MITOCHONDRIAL CALCIUM AND POSTMORTEM MUSCLE SHORTENING

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Received April 17,1975

SUMMARY

A study was made of cold shortening, the large, slow contraction which red mammalian muscles undergo when chilled early postmortem. Thin strips of bovine muscle, held at 2°, shortened very much less in oxygen than in nitrogen, and this aerobic suppression was completely overcome by uncoupling agents. The same uncouplers provoked no early length change at all in the bovine tissues at 20°, and white rabbit muscle did not shorten under their influence at either 2°, or 20°. The results indicate that mitochondria are involved in cold shortening. It is suggested that the calcium they release under postmortem anaerobic conditions cannot be removed rapidly enough by the sarcoplasmic reticulum, the calcium uptake of which is markedly reduced by low temperatures.

INTRODUCTION

Mammalian muscles vary widely in the extent of their slow contractile response to early postmortem chilling (1). Those which are obviously red in color - bovine and ovine muscles generally, rabbit semitendinosus and rat soleus - shorten considerably (1-4), some of them by up to 60% of initial excised length. In the paler muscles of the rabbit and rat, by contrast, cooling toward 0° provokes no shortening at all (1, 4). The length change is probably triggered by an increase in free calcium concentration (5), and it has been suggested (5-7) that this ion flux takes place from the sarcoplasmic reticulum (SR). To account for the cold effect and the difference in behavior between red and white tissues, two reported properties of the SR have been invoked (5, 8): its markedly reduced uptake of calcium at low temperatures (9), and its greater calcium-binding ability in white muscle than in red (10).

Preliminary results of a current study indicate mitochondrial participation in cold shortening, and suggest that the calcium prompting the length change is released from the mitochondria reacting to anoxia, rather than the SR responding to cold. The finding leads to a simpler and less conjectural explanation of the

phenomenon, the differences in cold shortening among muscles being reflections of their widely varying contents of mitochondria.

METHODS

Bovine sternomandibularis ("neck") muscles, removed from the carcass 20-30 min. postmortem, were cut longitudinally into strips about 2-3 mm across and 5-7 cm in length. Several strips, selected from the preparations for uniformity, were dipped briefly (3-5 sec.) into one of a number of test solutions (0.9% NaCl, either alone or with added reagent: see later). They were then laid out on light nylon mesh previously stretched tightly over an open frame, a method of support allowing atmospheric contact on all surfaces. A 70 mm scale was placed parallel to and between each pair of strips to facilitate length measurements. The entire assembly, holding up to 10 strips, was transferred to a large beaker, either in the laboratory (20^{o}) or in a cold room (2^{o}). The beaker was then covered tightly with clear plastic film to exclude air, and was flushed with water-saturated oxygen, air or nitrogen. Gas flow was continued throughout the experiment via an inlet tube to the bottom of the beaker. With three such units in use, concurrent observations could be made of the rates and extents of shortening induced by various combinations of temperature, atmosphere and added reagent. Although length measurements were made at intervals until 24 hours postmortem, results are usually quoted simply as percent shortening attained 1 hour after the dipping operation, to avoid any possible overlap between true cold shortening and the later rigor shortening observed on most occasions.

RESULTS

In the first series of experiments, muscle strips were exposed to either nitrogen or oxygen at either 2^{o} or 20^{o}. At the higher temperature and in both atmospheres, little or no shortening occurred during the first hour, but at 2^{o} a major difference in response to the two gases became evident. Strips held in nitrogen (2^{o}) shortened on average by 22% in 1 hour, a result entirely compatible with that of Locker and Hagyard (1), whose much larger samples would have remained internally anaerobic despite continuous air contact. In oxygen, however, the strips shortened very much less, the length change being sometimes undetectable and seldom more than 10% even after 5 hours; even this small shortening may have been a result of difficulties in preparation, since localized contraction could have occurred in zones where the strip had been left thicker (and therefore less oxygen-permeable) than elsewhere.

Further evidence that oxygen suppresses cold shortening was obtained by replacing oxygen (2^{o}) by nitrogen (2^{o}) after varying intervals of time. In every case (2-8 hours of oxygen exposure), shortening commenced within a few

minutes of the gas change, attaining an additional 8-12% of initial length within 1 hour and 17-22% within 3 hours. In a single experiment over a longer time-span, strips left in oxygen (2^{o}) for 25 hours shortened by 3%; following transfer to nitrogen (2^{o}), their shortening increased to 7% in $1\frac{1}{2}$ hours and to 12% in a further 3 hours.

With this indication that oxygen reduces or eliminates cold shortening, a study was made of the ability of several reagents to overcome the oxygen suppression. The following substances were used: dinitrophenol, ruthenium red, carbonyl cyanide m-chlorophenylhydrazone and dicumarol. The same dipping technique was employed, strips being immersed briefly in 0.9% saline plus reagent while control strips from the same muscle received only the saline treatment. Both treated and control samples were then transferred immediately to the same oxygenated container.

At 20^{o}, none of the reagents had any significant effect on strip length during the first 2 hours. At 2^{o}, however, the reagents caused rapid and intense responses. Within an hour of treatment with ruthenium red (.01 and .1 mM) or dicumarol (.6 mM), the strips shortened by an average of 24%, their entire pattern of length change resembling very closely that of saline-treated strips exposed to nitrogen at 2^{o}. Dinitrophenol (1mM) and carbonyl cyanide m-chlorophenylhydrazone (approximately .06 mM) stimulated considerably greater shortening, about 40% in the one-hour period, the rate of change during the first 15 min. being about three times that of saline-nitrogen (2^{o}) strips.

Two variations on these simple experiments provided further evidence that uncouplers in the cold cause markedly different effects from those produced at room temperature. In the first, saline-dipped strips were left in air at 2^{o} for 24 hours, during which time they shortened by 15%. They were then immersed briefly in saline/dinitrophenol (1 mM) and returned to 2^{o} air; within an hour their mean shortening had increased to 39%. In the second, repeated several times, strips were dipped in saline/dinitrophenol (1 mM) immediately after preparation 2 hours postmortem, and were left in 20^{o} oxygen for intervals of

from 0 to $5\frac{1}{2}$ hours before transfer to 2^{o} oxygen. Within 15 min. of their exposure to cold, shortening had increased from 0-5% of initial length to 29-45%.

Finally, a few key experiments were performed on rabbit psoas muscle at 2^{o}, in order to allow direct comparison of shortening responses to treatment in "red" and "white" muscles. Strips were prepared and treated exactly as had been done previously with the bovine muscle, and four sets of conditions were imposed on them: a saline dip followed by exposure to either nitrogen (2^{o}) or oxygen (2^{o}), a saline/dinitrophenol (1 mM) dip followed by oxygen (2^{o}), and a saline/ruthenium red (.01 - .1 mM) dip followed by oxygen (2^{o}). In all cases, shortening in 1 hour was either totally absent or very small, the highest observed value of 3% occurring in the dinitrophenol-treated strips.

DISCUSSION

Mitochondria have been ignored as a possible source of the calcium presumed to initiate cold shortening, yet for several reasons they appear to be a rather obvious and appropriate calcium reservoir for an effect which is confined to visibly red muscles. It is known that they can release and absorb calcium quite readily (11), and are much more abundant in red muscles than in white (12). They discharge calcium slowly when incubated anaerobically and much more rapidly when treated with uncoupling agents (13), a pattern of behavior paralleling our own observations of a relatively slow cold shortening in cold nitrogen and a much faster change in the presence of uncouplers in cold oxygen. Finally, mitochondrial calcium continues to receive attention as a possible initiator of contraction in both cardiac (14) and skeletal (15) muscle, the principal objection to this mechanism being its relative slowness (16). No such problem arises in the present hypothesis since, compared with contraction, cold shortening is an extremely sluggish process.

We propose, therefore, that cold shortening is a consequence of anoxia-induced calcium release from muscle mitochondria at a temperature which is low enough to prevent a fully compensating calcium uptake by the highly temperature-sensitive sarcoplasmic reticulum.

ACKNOWLEDGMENTS

The authors are grateful to Dr. M. L. Greaser for helpful advice and constructive criticism. Research supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison, and by the American Meat Institute Foundation. Muscle Biology Manuscript No. 88.

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