The Use of Osmotic Minipumps to Block Impulse Conduction in Peripheral Nerve and Spontaneous Activity in Denervated Skeletal Muscle

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Abstract

Two methods which allow the chronic block of nerve impulse and the chronic depression of the spontaneous activity induced by denervation in skeletal muscle are described. The techniques utilize osmotic minipumps to obtain a steady delivery of a substance, which by means of a catheter attached to the pump, is released at the level of nerve or skeletal muscle.

Key words: osmotic minipumps, nerve block, skeletal muscle.

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Many studies have been made with the aim to understand the complex interactions between spinal motoneurons and skeletal muscle fibres. These experiments clarified several aspects of the nervous influence on the development and differentiation of muscle fibres and revealed a reciprocal influence of muscle fibres on the growth and sprouting of nerve terminals [6, 8].

Among the various experimental models (denervation, cross-innervation, direct chronic stimulation of the muscle, cordotomy and others) of particular interest is the one which allows to block impulse conduction in the nerve, without damaging the nerve itself. This model helps to distinguish neuromotor from neurotrophic influences on skeletal muscle properties.

Robert and Oester [6] in the rabbit and subsequently Lømo [3] in the rat, were able to block the impulse conduction by positioning around the sciatic nerve at the trochanteric region, a silicon cuff mixed with an anaesthetic (Lidocaine or Bupivacaine).

This method was improved by the use of osmotic minipumps [1], which permit the storage of a fixed amount of a substance and its subsequent continuous and steady release. The delivery rate of the solution from the pump is regulated by body temperature and osmolality.

We are using the osmotic minipump technique to study the effects of chronic nerve block on muscle under particular experimental conditions (muscle regeneration after acute degeneration).

With the same technique we also developed a method which permits a chronic depression of the spontaneous activity (fibrillation) induced in skeletal muscle by denervation. This method proved to be of help in assessing the importance of fibrillation for the effects of denervation on muscle [5].

Materials and Methods

All experiments were carried out on adult male Wistar rats (300-400 gr.), according to the International Guiding Principles in the care and use of animals in research.

Chronic block of nerve impulse conduction

The osmotic minipump (Alzet 2002, Alza Corp., 200 µl capacity, mean pumping rate of solution 0.5 µl/h) is usually implanted intraperitoneally and is connected by means of a catheter to a cuff made with a longitudinally slit silicon tubing. The cuff is placed around the sciatic nerve exposed through a little incision of the skin at the trochanteric region [2, 6].

In our experiments the minipump, attached to a polyethylene catheter (Intramedic, Clay Adams, i.d 0.22 mm, o.d. 0.61 mm, lenght 100 mm), is implanted under the skin in the interscapular region. The catheter, via a subcutaneous tunnel, is then placed near the sciatic nerve exposed at the trochanter level as above described. In order to avoid the damage of the nerve as a consequence of the elastic recoil of the cuff, the longitudinally slit tubing is replaced with a rectangular silicon patch (5 mm wide, 15 mm long, 0.5 mm thick). The patch is carefully positioned

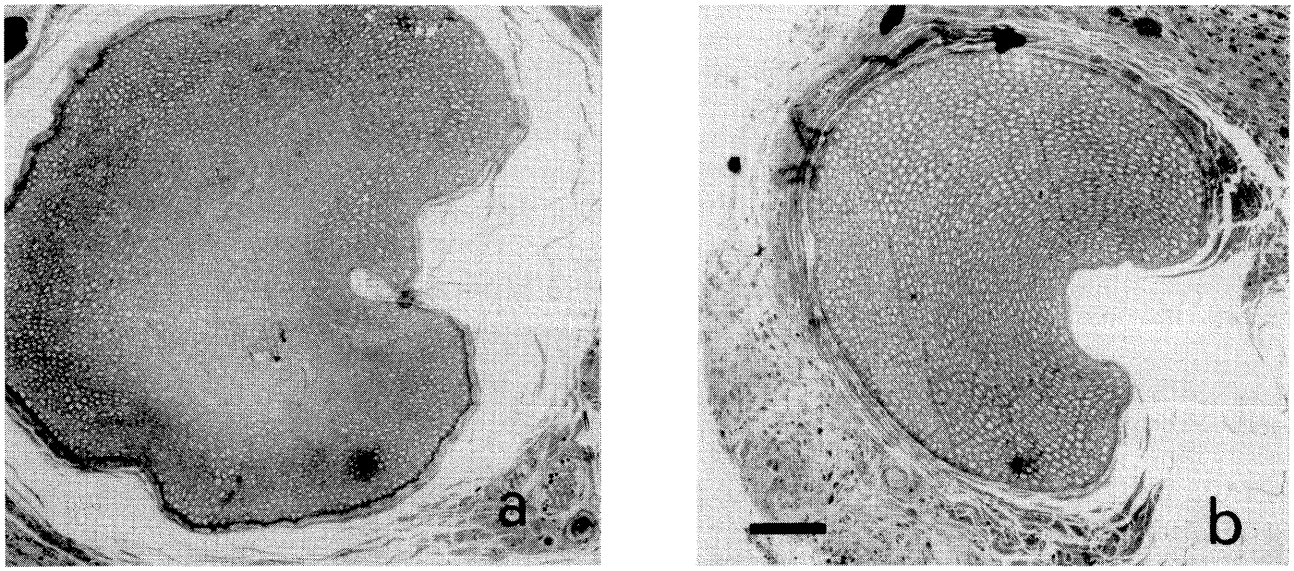

Figure 1. Cross sections of the sciatic nerve stumps above (a) and below (b) the silicon cuff. (Semithin sections fixed using the Karnovsky method and postfixed in osmium. Toluidine blue staining. X 32 - calibration bar: 312 μ).

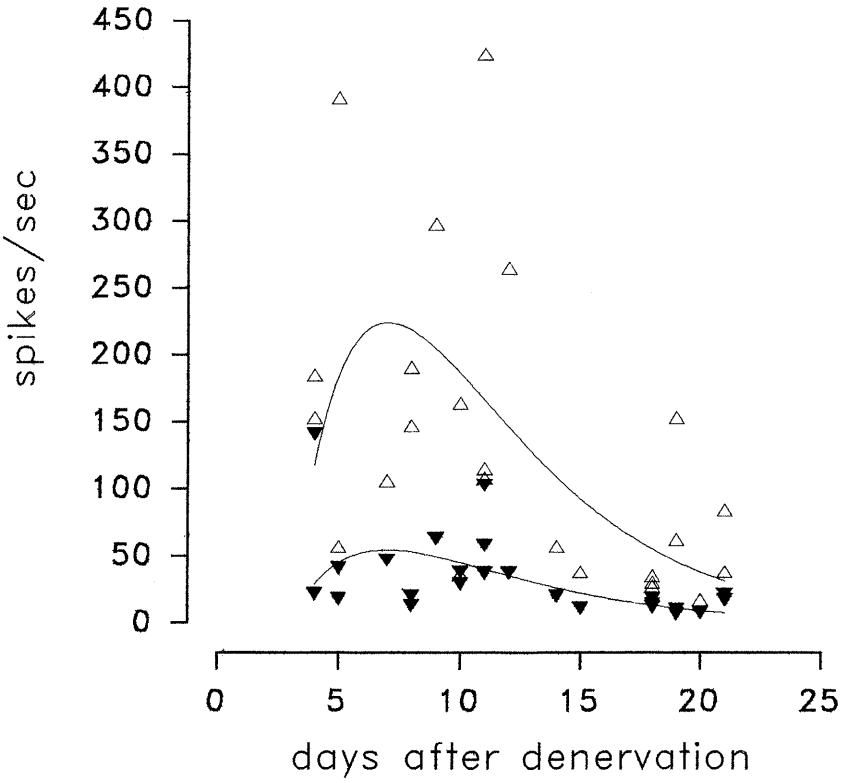

Figure 2. Fibrillation activity in the denervated soleus muscles of 9 rats. The left soleus was treated with procainamide (DPR, filled symbols), the right served as control (D, open symbols). Ordinate, spikes/s; abscissa, days after bilateral denervation. The differences between DPR and D muscles were highly significant ($P < 0.05$) (From: M.Midrio et al.: Pflügers Arch 1990; 420: 446-450).

underneath and around the nerve, and its extremities are firmly bound around the catheter tip with surgical silk. In this way we can easily adjust the diameter of the cuff to the diameter of the nerve, therefore reducing the possibility of a trauma to the nerve itself. Moreover, the catheter tip can be brought as close as possible to the nervous tissue.

The diffusion of the substance delivered by the catheter to the surrounding tissue is reduced by positioning a little piece of biocompatible surgical sponge (Spongostan, Ferrosan) between the cuff and the nerve.

The block of neural activity is achieved by superfusion of the nerve with tetrodotoxin (TTX, Sigma 250-500 μg/ml in sterile saline) and is checked daily by observing the absence of toe-spreading and withdrawal reflexes in the limb of the treated side [6]. At a 4-5 day interval, beginning on the 3rd day after surgery, electromyographic recordings are also taken from the muscles of the paralyzed leg to check for the presence of muscle fibrillation.

At the end of the experiment, before killing the animals, the nerve block is confirmed by the absence of neural activity below the silicon cuff upon stimulation of the sciatic nerve above the cuff. After killing the rats by neck dislocation under ether anaesthesia, the sciatic nerve stumps above and below the cuff, as well as the soleus nerve of the treated side, are removed and analyzed with light microscopy to evaluate the presence of axonal degeneration.

Chronic block of fibrillation in denervated muscle

Preliminary studies were devoted to test the antifibrillatory activity on the denervated muscle of drugs (verapamil, quinidine, lidocaine and procainamide) known to depress cardiac automatism through their membrane stabilizing properties. The acute antifibrillatory effect of these drugs was investigated following their injection in the iliac artery 5-7 days after section of the sciatic nerve, while recording electromyographic activity from the leg denervated muscles. These preliminary studies led to the exclusion of verapamil because it had no effects as well as of quinidine and lidocaine for their local and systemic effects. Consequently, we selected procainamide to continue the investigation.

The osmotic minipump (Alzet 2002, Alza Corp., 200 μl capacity, mean pumping rate of solution 0.5 μl/h) was inserted 3 days after bilateral sciaticotomy, i.e. at the onset of fibrillation activity [4].

The pump was filled with an aqueous solution of procainamide hydrocloride, containing 75 g drug in 100 ml solution. The pump was inserted in the subcutaneous tissue of the interscapular region, and it was attached to a polyethylene catheter (o.d 1.5 mm), which via a subcutaneous tunnel reached the lateral side of one leg. Through a small cutaneous incision, the tip of the catheter was exposed and, by sectioning the underlying superficial fascia, it was accurately positioned between the gastrocnemius and the soleus muscles. The fascia was subsequently sutured and the suture held firmly in place the catheter, as it was checked at the end of the experimental period.

Electromyographic records were taken from the soleus muscle of treated and control side beginning on 3rd-4th day after denervation and then were repeated in the same animal at 5- to 7-day intervals, with a total of 3-4 recordings for each animal.

Results and Discussion

In most animals in which the sciatic nerve was superfused with TTX, a block of nerve impulse conduction was observed. The block was only partial with the lower TTX concentration, while with the higher concentration the block appeared to be complete for 8-10 days.

The electromyographic recordings from the limb muscles of the blocked side showed only in few cases the appearance of spontaneous activity. At the following light microscopy analysis, the axons of the treated sciatic nerve appeared to be normal and myelinated above (Fig.1a) and below the cuff (Fig.1b); similar aspects were observed in the ipsilateral soleus nerve. In the cases in which a fibrillatory activity was previously recorded, the subsequent light microscopy analysis revealed the presence of degenerated axons and myelin sheaths between normal axons.

Concerning the use of osmotic minipumps to chronically deliver an antifibrillatory drug to denervated muscles, we were able to obtain a marked depression of fibrillation throughout the experimental period in an elevated percentage of treated animals. With respect to the contralateral denervated not treated muscles, fibrillation of treated muscles showed the greatest percentage reduction up to the 12th day after denervation, whereas in the subsequent days, in coincidence with the spontaneous reduction of fibrillation activity in denervated muscles [5], the difference became progressively smaller (Fig. 2). The amount of fibrillation activity during the whole experimental period was reduced, on average, in denervated treated muscles to 25 % of the value observed in the contralateral denervated muscle. A complete block of fibrillation was never observed. In control experiments the insertion of the catheter alone had no appreciable effects on fibrillation activity.

At histological analysis, no signs of pathological damage of the treated muscles were observed.

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