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Short Communications and Technical Notes

Changes in Resting Membrane Potential and Contractility of Innervated and Denervated Skeletal Muscle Free Grafts in the Rat

F. Vyskočil, B. Carlson, and E. Gutmann

Institute of Physiology, Czechoslovak Academy of Sciences, Prague; Dept. of Anatomy, University of Michigan, Ann Arbor

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Summary. In free orthotopic auto-grafts of the extensor digitorum muscle of rats a marked temporary decrease of resting membrane potential (RMP) of two superficial layers of muscle fibres is observed at 2 days with subsequent recovery 4 days after transplantation. Such a temporary decrease of the RMP is not observed in grafts of denervated muscle. This difference in change of RMP is apparently related to a temporary marked decrease or loss of contractility observed in innervated but not denervated graft and may explain in part the relatively more successful grafting of denervated muscles.

Key words: Membrane Potential — Contractility — Denervation — Transplantation.

Skeletal muscle may be transplanted after mincing or as a free graft of the entire muscle, the muscle fibres undergoing successively a degenerative reaction and a regenerative response (Studitsky, 1959; Carlson 1972) resulting in recovery of a muscle with normal isometric twitch characteristics of the muscle after minced (Salafsky 1971, Carlson and Gutman 1972) and free (Carlson and Gutmann 1973) muscle grafts. Attempts of grafting larger mammalian skeletal muscles have met with little success, unless the muscles had been denervated prior to grafting (Studitsky and Zhenevskaya 1967, Thompson 1971). The differences in success of free denervated grafts have been thought to be due to a "plastic" condition, involving a shift to anaerobiosis, increase of myoblasts and RNA in the denervated muscle (Studitsky 1963). In a previous paper (Carlson and Gutmann 1973) it could be shown, that there are basic differences between innervated and denervated free muscle grafts in respect to contractility in the early stages after transplantation. Innervated free grafts lose completely or almost completely their contractility 2 days and recover it 4 days after transplantation. Denervated free grafts do not exhibit this temporary complete or almost complete loss of con-

tractility and maintain the normal characteristics of an acutely denervated muscle throughout this period (Carlson and Gutmann 1973).

Free muscle grafts, i.e. intact or denervated superficial muscle fibres, survive temporarily in an avascular environment before revascularisation. This might result in changes in resting membrane potential, redistribution of internal K^+ ions, decrease in ionic permeability and structural changes of the muscle membrane which might differ in innervated and denervated grafts. Moreover, decrease of RMP has been observed after nerve section (Albuquerque and McIsaac 1970, Redfern and Thesleff 1971) and this has been related to alterations in the electrogenic sodium pump (Locke and Solomon 1967).

We have therefore investigated the changes in testing membrane potential in innervated and denervated free muscle grafts and correlated these with contractility during the earliest stages after free transplantation of muscle.

Material and Methods

Orthotopic auto-transplantation of the extensor digitorum longus (EDL) muscle of one-month-old rats was accomplished by severing the tendons of origin and insertion, removing the normal (innervated) or denervated muscle and resuturing their tendons to the corresponding previous tendon stumps. When using denervated free grafts the initial denervation was performed in the left leg and the denervated EDL was then transplanted into the bed of the removed EDL on the right side 14 days after denervation (see Carlson and Gutmann 1973). The muscles were removed 2 and 4 days after transplantation. Records of resting membrane potentials (RMP) were made from two layers of surface muscle fibres (the first superficial and the second underlying one) along the midline of the muscle. Intracellular recording was done with a glass microelectrode filled with 3 M KCl (10—15 MΩ), and the potentials were monitored on a oscilloscope. Continuous recording of microelectrode resistance was made during the experiments. The isometric twitch characteristics of the muscles were determined in vitro by the method of mass-stimulation (Sandow and Brust 1958) and with the help of an automatic analyser of muscle contraction (Carlson and Gutmann 1972). Maximal amplitude and contraction time (CT-time to peak) of the isometric twitch of innervated („normal") and denervated EDL graft were recorded 2 and 4 days after transplantation.

Results and Comments

Fig. 1 shows the overall changes in RMP of the muscle fibres in the first and second layer and both layers (taken together) observed in control muscles (A) and in grafted innervated muscle 2 days (B) and 4 days (C) after transplantation. The following bars show the corresponding changes in the denervated control muscles (D) in the denervated free grafts 2 (E) and 4 (F) days after transplantation. It can be seen, that 2 days after transplantation of the innervated EDL there is a decrease of RMP in both layers of the muscle fibres, being however much more marked in the second layer of muscle fibres (B). A marked increase is

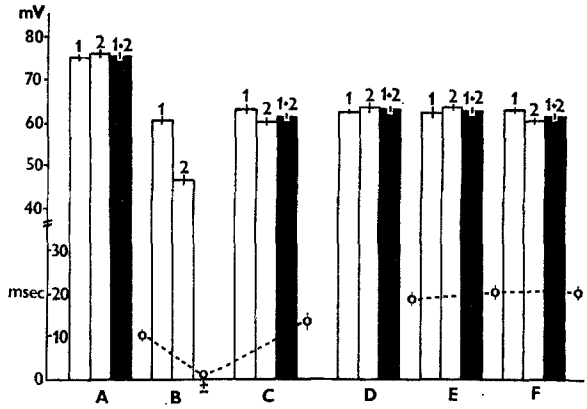

Fig. 1. Average values of the resting membrane potential (mV) in 2 superficial muscle fibres (1-first, 2-second, 1+2-average of both) recorded intracellularly of the innervated (normal) extensor digitorum longus (EDL) muscle of one-month old rats (control-above A), of the orthotopic innervated autografts 2 (above B) and 4 (above C) days after transplantation and of the EDL muscle; 14 days after denervation (above D) and of the 14 days denervated autografts 2 (above E) and 4 (above F) days after transplantation to the other leg. The circles (at the right of the corresponding bars) denote the corresponding values of contraction time (msec), i.e. of the normal EDL muscle (A), of the innervated autograft 2 days (B) and 4 days (C) after transplantation; of the EDL muscle 14 days after denervation (D) and of the denervated autograft, 2 (E) and 4 (F) days after transplantation (see interrupted line in lower part of graph)

observed in RMP 4 days after transplantation. In the denervated control muscle there is a decrease of RMP as compared with the normal control muscle (D) comparable to that observed before in denervated muscle (Albuquerque and McIsaac 1970). However, no change of RMP is observed in the first and second 2 layers of muscle fibres 2 (E) and 4 days (F) after transplantation of the denervated muscle.

The circles in Fig. 1 indicate the changes in contraction time (Carlson and Gutmann 1973). CT of the normal EDL of one months-old rats is 11.60 ± 0.60 msec. Two days after transplantation of an innervated muscle almost no contractibility was observed in the EDL. An isometric twitch, if elicited at all, could be produced only by using very high voltage and a pulse of long duration (5 msec) of direct muscle stimulation. The symbol is used to indicate this situation. Two days later, i.e. 4 days after transplantation, contractility is restored in the free graft. The CT is somewhat prolonged due to denervation. The isometric twitch is now recovered and elicitable at the usual threshold and duration of impulse (1 msec) though being always smaller than in the denervated muscle at these early stages after transplantation. CT in the muscle denervated for

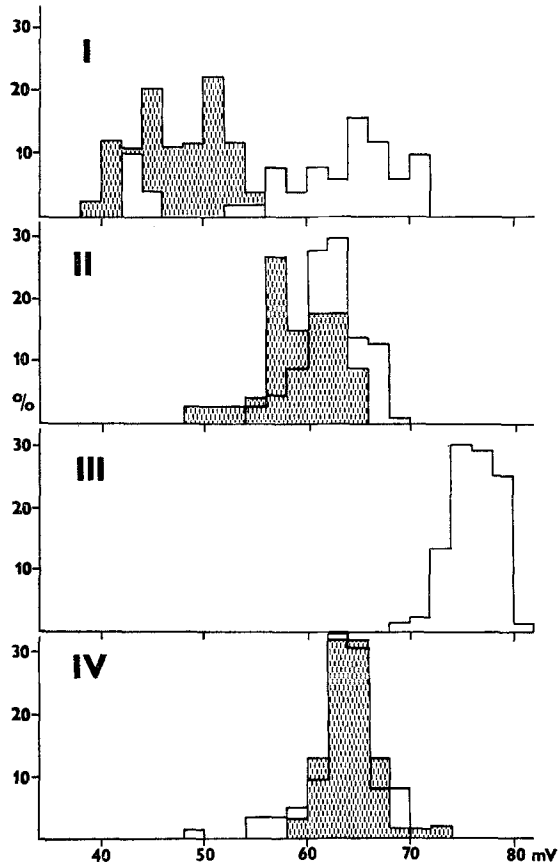

Fig. 2. Percentage histograms showing change in distribution of resting membrane potential in the superficial muscle fibres of the first (full) and second (stippled) layer of the extensor digitorum longus muscle 2 (I) and 4 days (II) after orthotopic transplantation of the innervated muscle, of the normal control EDL muscle (III) and of the EDL muscle denervated for 14 days, 2 days after transplantation into the bed of the EDL muscle of the other leg (IV). Ordinate: number of observations, abscissa: RMP (mV)

14 days is prolonged (D) but there is no change in CT 2 (E) and 4 days (F) after transplantation of the denervated muscle. The values of RMP during this period are the same in both layers of muscle fibres. There is also no change in other isometric twitch characteristics. The free denervated graft does not show the temporary loss or marked decrease in contractility as observed in innervated free grafts 2 days after transplantation. Fig. 2 shows the histogram of the RMP in the 2 layers of muscle fibres in the innervated EDL 2 and 4 days after transplantation

and in the normal control EDL muscle (I,II,III). The drop in RMP is less pronounced in the first layer of muscle fibres due apparently to better substrate supply. This is also shown by histochemical observations showing almost intact enzyme activities of the most superficial fibres (Carlson and Gutmann 1973). The shift of the histogram of the RMP of the denervated muscle fibre layers shows however no change 2 days after transplantation of the denervated graft (IV) and the histograms of first and second layer are practically identical.

The temporary complete or almost complete loss of contractility of the free innervated muscle grafts 2 days after transplantation is probably related to the marked drop in RMP, mechanical threshold of the muscle not being achieved. The increase of contractility 4 days after transplantation on the other hand is probably related to the subsequent increase in RMP of the muscle fibres. It is probable that those changes (in contractility and RMP) are due to subsequent avascularisation and revascularisation of the grafts producing a reversible ischemia with related redistribution of K ions. However, this ischemic change either does not take place in the denervated graft or is not decisive. Thus it is possible that the denervated muscle are less vulnerable to lack of oxygen supply and may be more adapted to anaerobiosis. However, it is also possible, that the increase in satellite cells which are important in the regeneration process and are observed in denervated muscle (Aloisi 1970, Hess and Rossner, 1970) may be an other factor explaining why denervated grafts are more favourable for transplantation and maintain their membrane characteristics and contractility after transplantation. The differences in stability of muscle fibres membrane of normal and denervated free grafts in the very early stages of transplantation may be one important factor in explaining the relatively greater success of denervated free grafts (Studitsky, Zhenevskaya and Rumyantseva, 1963; Thompson, 1971; Carlson and Gutmann, 1973).

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Prof. Dr. E. Gutmann
Czechoslovak Academy of Sciences
Budějovická 1083
Prague/Czechoslovakia