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Does the sequence of onset of rigor mortis depend on the proportion of muscle fibre types and on intra-muscular glycogen content?

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Abstract We examined the postmortem changes in the levels of ATP, glycogen and lactic acid in two masticatory muscles and three leg muscles of rats. The proportion of fibre types of the muscles was determined with NIH image software. The ATP levels in the white muscles did not decrease up to 1 h after death, and the ATP levels 1 and 2 h after death in the white muscles were higher than those in the red muscles with a single exception. The glycogen level at death and 1 h after death and the lactic acid level 1 h after death in masticatory muscles were lower than in the leg muscles. It is possible that the differences in the proportion of muscle fibre types and in glycogen level in muscles influences the postmortem change in ATP and lactic acid, which would accelerate or retard rigor mortis of the muscles.

Key words ATP · Glycogen · Muscle fibre types · Rigor mortis · Rat experiments

Introduction

Why rigor mortis progresses downwards from the jaw [1–3] is unknown although it is one of the important postmortem changes [4–7]. We have previously reported postmortem changes in the levels of ATP, ADP, AMP and lactic acid in five different rat muscles [8] and have shown that the changes in ATP, ADP and lactic acid were not the same in all the muscles. There was a greater decrease in the ATP level 2 h after death in the masseter muscle compared with the other muscles but the cause of this was not studied.

The slow postmortem formation of ATP and the rapid consumption of ATP cause the total decrease of ATP level after death, resulting in rigor mortis. Concerning the formation of ATP in muscles postmortem, oxidative phosphorylation is blocked by anoxia and ATP is formed exclusively by glycolysis, following glycogenolysis. It is believed that glycogenolysis influences both the level of ATP in a muscle postmortem and the progress of rigor mortis in a muscle [3, 9]. Myosin adenosine triphosphatase (myosin ATPase) plays an important role in the consumption of ATP in muscles postmortem.

Muscle fibres are not the same in all muscles and are divided into several types mainly by enzyme histochemistry and each type has its own metabolic, morphological and physiological features [10, 11]. Types I, IIA and IIB are well known classifications that are distinguished by the characteristics of their ATPase [12–14]. Type I fibres contain relatively many mitochondria and large amounts of myoglobin and perform oxidative phosphorylation to form ATP [15–17]. Muscle fibres of type IIB contain relatively more glycolytic enzymes and perform glycolysis [18]. The type IIA fibre has the advantages of both types I and IIB and performs both oxidative phosphorylation and glycolysis. The activity of myosin ATPase in type II fibres is higher than in type I [18] and this activity is associated with the speed of contraction of the fibre [19]. Rat muscles, which mainly consist of type I or IIA fibres, have a reddish appearance due to their myoglobin content (red muscle). Conversely, those consisting mainly of type IIB fibres have a whitish appearance (white muscle). Muscles consist of a mosaic of these types of fibres. The proportion of different fibre types differs according to the kind or part of the muscle [20–24] and it is well known that the proportion can vary according to age and repetitive exercise [25, 26].

It is possible that these differences in fibre types influence the formation and consumption of ATP in muscles after death and the onset or advance of rigor mortis. In this study, we selected the kinds or parts of muscle with different proportions of fibre types and examined the postmortem changes in ATP, glycogen and lactic acid levels in these muscles. The relationship between these changes, fibre types and rigor mortis is also discussed.

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Materials and methods

Muscle samples

For the experiment 40 male Sprague-Dawley rats, 9–10 months old, were used. The animals were rested and fed *ad libitum* until the start of the experiment, but they did not eat food at least 1 h before the experiment. They were anaesthetised with diethyl ether and bled from the heart. The following five muscles were examined:

1. Masseter (MA)
2. Temporalis (TE)
3. Red gastrocnemius (RG)
4. White gastrocnemius (WG)
5. Soleus (SO)

MA and TE are masticatory muscles and RG, WG and SO are lower leg muscles. The RG and WG consisted of the deepest and most superficial portions of the medial head, respectively [27].

The muscles of 20 rats were used for determining ATP and lactic acid levels. The five muscles were removed immediately after death from one side of each rat. Before a muscle was removed, the skin on the muscle was removed and the muscle quickly cooled in situ with liquid nitrogen to prevent contraction caused by removal of the muscles. The middle portion of the muscle was completely frozen with liquid nitrogen immediately after removal and pulverised (Cryo-press, Microtech Nition, Funabashi, Japan). The concentrations of ATP and lactic acid in the powdered frozen tissue were determined. Out of the 20 corpses 5 were placed in the supine position at 33° C for 1 h and the same five muscles were then removed without freezing from the unused side and prepared as above. In a similar manner, muscles were taken from corpses at 2, 3 and 4 h after death and prepared.

The muscles of the other 20 rats were used for determining glycogen levels and the proportion of muscle fibres. The muscle was removed without freezing and cut in half. One piece was reserved for determining glycogen, the other was prepared for a frozen section. The muscles removed 1, 2, 4 and 8 h after death were prepared to determine the concentration of glycogen in a similar manner. The proportion of fibre types in the muscles was measured only immediately after death.

Histochemistry

The proportions of fibre types were examined in 20 sets of muscle samples taken from the rats immediately after death. The block for sectioning was prepared by the method used by Dawson and Romanul [27]. Samples of two or three different muscles were removed from each rat at the same time and placed next to each other. The block was quickly frozen in isopentane cooled in liquid nitrogen and serial cross-sections 10 μm thick were cut in a cryostat.

The histochemical technique for detecting the ATPase reaction was based on the method of Brooke and Kaiser, using the acid lability of ATPase in type II fibres [12, 13, 28]. Following pre-incubation at pH 4.35–4.45, muscle fibres were identified as type I, IIA, or IIB by dark, light, and intermediate staining, respectively [27]. The total area of each fibre type in the muscle was measured semi-automatically by NIH image, an image analyser software package.

Measurement of ATP, glycogen and lactic acid

The concentration of ATP in the muscle was determined by a UV method using phosphoglycerate kinase [29]. The concentrations of glycogen and lactic acid were determined by the methods of Lo et al. [30] and of Livesley and Atkinson [31], respectively.

Statistical analysis

The number of muscle samples used to determine the concentrations of the substances was 20 at the time of death and 5 for the other times after death. The significance of differences for each of the following was analysed by one-sample *t* test:

1. The differences in the ATP and glycogen levels in the same type of muscle immediately after death and at each postmortem time.
2. The differences in the ATP, glycogen and lactic acid levels in two out of the five muscle samples at the same postmortem time.
3. The differences in the ATP level as a percentage of the initial level in two out of the five muscle samples. The ATP level as a percentage of the initial level was also calculated for each muscle of the same rat and for each postmortem interval.

Results

Fibre type

The average proportions containing each fibre type in the five muscles are shown in Table 1. TE and WG were white muscles in which type IIB fibres were dominant. Type IIA and type I fibres dominated in MA and SO, respectively and were red muscles. The average proportion containing type IIB fibres in RG was less than 20%, so RG was also regarded as a red muscle.

ATP

The average ATP level in each muscle for each postmortem time is shown in Fig. 1 where open symbols show the data for the white muscles. The following number corresponds to the number used in the statistical analysis which showed the following results:

1. The ATP level decreased 1 h after death in MA, RG and SO ($P < 0.01$) but did not significantly decrease in TE and WG.
2. The ATP level at the time of death was lower in SO than in the other muscles ($P < 0.01$), and was lower in RG than in MA, TE and WG ($P < 0.05$). The ATP level 1 and 2 h after death was higher in TE or WG than in MA, RG or SO ($P < 0.05$), except for the level in TE

Table 1 The proportion of fibre types (± standard deviation)in masseter (MA), temporalis (TE), red gastrocnemius (RG), white gastrocnemius (WG) and soleus (SO) muscles of male Sprague-Dawley rats immediately after death

Fibre type	Muscle				
	MA	TE	RG	WG	SO
Type I	0.0 ± 0.0	0.3 ± 0.7	38.2 ± 17.5	0.0 ± 0.0	82.4 ± 6.8
Type IIA	99.1 ± 2.8	6.7 ± 6.1	43.1 ± 16.1	0.1 ± 0.4	13.3 ± 5.9
Type IIB	0.9 ± 2.8	93.0 ± 6.5	18.8 ± 12.0	99.9 ± 0.4	4.3 ± 2.6

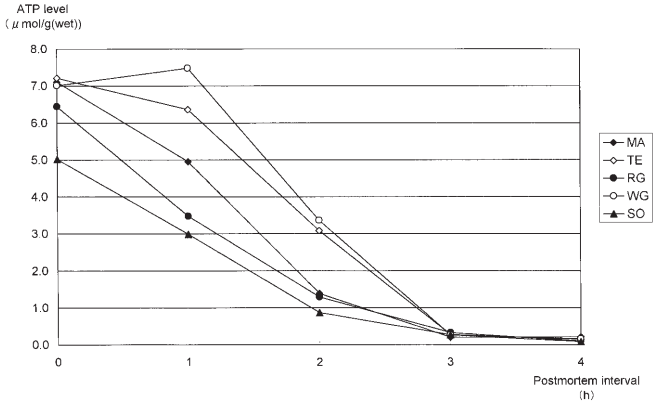

Fig. 1 Average ATP levels at 0 (20 rats), 1, 2, 3, and 4 h (5 rats each) after death in masseter (MA), temporal (TE), red gastrocnemius (RG), white gastrocnemius (WG), and soleus (SO) muscle of male Sprague-Dawley rats

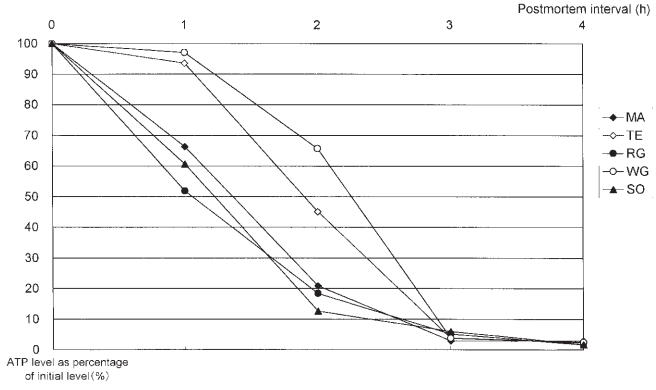

Fig. 2 Average ATP levels as percentage of the initial level at 1, 2, 3 and 4 h after death in five different muscles of five male Sprague-Dawley rats

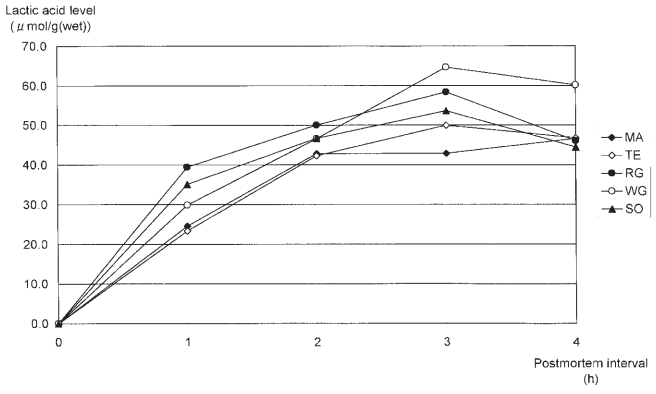

Fig. 3 Average lactic acid levels at 0 (20 rats), 1, 2, 3, and 4 h (5 rats each) after death in temporal (TE), red gastrocnemius (RG), white gastrocnemius (WG), and soleus (SO) muscle of male Sprague-Dawley rats

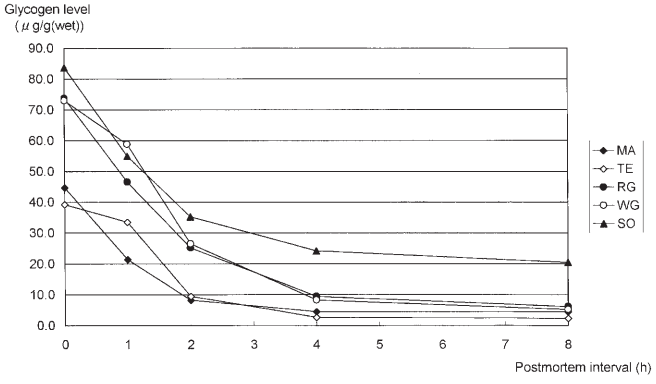

Fig. 4 Average glycogen levels at 0 (20 rats), 1, 2, 4, and 8 h (5 rats each) after death in temporal (TE), red gastrocnemius (RG), white gastrocnemius (WG), and soleus (SO) muscle of male Sprague-Dawley rats

1 h after death, which was not higher than in MA. The ATP level at death was much lower in SO than in the other muscles ($P < 0.01$), and remained significantly lower than in the other muscles except MA until 2 h after death ($P < 0.05$).

3. The data shown in Fig. 2 is the ATP level as a percentage of the initial level in each muscle of the same rat and for each postmortem interval. The percentage 1 and 2 h after death was higher in WG than in MA, RG and SO ($P < 0.05$). The percentage 1 h after death was higher in TE than in RG and SO ($P < 0.05$).

Lactic acid

The average lactic acid level in each muscle for each postmortem time is shown in Fig. 3. The level in the muscles at the time of death was not detected but in our previous report [8] the lactic acid level at death was not zero, suggesting an artefact caused by the contraction of muscle. The muscles usually contract when removed at death, but in this experiment the contraction was prevented by freezing in situ.

The lactic acid level 1 h after death was different in each muscle ($P < 0.05$), except that the level 1 h after death in TE was not different from that in MA. The levels 3 and 4 h after death were higher in WG than in the other muscles ($P < 0.05$).

Glycogen

The average glycogen level in each muscle for each postmortem time is shown in Fig. 4.

The glycogen level decreased in all muscles 1 h after death compared with the level at death ($P < 0.01$) except that the level in TE did not decrease 1 h after death.

The glycogen level at death was much lower in MA or TE than in RG, WG or SO ($P < 0.01$), and remained so until 2 h after death ($P < 0.05$) except that the level 2 h after death in MA was not lower than in RG. The glycogen level in SO was higher than in TE and RG from 0 to 8 h after death ($P < 0.05$), and was higher than in MA and WG 0, 1 and 2 h ($P < 0.05$), and 0, 4 and 8 h after death ($P < 0.05$), respectively. The difference between the glyco-

gen levels in RG and WG was not significant ($P > 0.05$) except at 1 h after death ($P < 0.05$).

Discussion

The decrease of ATP level in each muscle differed significantly, as in our previous report [8]. The level of ATP had not decreased 1 h after death in the white muscles, WG and TE, in which type IIB fibres dominate. The ATP level was higher in white muscles than in red muscles 1 and 2 h after death, with a single exception. Although RG and WG were portions of the same muscle, the difference in change in ATP level between them was very remarkable. Similarly, MA and TE were adjacent muscles, but the change in ATP level was very different.

The ATP level at the time of death was lower in red muscles than in white muscles which was previously reported by Edström et al. [32].

Glycogen is the most important substrate for ATP formation by glycolysis in postmortem muscles [3, 9, 33] and the final product of glycolysis is lactic acid. Postmortem glycogenolysis in a single muscle has been studied in detail [34, 35] but never in different muscles. The level of glycogen was lower at the time of death and 1 h after death in masticatory muscles (MA and TE) than in leg muscles. Moreover, the level of lactic acid was also lower 1 h after death in these masticatory muscles than in the leg muscles. This shows that ATP formation by glycolysis in the masticatory muscles was less than in the leg muscles, which is a special characteristic of masticatory muscles. Some characteristics of human masticatory muscles are known [36–38] but a low glycogen content has not yet been reported (although our experiment was on rat muscles) and it was not known how much this characteristic of the masticatory muscles affects the postmortem change in ATP level. The difference in the ATP level between masticatory muscles and leg muscles was not significant.

The relationship between postmortem glycogenolysis and fibre type was reported previously [39], but the difference in the glycogen level between red and white muscles was not significant. More studies are needed to identify what determines the difference in the change of the ATP level between red and white muscles and in the change of glycogen level between masticatory muscles and leg muscles.

The activity of human myosin ATPase is lower in type I fibre than in type II [18] however, the ATP level in the postmortem muscles in which type I fibre was dominant decreased more rapidly than in those in which type IIB fibre was dominant. Thus it seems that there are other factors influencing the activity of ATPase in each fibre in postmortem muscles.

Bendall showed that the onset of rigor mortis in rabbit psoas muscles depended not only on the ATP level but also the pH [40]. A large decrease in ATP level was needed for the onset of rigor mortis in muscles where the pH was comparatively low. The level of lactic acid was linearly related to the fall in pH [41]. If Bendall's theory is correct for all muscles, the onset of rigor mortis will need a marked decrease of ATP level in muscle in which much lactic acid is formed after death. Conversely, a smaller decrease in ATP level will cause rigor mortis in the muscle in which postmortem lactic acid formation is comparatively less.

In our experiment, the masticatory muscles had less glycogen than the leg muscles and the level of lactic acid 1 h after death was also lower in the masticatory muscles than in the leg muscles. Therefore, the pH 1 h after death would be higher in the masticatory muscles than in the leg muscles. In the masticatory muscles, rigor mortis might start when the ATP level was higher than in the leg muscles. The difference in pH among different muscles, which is associated with the difference in glycogen level, might be one of the causes of the sequence of rigor mortis, although this was not examined in human muscles.

Our study showed the difference in the postmortem changes in ATP level between red and white muscles of rats. Rigor mortis would start earlier in the red muscles than in the white muscles if the pH at onset of rigor mortis was the same.

Rigor mortis is measured manually by attempting to flex or extend each joint during autopsy. Many factors such as exercise, cause of death, temperature and nourishment affect the onset or progress of rigor mortis of the whole body [9, 33, 42–44]. Concerning rigor mortis in each joint, of course we have to consider that it is not just one muscle that moves a joint. The onset or progress of rigor mortis of each joint would be accelerated or retarded by the dominant fibre type in the comparatively large or dynamically influential muscle out of the muscle group that moves the joint.

In conclusion, it is possible that the sequence of rigor mortis depends on the difference in lactic acid level among different muscles, which corresponds to the difference in glycogen level, as well as on the difference in muscle fibre types.

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