Thermal Inactivation of the Calcium Regulatory Mechanism of Human Skeletal Muscle Actomyosin:
A Possible Contributing Factor in the Rigidity of Malignant Hyperthermia

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The muscular rigidity associated with anesthetically induced malignant hyperthermia has been attributed to an increase in myoplasmic free calcium concentration. However, previous in-vitro studies have shown that increased temperature can eliminate the calcium requirement for actin-myosin interaction. Therefore, the calcium dependency of human skeletal muscle actomyosin in response to temperature increases of the magnitude encountered in human muscle during hyperthermic episodes was investigated. Calcium dependency is expressed in terms of the ability of a calcium-chelating agent, EGTA, to inhibit the ATP-induced turbidity increase of actomyosin suspensions (superprecipitation). In the presence of millimolar concentrations of ATP and magnesium, EGTA completely inhibits superprecipitation at temperatures as high as 35 C. With further increase in temperature this inhibition is progressively reduced until, at 45 C, the extent of superprecipitation is independent of the calcium concentration. Loss of calcium control is potentiated by reduction in the ATP concentration. Since the muscular rigidity of malignant hyperthermia is associated with both an elevation of muscle temperature and a decline in muscle ATP content, it is evident that in this disorder conditions might exist for an increase in muscle tension that is independent of changes in intracellular free calcium concentration.

(Key words: Hyperthermia, malignant pyrexia; Ions, calcium, malignant pyrexia; Muscle, skeletal, malignant pyrexia.)

MALIGNANT HYPERThERMIA is an infrequent, but nonetheless catastrophic, complication of inhalation anesthesia. Although the precise etiology is unknown, most investigators now seem to agree that it is a pharmacogenetic disorder of skeletal muscle.1,2 An apparently identical syndrome occurs in certain breeds of swine.3

Apart from the alarming rise in body temperature, a striking feature of this disorder, seen in many cases, is the pronounced rigidity of skeletal muscle. Since an increase in muscle tension ordinarily suggests an increase in the intracellular free calcium concentration, the attention of investigators has focused on the excitation–contraction coupling system as the site of the defect. Several authors1,2,4 have proposed that in individuals prone to malignant hyperthermia the sarcomembranes that regulate calcium distribution are abnormally sensitive to certain inhalation anesthetics such as halothane. It is assumed that the anesthetic causes an increase in the calcium permeability of these membranes or a displacement of bound calcium. The resultant elevation of myoplasmic calcium concentration would stimulate sarcomembrane ATPase,5 glycolysis,6 and the actin-activated myosin ATPase.7 Secondarily, the excess calcium may also cause uncoupling of oxidative phosphorylation.8 All these reactions would account for massive heat production, and the increased actin–myosin interaction would account for the muscle rigidity.

The above hypothesis is supported by some indirect evidence. Defects in calcium transport by sarcomembranes isolated from susceptible human and porcine muscle have been detected.9,10 In addition, there have been several reports of successful arrest

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of hyperthermia and rigidity in humans and swine by membrane-stabilizing drugs (procaine, procainamide), which are known to reduce the calcium permeability of muscle membrane systems.\textsuperscript{11-13}

The aim of this communication is to call attention to another facet of this syndrome which has not been emphasized previously, namely, the effect of elevated muscle temperature on the contractile system. Under normal circumstances muscle is maintained in a relaxed state through the inhibitory effect of a regulatory protein complex (tropomyosin–troponin) bound to the actin filament.\textsuperscript{14} Contraction occurs when calcium ion is bound to troponin.\textsuperscript{15,16} Muhlrad and Hegyi\textsuperscript{17} first noted that the calcium requirement for activation of rabbit myofibrillar ATPase was eliminated as the temperature was raised—that is, the system behaved as if the tropomyosin–troponin complex were no longer present. This phenomenon was later studied in more detail by Murphy and Hasselbach\textsuperscript{18} and Hartshorne et al.\textsuperscript{19,20} Using superprecipitation of rabbit actomyosin as an \textit{in-vitro} model of contraction, Fuchs, Hartshorne, and Barns\textsuperscript{20} showed that in the presence of millimolar concentrations of ATP and magnesium, contraction could take place in the absence of calcium when the temperature was just a few degrees higher than the physiologic temperature. If one extrapolates to human muscle \textit{in vivo} it would seem reasonable to postulate that in the hyperthermic state a large fraction of the increased heat production and muscle tension may not be calcium-dependent. This report contains observations on the calcium requirement for superprecipitation of human skeletal actomyosin in the normal and hyperthermic temperature ranges (35–45°C).

\section*{Materials and Methods}

Human skeletal muscle actomyosin was prepared from autopsy material according to methods already described.\textsuperscript{21} Dr. Frederick J. Samaha, Department of Neurology, University of Pittsburgh School of Medicine, kindly provided the actomyosin used in this study.

The ATP-induced superprecipitation of actomyosin was assayed as described previously.\textsuperscript{13} All measurements were made with a Hitachi-Coleman 124 spectrophotometer equipped with a constant-temperature circulating system. Cuvette temperature was verified with a thermistor probe. The medium contained 60 mM KCl, 30 mM imidazole (pH 7.0), 0.5 mM EGTA; actomyosin, 0.26 mg/ml. The reaction was started by addition of 1 mM MgATP, as indicated by the arrow at time zero. The arrow at 2 minutes indicates addition of 0.5 mM CaCl\textsubscript{2}.

\begin{figure}
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\includegraphics[width=\textwidth]{fig1}
\caption{Representative records of superprecipitation of human actomyosin at different temperatures. Assay conditions: 60 mM KCl, 30 mM imidazole (pH 7.0), 0.5 mM EGTA; actomyosin, 0.26 mg/ml. The reaction was started by addition of 1 mM MgATP, as indicated by the arrow at time zero. The arrow at 2 minutes indicates addition of 0.5 mM CaCl\textsubscript{2}.}
\end{figure}

\section*{Results}

Representative records of actomyosin superprecipitation at four temperatures are shown in figure 1. Note that at 30°C the addition of 1 mM MgATP, in the presence of the calcium-chelating agent EGTA, caused a reduction in optical density or "clearing." This response is considered to reflect the dissociation of actin and myosin and corresponds to the "relaxed" state. Upon addition of sufficient CaCl\textsubscript{2} to elevate the free calcium concentration to \(\sim 10^{-3}\text{M}\), there was an im-
mediate increase in optical density beyond the baseline value. This increase is associated with an enhanced cycling of myosin cross-bridges and corresponds to the "contracted" state. Although not illustrated here, the increase in turbidity of the actomyosin suspension is coupled to an accelerated hydrolysis of ATP. It is evident that with an increase in temperature the clearing response is eliminated and replaced by superprecipitation despite the low level of free calcium in the medium. Evidence has been presented elsewhere that this is a reversible inactivation of the calcium control mechanism, not attributable to denaturation or sulphydryl oxidation.

A plot of the optical density changes induced by ATP in the presence and absence of calcium as a function of temperature is shown in figure 2. Under these experimental conditions optimal calcium control is observed at temperatures below 35°C. At higher temperatures the two curves converge, and at 45°C the extent of superprecipitation is essentially the same regardless of calcium concentration.

As shown elsewhere with rabbit actomyosin, the temperature at which calcium control is lost is dependent upon the ATP concentration. That is, with higher ATP concentrations a higher temperature is required for thermal inactivation of the calcium control system. The possible molecular explanation of this phenomenon is discussed elsewhere. In this respect, human actomyosin behaves in the same way as rabbit actomyosin does. Figure 3 shows the effects of temperature and ATP concentration on actomyosin turbidity in the absence of calcium. The temperatures and ATP concentrations chosen fall within the ranges that might be encountered in human skeletal muscle under normal and hyperthermic conditions. It is clear that at any given ATP concentration a two- or three-degree rise in temperature can cause a significant increase in actin–myosin interaction independent of changes in calcium concentration. Moreover, at any given temperature actin–myosin interaction is enhanced by a decrease in ATP concentration. It is significant that in malignant hyperthermia not only is muscle temperature increased, but muscle ATP content is diminished (see below).

**Discussion**

The data presented here show that the calcium control system of human actomyosin is inactivated by increases in temperature of the magnitude that might be encountered in intact muscle during severe hyperthermic episodes. Furthermore, the effect of increased temperature is potentiated by reduction in the ATP concentration. Although no data for man are available, several workers have...
have shown that in porcine muscle ATP content is significantly decreased during halothane-induced hyperthermia. Thus, conditions can exist for a development of muscle tension that does not require calcium. When excised amphibian or mammalian muscles, in the absence of any pharmacologic agent, are exposed to a temperature just a few degrees higher than their usual operating temperatures, they go into a state of "heat contracture."25-26 Fuchs et al.29 have proposed elsewhere that this phenomenon may be the result of a direct effect of temperature on the calcium regulatory mechanism.

With regard to the problem of malignant hyperthermia in man, two main considerations emerge. Firstly, an increase in muscle tension, by itself, cannot be taken as conclusive evidence of an increase in myoplasmic calcium concentration. Second, even if it is assumed that the thermogenesis and rigidity are initially induced by release of calcium, it is likely that at higher temperatures a large fraction of the extra energy released is not calcium dependent. Thus, as muscle temperature rises, the system would tend to go "out of control": this loss of calcium control may be a major factor in the "malignant" character of this disorder.

It remains for investigators to sort out the quantitative contributions of changes in muscle temperature, ATP content, and myoplasmic calcium concentration to the origin of the rigidity observed in malignant hyperthermia. Unfortunately, methods for the measurement of free intracellular calcium concentration in intact mammalian muscle are not available. Studies with isolated sarcotubular vesicles do not lend themselves to a simple physiologic interpretation. Kalow et al.9 reported that vesicles isolated from patients who had survived hyperthermia and rigidity demonstrated a reduced calcium uptake in the presence of concentrations of halothane that had no effect on vesicles obtained from normal subjects. Brucker et al.10 obtained similar results with porcine sarcotubular vesicles. On the other hand, Dhalia et al.27 found that vesicles isolated from a surviving human patient retained more calcium in the presence of halothane than controls, and Nelson et al.28 were unable to detect any difference between vesicles isolated from normal and susceptible pigs.

Harrison's data indicate that the onset of rigidity in the pig during halothane administration precedes the rise in muscle temperature. This finding is consistent with the hypothesis that calcium release is the triggering event responsible for both hyperthermia and rigidity. However, as Brittl has pointed out, there is a significant number of human subjects in whom hyperthermia develops without rigidity or in whom rigidity is a late event. Rigidity of late onset might be accounted for by a direct effect of increased temperature on the calcium regulatory mechanism. The origin of the increased heat production in such cases is still an open question. Kalow et al.9 and Brittl believe that human malignant hyperthermia is not a single disease entity.

The significance of ATP depletion in hyperthermic muscle has been discussed by Nelson et al.28 The reason for the decline in ATP levels has not been established but, considering the enhanced rate of ATP utiliza-
tion and the possible uncoupling of oxidative phosphorylation, such a finding is not unexpected. According to Berman and Kench, there were only small decreases in muscle ATP content at the onset of rigidity in the pig—large changes were observed only after prolonged periods of rigidity. However, this observation does not necessarily mean that ATP concentration can be discounted as a significant factor in the genesis of the rigid state. Bendall showed more than two decades ago that in excised rabbit psoas muscle at 37 C the onset of rigor was associated with a decrease in ATP content of only 15–20 per cent. It would seem reasonable to suppose that such a small change in ATP content of human muscle, coupled with a three- or four-degree rise in muscle temperature, could have a significant effect on muscle tension independently of changes in myoplasmic calcium concentration.

In summary, a complete understanding of malignant hyperthermia must take into account not only the function of the calcium-release mechanism but also the biochemical properties of the contractile system. The situation might be clarified by careful studies in the pig in which temperature, tension, ATP content, and calcium turnover are measured in the same muscle at various times after the induction of anesthesia. It would also be of interest to know whether there are differences in the thermal sensitivities of actomyosin from normal individuals and actomyosin from genetically susceptible individuals.

References

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Circulation

HYPOTENSION IN UREMIC PATIENTS

Eight uremic patients were studied to determine the cause of hemodialysis-induced hypotension and assess the effects of therapy by volume expansion vs. administration of norepinephrine. Hemodynamic responses to the Valsalva maneuver and inhalation of amyl nitrite were used to assess autonomic nervous system function. Peroneal nerve conduction velocities were determined for each patient. Hemodynamic studies were performed during hemodialysis at regular intervals and during hypotensive episodes. Two patients (Group 1) had apparently normal autonomic responses and normal peroneal nerve conduction, and their hemodynamic responses to hypotension and subsequent volume expansion suggested hypovolemia as the cause of hypotension. Six patients (Group 2) had depressed peroneal nerve conduction velocities and autonomic insufficiency. Volume expansion did not significantly improve their hemodynamics, whereas infusion of norepinephrine did. The authors conclude that autonomic insufficiency probably accompanies the generalized neuropathy seen in chronic uremia and may contribute to the pathogenesis of hemodialysis-induced hypotension. (Kersh, E. S., and others: Autonomic Insufficiency in Uremia as a Cause of Hemodialysis-induced Hypotension. N Engl J Med 290:650–653, 1974.) ABSTRACTER’S COMMENT: Thus, in uremic patients, one may or may not see the cardiovascular stability we expect with inhalation agents that normally stimulate the sympathetic nervous system.