Life Sciences Vol. 18, pp. 109-114 Pergamon Press
Printed in the U.S.A.

REGENERATION AND TRANSPLANTATION OF MUSCLES IN OLD RATS AND BETWEEN YOUNG AND OLD RATS

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(Received in final form December 9, 1975)

Summary

In order to compare the regenerative ability of skeletal muscle between young (5month) and old (26 month) rats, sliced or intact extensor digitorum longus muscles were freely autografted into young and old rats and also reciprocally grafted from young to old inbred animals and vice versa. Sixty days after grafting, the transplants were analyzed for contractile and histochemical properties. There was a relative similarity between the contraction times of both normal control muscles and of all groups of transplants, although the contraction time tended to be prolonged and histochemical fiber pattern was more often found to be uniform in grafts of senescent animals. All groups of transplants possessed histochemically heterogeneous fiber types at 60 days. The experiments demonstrate that skeletal muscle in old rats possesses a substantial degree of regenerative ability and that the free transplantation of entire muscles in old animals is feasible.

One of the lesser explored aspects of skeletal muscle regeneration and transplantation is the influence of age upon the success of the restorative process. Because it has been generally assumed that muscle in young animals regenerates better than muscle in old ones, virtually all experimental analysis of muscle regeneration has been carried out upon immature or young animals.

With the increasing use of free grafting techniques of muscle in clinical medicine (9), there is a need for basic infor- on the functional properties of transplanted muscles throughout the lifespan of an individual because free muscle grafts are sometimes performed on elderly individuals.

The present experiments were designed to determine the effects of old age upon the overall success of free muscle grafts in rats. To do this, we examined autografts of the extensor digitorum longus (EDL) muscle in young and old rats as well as reciprocal grafts of the EDL muscle between young and old inbred rats and vice versa.

Materials and Methods

These experiments were carried out on 63 male Wistar rats that had been inbred for over 60 generations at the animal colony of the Institute of Physiology in Prague. Animals of two ages were used: young rats (three months old at the time of grafting) and old rats (24 months old).

Two types of muscle grafts were performed. In one, the entire EDL muscle was freely grafted without prior denervation (3), and in the other the EDL was cut into 7-8 transverse slices at the time of grafting to eliminate the possibility of any surviving muscle fibers (5). All grafts were orthotopic, *i.e.*, placed into the bed of the EDL.

For both the free grafts and the sliced graft models there were three general groups.

1) Normal untreated young and old EDL muscles (controls).
2) EDL autografts in both young and old rats.
3) Reciprocal transplants of EDL muscles from young into old animals and from old into young animals.

All transplants were analyzed 60 days after grafting, at which time the contractile and histochemical properties of transplants are stable in the rat. The normal control muscles were analyzed at a similar age.

Contractile analysis. At the termination of the experiments the rats were anesthetized with ether, and the muscles were quickly removed and placed into an oxygenated tissue culture medium into which tubocurarine had been added. The muscles were then set up for the automatic recording of isometric contractile properties in the manner described previously (2,3). The following contractile properties were measured: twitch and tetanic tensions, latency period (LP), contraction time (CT - time to peak), and half relaxation time (HRT).

Histochemical analysis. After the measurements of contractile function, the transplants were frozen in liquid nitrogen and analyzed histochemically for succinic dehydrogenase [SDH] (7) and myosin adenosine triphosphatase [ATPase] (4,8) activity.

Results

Contractile Properties (Table 1)

Controls. In these experiments, there were only slight differences in contraction times between the normal EDL muscles of young (5 month) and old (26 month) rats, although the CT's and HRT's of young muscles were somewhat shorter than those of old muscles. The ages of these animals correspond to those in the transplant series at the end of the 60 day transplantation period.

Transplants. As a group, all of the transplanted muscles displayed certain general characteristics. Both twitch and

Table 1. Contractile Properties of Intact Free Grafts of the Extensor Digitorum Longus Muscle in Young and Old Rats

	Tw. T (gm)	LP (msec)	CT (msec)	HRT (msec)	Tet. T (gm)	Muscle Wt. (mg)
Young Control n=7	32.07 ±2.78	2.60 ±0.07	14.93 ±0.73	9.49 ±0.92	241.43 ±29.39	185. ±12.32
Old Control n=7	31.83 ±3.35	2.67 ±0.07	15.76 ±0.95	12.83 ±1.11	184.12 ±52.15	156.9 ±12.64
Young Autograft n=10	2.23 ±0.46	3.47 ±0.19	13.60 ±0.43	14.14 ±1.90	24.42 ±5.03	63.0 ±5.54
Old Autograft n=6	1.97 ±0.80	3.30 ±0.33	15.40 ±0.83	15.45 ±2.78	11.22 ±4.50	41.0 ±9.63
Young into Old n=3(5)[a]	0.40 ±0.30	3.53 ±0.38	16.30 ±1.21	14.30 ±1.01	2.40 ±1.62	72.3 ±15.34
Old into Young	1.43 ±1.07	3.64 ±0.32	20.34 ±3.23	20.30 ±4.78	6.98 ±4.86	55.2 ±14.97

tetanic tensions were significantly less than those of the controls, usually between 5-10% of normal values. The LP's were consistently longer than that of normal muscle, and both the CT's and HRT's were slightly prolonged. As has been already reported (5), there was no difference between experiments involving intact free grafts and sliced grafts of muscles except that the contractile strengths of sliced grafts were less than those of intact grafts. Because of the similarity, only the data on intact free grafts are presented in tabular form.

1) Autografts in young and old muscles. As was the case in the control muscles, the contraction times suggest a slight difference between EDL autografts in young and old rats. However, the mean tetanic tension of grafts in young animals was about double that of grafts in old rats.

2) Reciprocal grafts from young into old and from old into young rats. The LP's in these two groups were virtually the same, but both the CT and HRT values of the old muscles grafted into young rats were prolonged as compared with those of young muscles grafted into old rats. The mean twitch tension of the transplants in the young into old group was low. In fact, two of the grafts were so poor that contractile properties could not be measured.

Histochemical Properties

FIG. 1
Sixty day sliced EDL autografts in old rat. Heterogeneous staining of muscle fibers is present. ATPase. 100X.

Sixty day autografts as well as reciprocal grafts of the EDL muscle were for the most part histologically mature and may demonstrate heterogeneous staining of muscle fibers after staining for both ATPase (Fig. 1) and SDH activity (Fig. 2). Both type I and type II fibers could be identified, and type II fibers, with high ATPase activity, predominated in the grafts (Fig. 1). In some grafts there were areas of very thin histochemically undifferentiated muscle fibers (Fig. 2), which resembled the muscle fibers found in denervated muscle grafts (Carlson and Gutmann, unpublished).

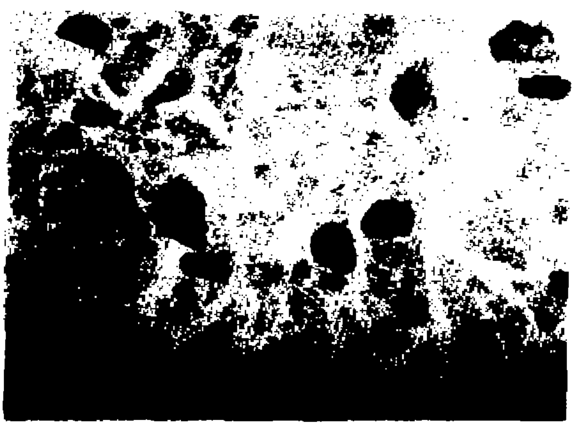

FIG. 2
Sixty day graft of intact EDL from an old into a young rat. Two populations of muscle fibers seem to be present. One contains large fibers with

heterogeneous staining. The other (arrow) contains atrophic, histochemically undifferentiated fibers. SDH. 100X.

Discussion

The major finding of these experiments is that at least between the two age groups tested (5 and 26 months) there was a high degree of similarity in the contractile properties of the muscles. This held true for both normal and grafted muscles. As has been previously shown (6), there is a substantial difference between the more rapidly contracting EDL muscle of one month old rats and that of senile animals, but the size restrictions imposed by the reciprocal grafting experiments necessitated the use of rats no less than three months old.

The pattern of contraction times in these experiments closely followed that seen in previous experiments on regenerating muscle (2,3). The LP is consistently prolonged in regenerated muscle. The CT's of regenerated muscles are normally within 10-15% of normal values, but the HRT's tend to be longer. Particularly in the experiments involving the grafting of old muscles (Table 1), the long HRT's may be due, in part, to the greater amount and inelasticity of the connective tissue stroma of the old muscle.

The low tension exerted by the grafts is again a typical characteristic of regenerated and freely grafted muscles in the rat, and it is a farily accurate reflection of the functional mass of the transplant. The weights of long term muscle grafts are of little value as an expression of the success of regeneration because of the great variability in the amount of connective tissue present. The data on twitch and tetanic tensions of the grafts suggests that the muscle bed of an old rat provides a somewhat less favorable environment for the healing and regeneration of a free graft that does that of a younger animal. The relatively small differences in contractile properties and the possibility of differentiation of histochemically demonstrable muscle fiber types in young and old grafts shows that the muscle that does regenerate in old animals is qualitatively only slightly different from that regenerating in younger animals. However, in some cases of grafting in senescent animals deficiencies in the maturation process may be observed, the CT being prolonged and the histochemical muscle fiber pattern remaining predominantly uniform (10). Accentuation of these deficiencies would be expected in older rats.

One possible explanation for the decreased contractile strength in the reciprocal grafts is that some form of immunological rejection has occurred. However, morphological evidence of lymphocytic infiltration was seen in neither these experiments nor in experiments involving the reciprocal grafting of minced gastrocnemius muscles in 60 generation inbred Wistar rats (1).

Pockets of atrophic looking muscle fibers (Fig. 2) are frequently encountered in muscle grafts in several species of mammals. It is likely that motor nerves fail to reach some muscle fascicles, possibly because of dense connective tissue sheaths surrounding them.

These experiments demonstrate the feasibility of freely grafting muscles in rats of advanced age as well as the occurrence of substantial amounts of regeneration in old muscle. Because of the close functional connection between nerve and muscles with respect to both functional and histochemical properties of muscle, the present experiments are a reflection of the total environment in which the transplanted muscles develop. Other experiments in our laboratories are examining the purely myogenic properties of regenerating and transplanted muscles at opposite ends of the life spectrum.

Acknowledgments

Part of this research was carried out under the auspices of a scientific exchange between the Academies of Sciences of Czechoslovakia and the USA. Supported in part by a grant from the Muscular Dystrophy Association.

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