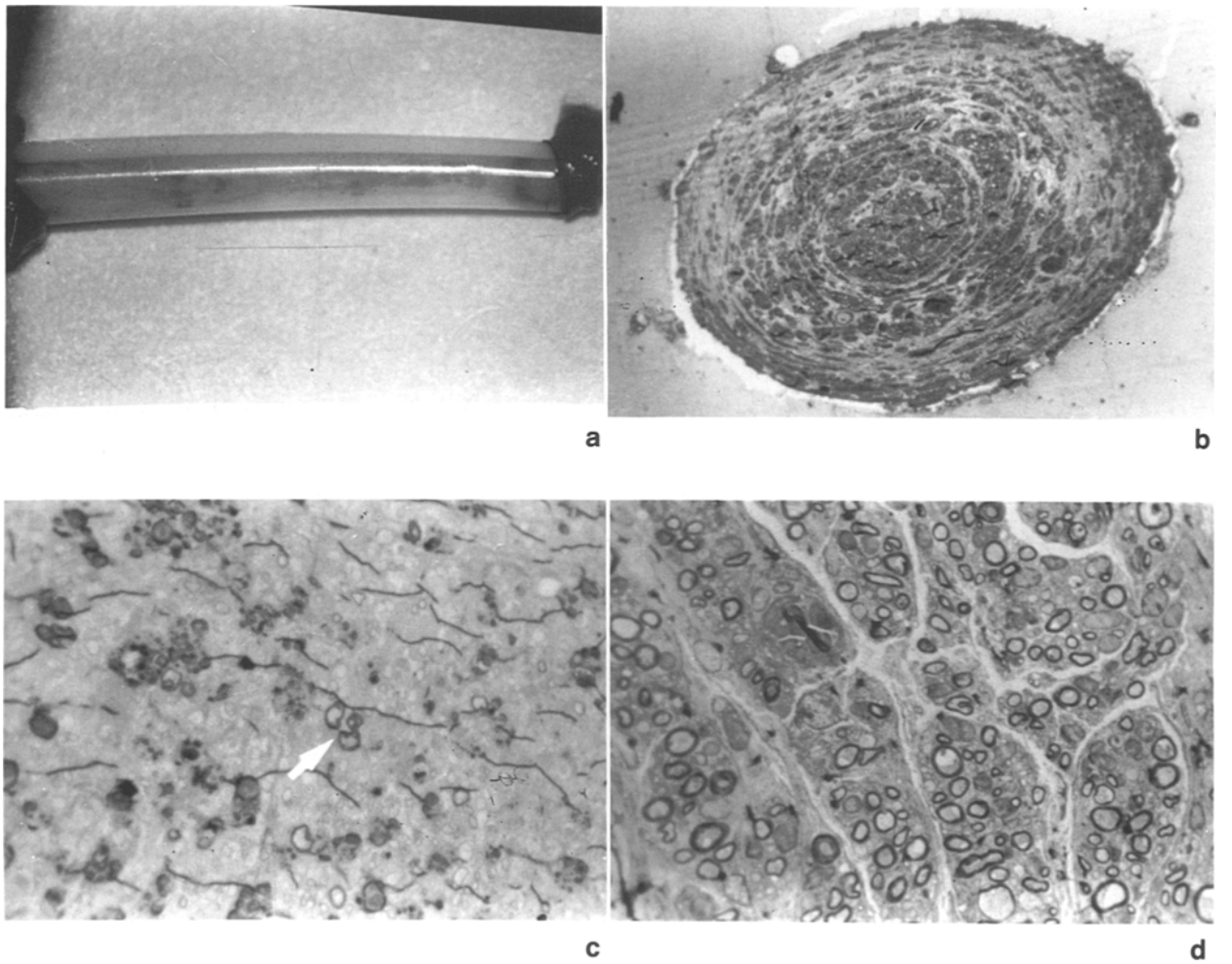

Fig. 3. Pathological study of centrocentral anastomosis (CCA) in group II. Macroscopic appearance of silicone chamber without nerve graft after 8 weeks of regeneration. b) Microscopic section from the end of a silicone chamber without nerve graft after 2 weeks, showing an irregular pattern of regeneration surrounded by some layers of connective tissue. These structures are surrounded by an amorphous fluid (× 10). c) Section from the center of a 4 weeks silicone chamber without nerve graft after 4 weeks, showing a regenerative pattern (× 40). d) Section of the center of a 8 weeks silicone chamber without nerve graft, showing a typical regenerative pattern with axons grouped in fascicles (× 40)

bouring tissues during wallerian degeneration have an important role in the regulation of the NGF-synthesis[4,7]. After a nerve injury macrophages invade the nerve increasing NGF-mRNA levels in Schwann cells and fibroblastic-like cells. This actions seems to be mediated by the interleukine-1 produced by macrophages. The levels of NGF-mRNA increase in the distal stump just distal to the axon sprouts guiding therefore the nerve regeneration peripherically.

In the CCA model with silicone tubes the tube isolated the degenerative-regenerative process from the neighbouring tissues and, in some way from the rest of the organism. Consequently macrophages are not allowed to arrive at the nerve stumps and interleukin-1 is not released. Moreover, into the chambers there are no (or in a less amount) target cells capable of producing NGF. These factors could explain the remarkably reduced regeneration capability in CCA with silicone tubes. In regular CCA with nerve grafts macrophages can arrive and target cells produce NGF. However, the regeneration coming from both sides, the time sequence and spatial organization of the process would be inadequate to ensure a satisfactory regeneration. On the other hand, in parent nerve branches proximally to the suture lines, wallerian degeneration does not occur and the NGF-synthesis would not be activated. This lack of guiding substratum could explain how axonal regeneration is reduced in the CCA.

In conclusion, following centrocentral anastomosis the axonal sprouts crossing the first suture line penetrate into the nerve graft where they grow freely, stopping in front of the second suture line. Axonal sprouts are well myelinated by Schwann cells and protected against the irritating perineural scar. The reduction in regenerative capability in CCA seems to be related to the lack of an adequate directional force to the peripheral targets.

References

1. González-Darder JM, Barberá J, Abellán MJ, Mora A (1985) Centrocentral anastomosis in the prevention and treatment of painful terminal neuroma. An experimental study in the rat. J Neurosurg 63: 754–758
2. Gorkisch K, Boese-Landgraf J, Vaubel E (1984) Treatment and prevention of amputation neuromas in hand surgery. Plast Reconstr Surg 74: 293–296
3. Lagarrigue J, Chavoin JP, Belahovari L, Savazza R (1982) Traitment des nevromes doloreux par anastomoses nerveus en anse piege a neurone. Neurochirurgie 28: 91–92
4. Lindholm D, Heumann R, Meyer M, Thoenen H (1987) Interleukin-1 regulates synthesis of nerve growth factor in non-neuronal cells of rat sciatic nerve. Nature 330: 658–659
5. Lundborg G, Hansson HA (1979) Regeneration of peripheral nerve through a preformed tissue space. Preliminary observations on the reorganization of regenerating nerve fibres and perineurium. Brain Res 178: 573–576
6. Lundborg G, Dahlin LB, Danielsen N, Gelberman RH, Longo FM, Powell HC, Varon S (1982) Nerve regeneration in silicone chambers: influence of gap length and of distal stump components. Exp Neurol 76: 361–375
7. Perry VH, Brown MC, Gordon S (1987) The macrophage response to central and peripheral nerve injury. J Exp Med 165: 1218–1223
8. Samii M (1981) Centrocentral anastomosis of peripheral nerves. A neurosurgical treatment of amputation neuromas. In: Siegfried J, Zimmermann M (eds) Phantom and stump pain. Springer, Berlin Heidelberg New York, pp 123–125
9. Seckel BR (1984) Treatment and prevention of amputation neuromas in hand surgery: Discussion. Plast Reconstr Surg 74: 297–299
10. Slooff ACJ (1978) Microsurgical possibilities in the treatment of peripheral pain. Clin Neurol Neurosurg 80: 107–111

Correspondence and Reprints: J. M. González-Darder, Department of Neurosurgery, Faculty of Medicine, 11002-Cádiz, Spain.