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Onset of rigor mortis is earlier in red muscle than in white muscle

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Abstract Rigor mortis is thought to be related to falling ATP levels in muscles postmortem. We measured rigor mortis as tension determined isometrically in three rat leg muscles in liquid paraffin kept at 37 °C or 25 °C – two red muscles, red gastrocnemius (RG) and soleus (SO) and one white muscle, white gastrocnemius (WG). Onset, half and full rigor mortis occurred earlier in RG and SO than in WG both at 37 °C and at 25 °C even though RG and WG were portions of the same muscle. This suggests that rigor mortis directly reflects the postmortem intramuscular ATP level, which decreases more rapidly in red muscle than in white muscle after death. Rigor mortis was more retarded at 25 °C than at 37 °C in each type of muscle.

Key words Rigor mortis · Muscle fiber types · Liquid paraffin · Temperature · Rats

Introduction

We previously reported that postmortem changes in ATP levels in various muscles were different and that one of the causes might be the difference in the proportion of different muscle fiber types [1,2]. ATP decreased more in red muscle than in white muscle, but we did not establish that the difference in the ATP level was related to the difference in progress of rigor mortis. Measurements of rigor mortis in different muscles are needed to show that the progress of rigor mortis does in fact vary among different muscles. However, it is difficult to measure and compare postmortem dynamic changes in different muscles.

Many researchers have measured rigor mortis of muscles *in situ*. Krompecher and co-workers [3–8] measured rigor mortis in whole limbs of rats. Forster measured rigor mortis of gastrocnemius muscles in rat cadavers isotonically and isometrically [9,10] by exposing only the calcaneal tendon. Schuck et al. [11] and Vain et al. [12,13] measured rigor mortis in human muscles *in situ*. Other researchers measured rigor mortis in muscles taken from cadavers. Bate-Smith [14], Bate-Smith and Bendall [15,16], Bendall [17,18] and Doering et al. [19] measured rigor mortis in one muscle in moist air isotonically by repeated extension and release. Single or skinned fibers have generally been used in recent experiments on muscle rigor caused by lack of ATP [20–23], but these were models of ischemia and not of rigor mortis. Although many methods have been used to quantify rigor mortis, they are not appropriate for comparing rigor mortis in various muscles.

We have developed a new method for measuring rigor mortis isometrically that made it possible to measure and compare rigor mortis in various muscles easily. We measured rigor mortis in three muscles of a rat leg, red gastrocnemius, white gastrocnemius and soleus. The proportions of fiber types and biochemical postmortem changes were examined previously [2] and significant differences were found. Red gastrocnemius and soleus are red muscles, in which type I and/or IIA muscle fibers predominate, whereas white gastrocnemius is a white muscle composed almost entirely of type IIB fibers. This paper discusses the relationship between the course of rigor mortis and muscle fiber types.

Materials and methods

Muscle samples

For the experiment, 9-month-old Sprague-Dawley rats were rested and fed ad libitum and then injected with a total of 200–250 mg/kg mephenesin intraperitoneally, according to the method of Bate-Smith and Bendall [16]. The mephenesin stopped movement of all skeletal muscles except those used in ventilation for 30 min. This stabilizes the muscles and excludes the influence of antemortem muscle exercise. The rats were then bled from the heart. We then examined the red gastrocnemius (RG), white gastrocnemius (WG) and soleus (SO) muscles. The difference in proportion of fiber

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types and postmortem changes in ATP, lactic acid and glycogen levels in these muscles have been reported previously [2].

The muscles were removed immediately after the rats were killed and sections cut parallel to the muscle fibers into strips of about 1.2 × 0.4 × 0.4 cm in liquid paraffin under a stereomicroscope. All surfaces of the samples were trimmed with a blade and the tendons and fascia were removed as far as possible.

Measurement of rigor mortis

The preparations were mounted vertically in a 20 ml water-jacketed tissue bath, containing liquid paraffin (Wako, Osaka, Japan) and the temperature was kept at 37 °C or 25 °C. The lower end of the preparation was attached to the bottom of the bath and the other end was connected to an isometric sensor (Star Medical, IM-20BS, Tokyo, Japan) by silk threads. The isometric tension was recorded on a recorder (Graphtec, Thermal arraycorder WR 7300, Yokohama, Japan) using a preamplifier (Star Medical PA-001, Tokyo, Japan). The first 10 min postmortem were used to set up the apparatus and no measurements were made during this time. At the start of measurement, the muscle was stretched by a 1 g weight to tense the thread and enable sensitive measurement of the tension. The change of tension was then measured and recorded over an 8 h-period for all samples.

Statistical analysis

There were four samples of each muscle for each temperature. The tension every 5 min up to 8 h postmortem was converted into a percentage of the maximum level in each sample and the initial tension was taken as 0%. The significance of the differences for each of the following was analyzed by a one-sample *t* test as follows:

1. Differences in the postmortem interval in which the rigor mortis reached 10%, 50%, and 100% of maximum tension at 37 °C or 25 °C. These were regarded as onset, half, and full rigor mortis, respectively.
2. Differences in the time taken for muscle rigor to resolve from 100% to 70% at 37 °C and 25 °C, or to 50% at 37 °C.

Results

In all muscles, tension increased and then decreased over the 8 h postmortem period both at 37 °C and 25 °C. The average tension as a percentage of the maximum tension in the course of rigor mortis at every 5 min postmortem is shown in Fig. 1. The average postmortem intervals in

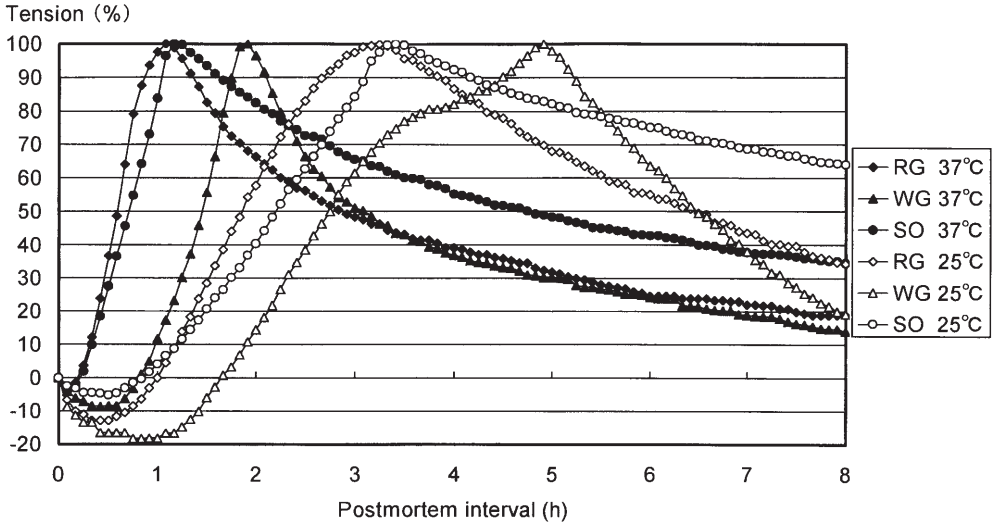

Fig. 1 Average tension as a percentage of the maximum tension in the course of rigor mortis at every 5 min postmortem in red gastrocnemius (*RG*), white gastrocnemius (*WG*) and soleus (*SO*) muscle of male Sprague-Dawley rats at 37 °C and 25 °C

Table 1 The average interval ± standard deviation (min) from death to the time for muscle tension to reach 10%, 50% and 100% of maximum tension in the course of progress of rigor mortis and from the start of resolution of rigor mortis to 70% and 50% in the course of resolution in red gastrocnemius (*RG*), white gastrocnemius (*WG*) and soleus (*SO*) muscles of male Sprague-Dawley rats at 37 °C and 25 °C

	Temperature					
	37 °C			25 °C		
	RG	WG	SO	RG	WG	SO
Progress						
0–10%	18 ± 2[c]	59 ± 1[a,e]	21 ± 4[c]	70 ± 9[c,g]	115 ± 14[a,e,g]	73 ± 15[c,g]
0–50%	36 ± 5[c,f]	89 ± 4[a,e]	43 ± 5[b,c]	112 ± 5[c,f,g]	166 ± 18[a,e,g]	133 ± 13[b,c,g]
0–100%	62 ± 8[c]	112 ± 5[a,e]	69 ± 4[c]	186 ± 12[c,f,g]	293 ± 5[a,e,g]	205 ± 7[b,c,g]
Resolution						
100–70%	46 ± 17[d,f]	29 ± 5[b,f]	92 ± 32[b,d]	97 ± 13[d,f,h]	50 ± 19[b,f,h]	197 ± 68[b,d,h]
100–50%	121 ± 53[d,f]	66 ± 15[b,f]	270 ± 144[b,d]	–	–	–

[a] Different from time in RG at same temperature ($P < 0.01$)
[b] Different from time in RG at same temperature ($P < 0.05$)
[c] Different from time in WG at same temperature ($P < 0.01$)
[d] Different from time in WG at same temperature ($P < 0.05$)
[e] Different from time in SO at same temperature ($P < 0.01$)
[f] Different from time in SO at same temperature ($P < 0.05$)
[g] Different from time in same kind of muscle at 37 °C ($P < 0.01$)
[h] Different from time in same kind of muscle at 37 °C ($P < 0.05$)

which rigor mortis reached 10%, 50%, and 100% of the maximum tension and the average time to go from 100% to 70% or 50% of the maximum tension in the course of resolution are shown in Table 1.

At 37 °C, the times to onset, half, and full rigor mortis were each shorter in RG and SO than in WG. There was a significant difference only in the time to half rigor mortis between RG and SO. The time taken for resolution from 100% to 70% and 50% of the maximum tension were shorter in WG than in RG and shorter in RG than in SO.

At 25 °C, onset, half and full rigor mortis in each kind of muscle took longer than at 37 °C. The time to onset, half and full rigor mortis were each shorter in RG and SO than in WG, as was the case at 37 °C. The times to half and full rigor mortis were both significantly shorter in RG than in SO. The time for resolution from 100% to 70% of the maximum tension was longer at 25 °C than at 37 °C in RG and SO, shorter in WG than in RG, and shorter in RG than in SO.

Discussion

To measure rigor mortis in muscles taken from a rat cadaver, we used liquid paraffin, which is generally used to prepare skinned fibers [24]. Various solutions have been used for physiological muscle experiments, but these are not appropriate for experiments on rigor mortis because various substances, including ATP and electrolytes, diffuse from the muscle to the solution and vice versa. Many studies measured rigor mortis of muscles in moist air. However, some degree of drying of such samples could not be avoided and the temperature was unstable. Liquid paraffin can prevent both diffusion of intramuscular and extramuscular substances and drying of muscle samples and the temperature can be controlled easily with a water-jacketed tissue bath.

When rigor mortis is measured manually at autopsy, it is measured isotonically. However, repeated extension and release of muscle in experimental isotonic measurement of rigor mortis is not natural because muscles are not usually repeatedly extended in human cadavers and such repeated extension of muscle can resolve rigor mortis. Rigor mortis has three characteristics, shortening and loss of elasticity and plasticity [9, 10]. Tension, as measured in our experiment, reflects only one of these characteristics *i.e.* shortening, but the isometric measurements could be performed in the natural course of rigor mortis in the muscles of cadavers.

In our previous study [2], we showed that the ATP level was lower at death and decreased more rapidly in RG and SO than in WG. In this paper, we showed that rigor mortis progressed significantly more rapidly in RG and SO than in WG. There was an especially notable difference between RG and WG even though they are portions of the same muscle. These data suggest that rigor mortis may directly reflect the decrease in the postmortem intramuscular ATP level, which differs between red and white muscle.

The cause of the more rapid decrease in ATP levels in postmortem red muscle is not clear. Glycolysis following glycogenolysis is the main process in the formation of ATP in living type IIB muscle fibers, which predominate in white muscle. Oxidative phosphorylation, which is mainly performed in type I red fibers, stops after death because of anoxia. Therefore ATP would be formed by glycolysis after death much more readily in white muscle than in red muscle, and ATP levels would fall more rapidly in red muscle than in white muscle. However, in our previous study, the level of lactic acid which is formed as a result of glycolysis, was lower in WG than in RG or SO 1 h postmortem. So the difference in postmortem changes in ATP levels between red and white muscle cannot be explained only by the difference in postmortem activity in glycolysis. The level of creatine phosphate is higher in white muscle than in red muscle [25] and this would retard the decrease in ATP level in white muscle. It is possible that postmortem ATPase activity is different between red muscle and white muscle.

The decrease in tension after full rigor mortis was faster in WG than in RG and faster in RG than in SO both at 37 °C and 25 °C, suggesting that this process might be related to the resolution of rigor mortis which is regarded as a denaturation process, namely, autolysis [8]. Bendall [18] suggested that this process was pH dependent and did not occur at comparatively high pH values. In our previous study on muscles in rat cadavers kept at 33 °C, the lactic acid was lower level 1 h postmortem, but the levels 3 and 4 h postmortem were much higher in WG than in RG and SO. Particularly in WG, a rapid decrease in tension was observed and one of the causes might be low pH caused by a high lactic acid level in WG.

The decrease in shortening after full rigor started in 2 h postmortem at 37 °C in our experiment. However, this started faster compared with resolution of rigor mortis in rat whole hind limb measured isotonically at 37 °C by Krompecher [8] and it started between 3 h and 4 h postmortem. Even if the muscles lengthen again after full shortening, the decrease of elasticity of muscles, which is another characteristic of rigor mortis measured isotonically, might not return in the same time and rigor mortis might not resolve when measured isotonically.

This experiment also showed that temperature influences the progress of rigor mortis considerably, as other researchers have pointed out [8,16]. The progress of rigor mortis in human hands or feet is slow and one of the causes of this might be the temperature, which falls rapidly in these body parts in a corpse, as assumed by Krompecher [8].

We showed that rigor mortis progresses more rapidly in red muscle than in white muscle, but this does not explain the downward progress of rigor mortis from the jaw. Data on the proportion of fiber types in human muscles [26–28] showed that the proportion of type I fibers is not as high in human masticatory muscles although type IIA and IIB were not distinguished in these reports. However, in human masticatory muscles, the size of type I fibers is much greater than that of type II fibers and there are also

intermediate fibers, which are not present in the biceps brachii [27–31]. These are unique characteristics of masticatory muscles and it is possible that they are related to early onset of rigor mortis in the jaw joint. Further study is needed to identify the cause of the downward progress of rigor mortis.

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