Differential microvascular response to disuse in rat hindlimb skeletal muscles

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Tyml, Karel, Odile Mathieu-Costello, Linong Cheng, and Earl G. Noble. Differential microvascular response to disuse in rat hindlimb skeletal muscles. J. Appl. Physiol. 87(4): 1496–1505, 1999.—The aim of the study was to address discrepant findings in the literature regarding coupling between decreased functional demand during disuse and reduced capillarity. We previously reported [K. Tyml, O. Mathieu-Costello, and E. Noble, Microvasc. Res. 49: 17–32, 1995] that severe disuse of rat extensor digitorum longus (EDL) muscle caused by a 2-wk application of tetrodotoxin (TTX) on the sciatic nerve is not accompanied by capillary loss. Using the same animal model, the present study examined whether this absence of coupling could be explained in terms of 1) too short a duration of disuse and 2) muscle-specific response to disuse. Fischer 344 rats were exposed to either no treatment (control) or to 2- or 8-wk TTX applications. Fiber size, capillary density per fiber cross-sectional area, and capillary-to-fiber (C/F) ratio were determined by morphometry in the EDL muscle (control, 2- and 8-wk groups) and in the superficial portion of medial gastrocnemius (Gas) muscle (control, 2 wk). In both muscles, microvascular blood flow was evaluated by intravital microscopy [red blood cell velocity in capillaries (V_{RBC})] and by laser Doppler flowmetry (LDF). Regardless of duration of TTX application or muscle type, TTX-induced disuse resulted in a significant reduction of fiber area (44–71%). However, capillary density increased in EDL muscle (both at 2 and 8 wk) but not in Gas muscle. C/F ratio decreased in EDL muscle at 8 wk (18%) and in Gas muscle (39%). This indicates that the effect on capillarity depended on duration of disuse and on muscle type. V_{RBC} and LDF signal were significantly larger in EDL than in Gas muscle. Analysis of change in capillarity vs. V_{RBC} suggested that the outcome of disuse may be modulated by blood flow. We conclude that the duration of skeletal muscle disuse per se does not dictate capillary loss, and we hypothesize that different findings of coupling between functional demand and capillarity could be due to the presence/absence of flow-related angiogenesis superimposed on the capillary removal process during disuse.

capillary density; blood flow; atrophy; capillary damage; angiogenesis

SKELETAL MUSCLE RAPIDLY ADAPTS to a chronic increase in functional demand by an increase in anatomic capillary density (10, 11, 20). Much less is known about the coupling between the microvasculature and a decreased functional demand, such as that which accom-panies muscle disuse and atrophy. In frog muscle, we found a reduction in number of perfused capillaries and increased capillary endothelial damage with muscle atrophy (27). Others have also noted a coupling between decreased muscle use and reduced muscle capillarity (2, 12, 13). In contrast, in a rat model of muscle disuse [tetrodotoxin (TTX) superfusion of sciatic nerve for 2 wk], not only was the capillary network of the extensor digitorum longus (EDL) muscle maintained, there was, in fact, some evidence of angiogenesis in the disused muscle despite a 40.5% loss in muscle weight (28). Similar observations of maintained microvasculature after muscle disuse have been made in rat muscle that had undergone other forms of disuse atrophy, including denervation, hindlimb suspension, and space flight (7, 11, 18). The reasons for the discrepant responses of the microvasculature to muscle disuse noted above are unclear. They could arise from differences in the severity or duration of disuse or from differences in responsiveness to metabolic or hormonal stimuli among the muscles examined.

In a previous study (28), we used a 2-wk TTX model of disuse to produce a severe atrophy in the rat EDL muscle. Surprisingly, no coupling between decreased functional demand and reduced muscle capillarity was observed. There were two possible explanations for this observation. First, the 2-wk TTX application could have been too short a period for this coupling to occur (28). Second, the effective functional sympathectomy associated with TTX application could have masked this coupling. Because TTX application elevated red blood cell velocity (V_{RBC}) in capillaries (28), the resulting increased shear stress on the capillary wall could have affected capillary structure by inducing angiogenesis and thereby maintaining capillarity (4, 19).

Thus the overall aim of the present study was to specifically address the surprising finding of lack of coupling between muscle use and capillarity in terms of these two possibilities. The first objective was to establish whether the occurrence of coupling depends on the duration of disuse per se. The second objective was to determine whether a coupling occurs when blood flow is not elevated after the application of TTX. We pursued this objective on the basis of the reports in the literature (5, 15) that mechanical denervation causes differential effects on muscle blood flow among different muscles and among portions of muscles. For example, flow to the rat soleus muscle was reduced by denervation by nearly 90%, whereas flow to the middle portion of the lateral head of gastrocnemius (Gas) was unaffected (5). Apparently, flexor muscles like the EDL may
be more sensitive to sympathetic nervous influences than are the antigravity ankle extensor muscles, such as the Gas (15). Our preliminary experiments along these lines indicated that blood flow at the surface of the medial portion of the Gas in rats was indeed not elevated after 2-wk TTX application; this suggests that this muscle was a purer model of disuse than was EDL (i.e., without the confounding effect of increased flow). Accordingly, we hypothesized that, unlike results in the EDL muscle, 2-wk TTX-induced disuse in the Gas would result in coupling between decreased muscle use and reduced capillarity.

**METHODS**

Animal preparation. The animal protocol was approved by the Council on Animal Care at the University of Western Ontario. Male Fischer 344 rats (2–4 mo old) were divided into four groups. Rats in the first group (2wD, n = 25) and the second (8wD, n = 14) group were subjected to unilateral disuse of the hindlimb muscles for 2 and 8 wk, respectively. Rats in the third group (2wC, n = 24) and the fourth group (8wC, n = 16) were subjected to no treatment and served as 2- and 8-wk time-matched control groups for the 2wD and 8wD groups, respectively. Disuse was achieved by superfusion of the right sciatic nerve with TTX, a procedure described in detail previously (28). Briefly, after pentobarbital sodium (Somnotol, MTC Pharmaceuticals; 65 mg/kg ip) anesthesia was induced, an osmotic pump (2002 Alzet) was filled with a TTX solution (300 µg/ml of saline), placed under the upper back skin, and connected via tubing under the skin to the nerve. During the period of disuse, the effect of TTX was verified by the absence of the toe-spreading reflex on hindlimb elevation and by observation of the animal’s limping gait. At the time the animal was killed, confirmation of the efficacy of TTX-induced disuse was obtained by means of stimulation of the sciatic nerve above and below the nerve cuff. It was observed that nerve transmission was blocked when stimulation was performed above the cuff. The amount of TTX solution in one pump was sufficient for 2 wk. Therefore, for each rat in the 8wD group, the pump was replaced (while the animal was under Somnotol anesthesia) by a new pump with fresh TTX solution after 2, 4, and 6 wk. In the present study, we used nontreated rather than sham-operated rats (pumps filled with saline) as controls. Our preliminary study (5 nontreated vs. 5 sham-operated rats for 2 wk) indicated that there was no difference between these groups in terms of the right EDL muscle weight (104 ± 5 vs. 106 ± 2 (SE) mg), density of perfused capillaries (CDper, 27 ± 1 vs. 25.2 ± 0.8 cap/mm), and blood flow [as measured by laser Doppler flowmetry (LDF): 0.26 ± 0.02 vs. 0.29 ± 0.05 V]. Measurements were made by intravital methods described below.

At the end of the treatment period, control and TTX-treated rats were anesthetized with Somnotol, weighed, and placed on a heated stage of an intravital microscope (Leitz model ELR) equipped with a ×10/0.22 numerical aperture objective and a ×6.3 eyepiece. The trachea was intubated, and the left carotid artery was cannulated for measurement of blood pressure. The middle of the right EDL muscle was exposed for intravital microscopic visualization according to a published procedure (24). The proximal one-third of the medial surface of the right Gas was similarly prepared for visualization. The surface of either muscle was covered by a glass coverslip, and the microcirculation at the surface was video recorded (final magnification, ×240) by using an MTI camera, a Mitsubishi U82 tape recorder, and a Panasonic WV5410 monitor.

Experimental protocol. To address the first objective (effect of duration of TTX-induced disuse), we used two approaches to determine capillarity (i.e., via intravital microscopy and histology) and blood flow (via intravital microscopy and LDF) in EDL muscles in the four groups. From video recordings of three randomly chosen fields of view in each muscle (field size, 1.05 × 0.78 mm) capillarity was analyzed in terms of the density of capillaries with moving (CDmov) and stationary (CDstat) red blood cells. In practice, we counted capillaries visible at the muscle surface that crossed a perpendicularly drawn test line on the video monitor. Capillarity measurement from histological sections is described in the next section. In the three recorded fields, we also measured the Vrybc, an index of blood flow, in randomly chosen capillaries (4 capillaries per field) by means of the flying spot technique (25). The second approach for assessment of microvascular flow was the LDF that provides a signal proportional to Vcrybc in capillaries found in a surface volume of ~1 mm³ (26). In practice, we measured this signal on-line in three randomly selected regions in the center part of the exposed muscle by using a LDF (model Pf 1d Perimed).

To address the second objective (differential response between EDL and Gas muscles), analysis of capillarity and blood flow was carried out similarly at the surface of medial Gas muscle in the 2wC and 2wD rat groups.

At the end of the intravital experiment, the exposed muscle (either EDL or Gas) was dissected out, blotted to remove excess moisture, and weighed. In a separate subgroup (n = 5) in each of the 2wC and 2wD groups, both the right EDL and the right Gas muscles were prepared for light and electron microscopic examinations. In a separate subgroup (n = 5) in each of the 8wC and 8wD groups, the right EDL muscle was also prepared for light and electron microscopy.

Light and electron microscopy. For morphometric analysis of capillarity, we followed a previously described procedure (28). The EDL muscle was fully exposed, superfused with glutaraldehyde fixative solution (6.25% glutaraldehyde in 0.1 M sodium cacodylate buffer) for 30 min, excised whole, and stored in the fixative solution in the refrigerator. In regard to the Gas muscle, the exposed surface (including the area analyzed by intravital microscopy) was superfused with the fixative solution for 30 min. At this time, a 1- to 2-mm-thick layer was sliced away from this surface, stored in the fixative solution, and refrigerated. The middle one-third along the length of the excised EDL and Gas muscles was then cut into small blocks, postfixed in OsO4, dehydrated in alcohol, and embedded in epoxy resin. Four blocks per muscle were analyzed. The fixative solutions and tissue processing were the same as used previously (17, 28). They lead to an adequate preservation of skeletal muscle ultrastructure (8) and a minimal amount of tissue shrinkage (14, 30).

Cross sections (1-µm thick) to the muscle fibers were cut on an LKB Ultratome III, stained with 0.1% toluidine blue solution, and analyzed by light microscopy. Morphometric measurements of fiber cross-sectional area (size of analyzed field, 135 × 190 µm) and of capillarity (size of field, 250 × 250 µm) were performed by light microscopy on sections, as described previously (14, 24). Specifically, capillary density (i.e., capillary number per fiber cross-sectional area, interstitial space excluded, or Qw,0) was measured by point counting with a 100-point test grid at a magnification of ×400. Fiber cross-sectional area (A(f)) and the mean number of capillaries around a fiber (Ncap,f) were measured with an image analyzer (Videometric 150, American Innovison) at a magnification of ×1,360. On average, 14 ± 1 (SE) fields were measured per
sample to estimate \( Q_A (0) \), 168 ± 26 fibers/sample for \( A(f) \), and 137 ± 25 for \( N_CAF \) (size of field: 135 × 190 µm), yielding a SE of the vast majority of the estimates of <10%. Capillary-to-fiber ratio, \( N_{CAF} (c,f) \), was computed as the product of \( Q_A (0) \) and \( A(f) \). The sharing factor was computed as \( N_CAF \) divided by \( N_{CAF} (c,f) \).

To assess capillary damage that may be associated with muscle atrophy (27), ultrathin sections (50–70 nm) of the same samples of EDL muscles (2wC, \( n = 5 \); 2wD, \( n = 5 \); 8wC, \( n = 3 \); 8wD, \( n = 3 \)) and Gas muscles (2wC, \( n = 5 \); 2wD, \( n = 5 \)) were contrasted with uranyl acetate and bismuth subnitrate and were examined with a Zeiss 10 electron microscope. Capillary wall damage was assessed from these sections at magnifications of ×8,000–12,500 by using the same qualitative indexes (endothelial thickening, and increased size and frequency of both cytoplasmic folding and projections) as used previously (27, 28).

**RESULTS**

**Morphology.** After the TTX application on sciatic nerve for 2 wk, rat body weight was significantly less (8%) compared with the control (291 ± 9 g vs. 267 ± 7 g in 2wC vs. 2wD group, respectively). Similarly, TTX application for 8 wk resulted in a 13% difference in body weight (344 ± 8 g vs. 299 ± 8 g in 8wC vs. 8wD group, respectively). The TTX applications for 2 and 8 wk resulted in significant reductions in EDL muscle weight (35 and 43%, respectively; Fig. 1) and in A(f) (44 and 71%, respectively; Fig. 2). Both of these reductions established the presence of disuse in the EDL muscle. The TTX application for 2 wk also decreased the Gas muscle weight by 53% (Fig. 1) and the A(f) by 53% (Fig. 2).

In regard to the TTX-induced muscle atrophy in the EDL muscle (Fig. 2, top), there were corresponding increases in the anatomic density of capillaries for both treatment durations (Fig. 3, top). This suggested that the density increase was related to the smaller fiber size. However, this effect was not observed in the Gas muscle, as no increase in capillary density occurred despite the 53% atrophy (Figs. 2 and 3, bottom), thereby indicating that capillaries were lost in the atrophied Gas muscle. Examination of capillary per fiber number (Figs. 4 and 5) confirmed the reduced capillarity in the Gas muscle. Figures 4 and 5 also show increased C/F ratio in the EDL muscle after the TTX treatment for 2 wk but reduced capillarity in the EDL muscle after treatment for 8 wk. This indicates that the duration of treatment can affect capillarity. As expected, the capillary loss is corroborated by examination of the computed values for the sharing factor, which increased significantly in Gas muscle (from 3.3 ± 0.3 in 2wC group to 4.2 ± 0.1 in 2wD group). Computed values for the EDL muscle were 4.6 ± 0.3 and 3.8 ± 0.2 in 2wC and 2wD groups, respectively (P < 0.05) and 3.4 ± 0.1 and 3.3 ± 0.2 in 8wC and 8wD groups, respectively.

Figures 6 and 7 are examples of light and electron micrographs of EDL muscle cross sections at low and high magnifications in 2wC, 2wD, and 8wD groups and from Gas muscle cross sections in 2wC and 2wD groups. Although all TTX-treated rats exhibited muscle atrophy, surprisingly, none of the groups demonstrated significant damage of the capillary wall. Of 149 randomly sampled capillaries in the EDL 2wC group, no capillary was judged to be damaged according to the previously published criteria of endothelial thickening and increased size and frequency of both cytoplasmic folding and projections (28). A similar negligible occurrence of damage in 2 of 185, 0 of 79, and 1 of 92 capillaries was found in the 2wD, 8wC, and 8wD EDL muscle groups, respectively. In Gas muscle groups 2wC and 2wD, damage was detected only in 1 of 138 and 2 of 138 capillaries, respectively.

**Hemodynamics.** Systemic blood pressure in rats of the 2wC group was 124 ± 4 mmHg; that of rats in the 2wD, 8wC, and 8wD groups did not differ. Based on the intravital approach, the TTX treatments for 2 wk and for 8 wk resulted in 36 and 50% increases, respectively.
be explained by the fact that LDF signal may be sensitive not only to \( V_{RBC} \) but also to vascular architecture factors (21). Thus increased capillary density in the 8wD group (Fig. 3) could possibly contribute to the LDF signal increase in the 8wD group (Fig. 11).

Although the TTX treatment affected blood flow in the resting steady state (Figs. 10 and 11, top), it did not alter the hyperemic response to short-term ischemia. Within 1–2 min after the tourniquet was released, the LDF signal peaked significantly above the resting level and then returned back to this level in ~20 min in all groups. In particular, for EDL muscles, the peak levels were (in V) 0.77 ± 0.09 (2wC), 0.85 ± 0.07 (2wD), 0.79 ± 0.09 (8wC), and 0.76 ± 0.06 (8wD), whereas for Gas muscles the peak levels were 0.79 ± 0.15 (2wC) and 0.97 ± 0.05 (2wD).

**DISCUSSION**

The overall aim of the present study was to address discrepant findings regarding coupling between the decreased functional demand during disuse and reduced capillarity. The first objective was to examine the effect of duration of TTX application on capillarity in the EDL muscle. We found that TTX application for 8 wk, but not for 2 wk, resulted in capillary loss during disuse. Thus duration of disuse may account for the coupling between decreased functional demand and reduced capillarity. The second objective addressed the

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**Fig. 2.** Effect of 2 and 8 wk of disuse on fiber cross-sectional area in EDL muscle (top) and of 2 wk of disuse on fiber area in gastrocnemius muscle (bottom). Nos. in parentheses, nos. of muscles used. *Significant difference between control (C) and disuse (D) groups, \( P < 0.05 \).

**Fig. 3.** Effect of 2- and 8-wk disuse on anatomic capillary density per fiber cross-sectional area \( Q_A(0) \) in EDL muscle (top) and of 2-wk disuse on \( Q_A(0) \) in gastrocnemius muscle (bottom). Nos. in parentheses, nos. of muscles used; cap, capillaries. *Significant difference between control (C) and disuse (D) groups, \( P < 0.05 \).
hypothesis that the 2-wk TTX application reduces capillarity in the Gas muscle. Capillary per fiber number data in this muscle demonstrated capillary loss and thus established coupling between reduced demand and capillarity in this tissue at 2 wk. The 2-wk TTX data also indicated that, despite large atrophy in both EDL and Gas muscles, there was a differential response in capillarity to disuse.

Model of TTX-induced disuse. The TTX model entails both muscle disuse and functional sympathectomy, because application of TTX on the sciatic nerve blocks Na\(^+\) entry in both motor and sympathetic nerves (16, 22). The present data (Fig. 1) confirm the pronounced effect of TTX-induced disuse on EDL and Gas muscle weights (23, 28). Although the TTX model produces severe atrophy in a short time, the functional sympathectomy and the resulting elevated blood flow at 2 wk may affect the capillarity response to muscle disuse. Using two independent approaches to assess blood flow, we have established that the TTX application yields a differential flow response in EDL and Gas muscles (see 2wC and 2wD groups, Figs. 10 and 11), a finding consistent with an earlier study (5). Thus the present study allowed us to examine the effect of disuse with and without the confounding effect of elevated flow. The mechanism of the differential flow response, which is unknown, may involve differences in \(\alpha\)-adrenergic receptor sensitivity and/or density in the vasculature (5). Future studies that examine the vascular response to catecholamines in these two muscles could contribute to the elucidation of the mechanism of differential flow response.

Effect of 2- and 8-wk TTX applications on EDL muscle. For short-term disuse, the reported increase in cross-sectional capillary density has been accounted for by muscle atrophy (7, 11). This indicates that the total number of capillaries within the muscle remained unchanged. In the present study involving TTX application at 2 wk, the increase in anatomic capillary density (Fig. 3, 2wC and 2wD groups) tended to be larger than the reduction in fiber area (Fig. 2). As in our previous study (28), this was due to a significant increase in the C/F ratio (Fig. 4) that suggests angiogenesis. This finding is surprising, because the opposite tendency, i.e., a capillary loss, was expected during disuse. The use of markers for angiogenesis (e.g., proliferating cell nuclear antigen), if positive, would provide conclusive evidence that the increase in C/F ratio was associated with growth of new capillaries in the EDL muscle.

On the basis of 1) the lack of significant rarefaction of capillary bed, 2) the negligible ultrastructural damage of the capillary wall, and 3) the maintained reactive hyperemic response, it appears that the rat EDL muscle microvasculature was remarkably resistant to changes during the 2-wk TTX-induced disuse. A possible mecha-
nism for the maintenance of vascular structure and function could be a dissimilar rate of muscle fiber vs. capillary endothelium adaptation to disuse. For example, the rate of production of de novo contractile proteins could be reduced much more than that of endothelial structural proteins. In this regard, the only available information relates to muscles subjected to an increased load. Over a 30-day period of compensatory overload, Plyley and co-workers (20) recently showed that increases in the mean $N_{\text{CAF}}$ and in fiber area corresponded to similar half-lives of 10.1 and 11.2 days, respectively. Thus, in muscle undergoing increased use, the time course of muscle fiber and endothelial adaptation appears to be similar. This observation is at odds with the proposal of dissimilar rates to explain the maintenance of the microvasculature during the 2-wk disuse.

In regard to the long-term disuse, the significant decrease in $C/F$ ratio and $N_{\text{CAF}}$ with 8-wk TTX treatment (Figs. 4 and 5) is consistent with reports of greater capillary loss with increased duration of disuse (3, 13). Because $V_{\text{RBC}}$ was no longer significantly elevated at 8 wk (Fig. 10), the outcome of 8 wk of muscle disuse was not confounded by the effect of elevated flow due to functional sympathectomy. The pattern of increased $V_{\text{RBC}}$ at 2 wk and the subsequent decline toward the control level at 8 wk (Fig. 10) is similar to that noted for blood flow in a muscle-denervation model.
In the face of unaltered systemic blood pressure, this pattern suggests an adaptation of vascular tone to reestablish normal microvascular perfusion after muscle disuse and/or functional sympathectomy.

Comparison of EDL and Gas muscles after TTX application. Comparison of C/F ratios in EDL and Gas muscles in the 2wD groups suggests that duration of disuse alone was not solely responsible for loss of capillaries. Because the same duration of 2-wk TTX application caused increased as well as decreased capillarity (Fig. 4), it appears that coupling between decreased functional demand during disuse and reduced capillarity was muscle specific. Muscle fiber atrophy was larger in Gas than in EDL (Fig. 2; ANOVA of interaction between muscle type and TTX treatment). This raises the possibility that the differential response in capillarity (Fig. 4) depended on the degree of atrophy. In another model of disuse (hindlimb suspension), Desplanches and co-workers (6) found no change in capillarity in EDL with a smaller degree of atrophy (22%), but they found a large change in capillarity (49% reduction) in soleus muscle with large atrophy (63%).

**Fig. 7.** Electron micrographs showing examples of normal (A) and damaged capillaries (B) in gastrocnemius muscle (2wD group). In general, negligible capillary damage was detected in both EDL and gastrocnemius disused muscles (see RESULTS for details).

**Fig. 8.** Effect of 2- and 8-wk tetrodotoxin-induced disuse (2wD and 8wD, respectively) on density of capillaries with moving (CD<sub>per</sub>) and stationary (CD<sub>stat</sub>) red blood cells, as visualized at the surface of EDL and gastrocnemius muscles. Nos. in parentheses, nos. of muscles used. *Significant difference in CD<sub>per</sub> between C and D groups, P < 0.05.

**Fig. 9.** Relationship between morphological and intravital microscopic assessment of capillary density (CD) in EDL and gastrocnemius (Gas) muscles in various groups. y-Axis, square root of Q<sub>W(0)</sub> predicted to be visible at the muscle surface; x-axis: actual measurement of visible capillaries, i.e., total CD (CD<sub>tot</sub>) = CD<sub>per</sub> + CD<sub>stat</sub> at the surface. Line represents line of unity.
Data in the 8wC and 8wD groups (Figs. 2 and 4) show, however, that the degree of muscle fiber atrophy per se cannot account for the differential response, because the largest atrophy (71%) was not associated with the largest loss in capillarity.

One of the proposed mechanisms that link increased demand (muscle contraction) and capillarization is that of increased shear stress and blood-endothelial cell interaction during contraction-induced increased flow (4, 19). In the present study, the 2-wk TTX-induced disuse of the EDL muscle was associated with increases in both capillarity (Fig. 4) and \( V_{\text{RBC}} \) (Fig. 10). As discussed below, we explored whether the same mechanism linking capillarization and flow could be operational in the present experiments. Figure 2 shows the degree of muscle fiber atrophy in all three experimental conditions. If capillary density changed only passively due to this atrophy, then one could directly predict this density on the basis of the degree of atrophy. For example, because the EDL muscle fibers atrophied by a factor of 1.783 after 2-wk TTX application, the predicted capillary density would be 1.783 \( \times 821 \) (i.e., density in the 2wC group, Fig. 3) or 1,464 capillaries/mm\(^2\). We identified the difference between the measured capillary density (i.e., 1,871 capillaries/mm\(^2\) in the 2wD group; Fig. 3 top, left) and the predicted capillary density as “differential capillarity” (i.e., 1,871 – 1,464 = 407 capillaries/mm\(^2\)). Figure 12 shows a dependence of differential capillarity on \( V_{\text{RBC}} \) and suggests that the outcome of disuse-induced change in capillarity may be modulated by blood flow. A similar modulation during EDL muscle disuse (peroneal nerve-crush model) was reported by Zaida and co-workers (29).

Fig. 10. Effect of 2wD and 8wD on velocity of red blood cells (\( V_{\text{RBC}} \)) in capillaries in EDL muscle (top) and in 2wD on \( V_{\text{RBC}} \) in gastrocnemius muscle (bottom). Nos. in parentheses, nos. of muscles used. In each muscle, velocity was obtained as an average computed from measurements in 12 randomly selected capillaries. *Significant difference between C and D groups, \( P \leq 0.05 \).

Fig. 11. Effect of 2wD and 8wD on laser Doppler flowmetry (LDF) signal (a measure of microvascular flow, given in volts) in EDL muscle (top) and in 2wD on the signal in gastrocnemius muscle (bottom). Nos. in parentheses, nos. of muscles used. *Significant difference between C and D groups, \( P \leq 0.05 \).

Fig. 12. Dependence of “differential capillarity” (\( \Delta \) capillaries) in disused EDL and gastrocnemius muscles on \( V_{\text{RBC}} \) in these muscles. Differential capillarity was computed as difference between measured \( Q_{\text{A}}(0) \) in disused muscle and predicted anatomic density on the basis of density in control muscle and degree of fiber atrophy. See DISCUSSION for details. Line represents a line of best fit through the 3 points.
Capillarity increased after a chronic administration of prazosin that is known to elevate blood flow (3, 29). Although other as-yet-undiscovered factors [such as a differential susceptibility to sympathectomy (15)] could also contribute to the differential response in capillarity seen in EDL and Gas muscles, the present data are consistent with the proposed mechanism that links capillarity and flow (4). Clearly, studies where flow can be experimentally manipulated during disuse must be carried out to further substantiate this possible mechanism.

In view of the capillary loss in disused Gas muscle (Fig. 3), the present findings of negligible capillary wall damage and of maintained hyperemic response in this muscle were not expected. In our previous study, an amphibian skeletal muscle that showed a 64% disuse-induced atrophy exhibited both capillary damage and absence of hyperemia (27). It is possible that the endothelial degeneration and capillary removal processes in the amphibian tissue occurred at a slower rate than in the mammalian tissue, possibly because of the lower body temperature in amphibians. The present findings suggest that the removal of ~39% of capillaries (drop in C/F ratio; Fig. 4) in disused Gas muscle was completed before the end of the 2-wk period of TTX application. This may be indicative of relatively fast plasticity of the mammalian capillary network in both angiogenic (10, 20) and degenerative directions (2, 13, 14).

In conclusion, the present study demonstrated for the first time that a 2-wk TTX application on sciatic nerve can result in consistent loss in muscle mass but in either an increase or decrease in capillarity in different muscles. Although our data indicate that neither the duration of disuse nor the degree of muscle fiber atrophy is solely responsible for capillary loss, they suggest that the outcome of disuse-induced change in capillarity in a given muscle may depend on blood flow.

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