breaking up of cellular or subcellular structures may considerably affect the properties of membrane ATPases. This fact must be borne in mind in any attempt to interpret the regulatory effects of calcium on ion exchanges by an approach at the enzymatic level.

The (Mg^{2+}-Ca^{2+})-activated ATPase described here is not likely to be bound to mitochondria since mitochondrial ATPase was never found to require both Mg^{2+} and Ca^{2+} for full activity. However, it is not possible to decide whether the ($Mg^{2+} + Ca^{2+}$)-stimulated enzyme system originates from plasma membranes or from endoplasmic reticulum of electroplaxes. On the other hand, the above findings suggest that low amounts of Ca^{2+} can exert a regulating effect on the ($Na^+ + K^+$)-stimulated ATPase provided subcellular structures are preserved.

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Sugar uptake in acutely denervated levator ani muscle of rat

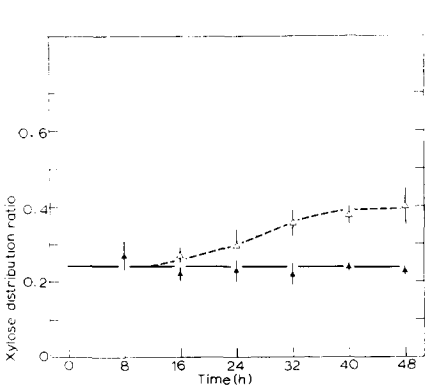
Previous experiments[1] showing that acute denervation specifically blocks testosterone effect on xylose uptake in the rat levator ani, a completely white muscle[2], prompted us to study further the regulation of sugar transport in this muscle after denervation.

No effect on the basal transport rate was shown after 16 h (ref. 1), but a rise in sugar uptake could be expected, at least in the white muscle, after more than 1 day, on the basis of the control of sugar transport by the energy metabolism[3]. In fact, at that time the denervated white muscle showed an increase in active transport of amino acids[4] and in O_2 consumption (see ref. 4). Decreases of glycogen (see ref. 5) and phosphagen content[6] and an increase of glycogen phosphorylase activity[7] have also been reported.

Experiments were therefore performed on the intact levator ani preparation[8] obtained from random groups of sexually immature male albino rats previously submitted to lumbosacral spinal cord destruction under light ether anesthesia. Muscles were incubated at 37° in 2 ml Krebs–Ringer bicarbonate medium (pH 7.4), O_2–CO_2 gas (95:5, v/v) containing inulin (7.5 mg/ml), [$^{14}C_6$]xylose (15 mM, specific activity 0.016 mC/mmole, The Radiochemical Centre, Amersham) and pyruvate (10 mM) or glucose (10 mM) (see ref. 1). Direct stimulation and anoxia were obtained as described[1]. Bovine insulin (Lilly, Lot PJ 4609, 0.1 unit/ml) and NaCN (1 mM) were sometimes added. [$^{14}C_6$]Xylose was extracted and counted as described[1]. Results are expressed as the distribution ratio of radioactivity between intracellular water and the incubation medium (see ref. 1).

Our results show that sugar uptake by the rat levator ani muscle rises between 24 and 32 h after denervation (Fig. 1). Increased permeability of the sarcolemma (see ref. 9) may be of some importance in explaining this phenomenon (xylose uptake is higher also in the presence of phloridizin (3 mM) and L-glucose space is rising (unpublished)). The slight decrease in volume of the fibers and the relative increase of the extracellular space (unpublished) and the consequent greater availability of sugar to transport mechanisms have to be taken into account too.

The increase of xylose uptake by the denervated muscle is additive with the enhancement caused by anoxia (Fig. 2). However, this result does not contrast with previous observations showing lack of additivity between stimuli enhancing sugar transport when at least one stimulus is exerting maximal effect (see ref. 1); in fact, the maximal sugar penetration rates attained by denervated and control muscles (see Fig. 3, 30 min of anoxia) are not significantly different. Therefore, additivity is only apparent and it is due to the shortening of the time lag required for anoxia to stimulate sugar transport in the denervated muscle (see Fig. 3, sugar uptake is significantly increased after 20 min of anoxia in the denervated muscle whereas only after 30 min in the control; longer time lags (60–90 min) have been reported[10] for



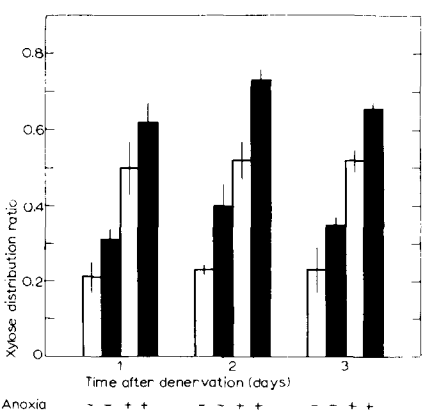


Fig. 1. Xylose uptake in levator ani muscle of intact (▲) and denervated (△) rats. Muscles were incubated 40 min. Each point represents the mean of at least 5 cases; vertical bars represent 1 or 2 × S.E.

Fig. 2. Effect of anoxia on xylose uptake in levator ani muscle of control rats (□) and of rats denervated 1, 2 or 3 days before the experiments (■). Incubation lasted 50 min. Means of at least 6 cases ± S.E. are given. After the 2nd day both anoxia and denervation effects were significant ($P < 0.01$), whereas interaction between treatments was not.

normal frog muscle). So, we can conclude that the mechanisms of control of sugar uptake by this denervated muscle are more sensitive to anoxia than normal. This result is likely to be of general relevance for white muscles, as it agrees with the

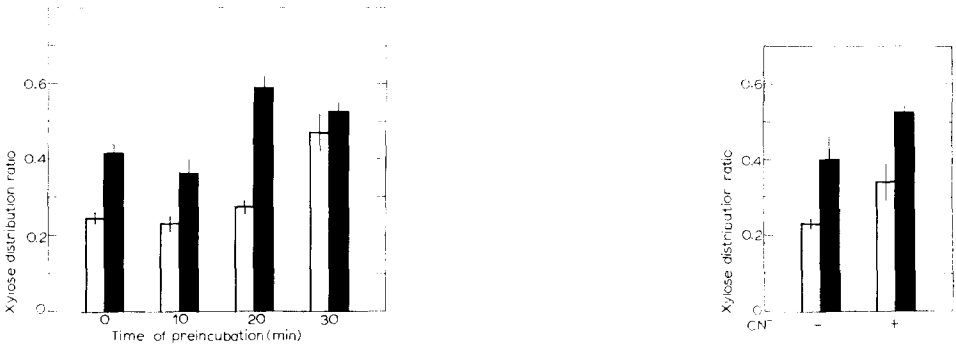


Fig. 3. Effect of anoxia on xylose uptake in normal (□) and denervated muscles (■). Muscles were preincubated under anaerobiosis for 10, 20 or 30 min and then incubated under anaerobiosis in the presence of [$^{14}C_6$]xylose for 40 min. Means of at least 4 cases ± S.E. are given. Analysis of variance (F test) showed that effects of both denervation and anoxia as well as the different behavior of denervated and control muscles as a function of anoxia duration were statistically significant ($P < 0.01$).

Fig. 4. Effect of CN^- (1 mM) on xylose uptake in control (□) and in 2-days-denervated (■) muscles. Incubation lasted 50 min. Means of 6 cases ± S.E. are given. Both denervation and cyanide effects were significant ($P < 0.05$); interaction between treatments was not.

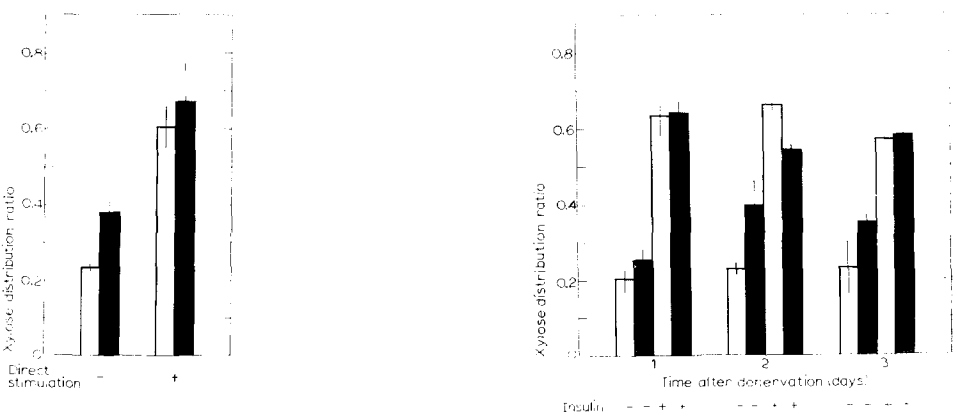


Fig. 5. Effect of direct stimulation on xylose uptake in control (□) and in 2-days-denervated (■) muscles. Medium contained pyruvate (10 mM) instead of glucose. Incubation lasted 50 min. Means of at least 6 cases ± S.E. are given. Both denervation and direct stimulation effects were significant ($P < 0.01$), whereas interaction between treatments was not.

Fig. 6. Effect of insulin on xylose uptake in normal (□) or 1-, 2- and 3-days-denervated muscles (■). Incubation lasted 50 min. Means of at least 5 cases ± S.E. are given. Effects of both denervation and insulin are significant 2 and 3 days after denervation ($P < 0.01$). Interaction between treatments is significant too ($0.01 < P < 0.05$). Xylose distribution ratio in the 2-days-denervated muscle is significantly lower ($0.01 < P < 0.05$) than in the control in the presence of insulin.

increase in their consumption of O_2 after denervation[4]. On the contrary, it is doubtful if denervated red muscles can react to anoxia in a similar way, as they show no increase in O_2 consumption[11] and early and marked mitochondrial damages have been described[12, 13].

Results similar to those described for anoxia are obtained in the presence of CN^- (Fig. 4). Direct stimulation *in vitro* produces an increase in sugar transport in both the 2-days-denervated and the intact muscle (Fig. 5).

Insulin increases xylose uptake to the same levels (with the possible exception of the 2nd day) both in the control and in the denervated muscle (Fig. 6) and consequently enhancement is smaller after denervation. Thus levator ani muscle *in vitro* behaves differently from the cut diaphragm preparation[14].

This may be due to differences in fiber type, as a difference in response to denervation has been already shown between white and red muscles as regards amino acid transport[15]. Also it is to be noted that the denervated hemidiaphragm shows a considerable hypertrophy till at least the 7th day (*cf.* refs. 16, 17) and that no other muscle reacts to denervation in such a way[16].

We may conclude that the denervated levator ani muscle shows enhanced basal sugar uptake and greater sensitivity to anoxia and that it can react to various sugar transport-enhancing stimuli with the exception of testosterone until after the onset of muscle atrophy. However, the all-or-none block exerted by denervation on testosterone effect in this muscle[1] is clearly related to the peculiar sensitivity of levator ani muscle to androgens.

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