Short Communication

Effect of Fasting on the
Endogeneous Inhibitor of
Calcium Activated Factor in the
Skeletal Muscle of RatsMasako TAKASHIMA, Tadao KURATA
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The effect of fasting on calcium activated factor (CAF) in the skeletal muscle of rats was described in the previous paper.¹⁾ CAF activity in the skeletal muscle of rats was found to increase significantly in response to fasting, although some differences were also observed in the CAF activity increase due to different strains and ages of rats.

The existence of an endogeneous inhibitor of CAF (CAF inhibitor) in various animal tissues has been found and some properties of this CAF inhibitor have been reported.^{2~4)} It is described that the formation of the CAF-CAF inhibitor complex was made under the existence of Ca ions. CAF activity may be regulated by this CAF inhibitor which binds the CAF-Ca²⁺ complex (representing the active form) to make the CAF-CAF inhibitor complex. Moreover, the CAF inhibitor has been described to inhibit CAF specifically without any effect on such other proteases as trypsin, chymotrypsin and papain.

In this experiment, both CAF activity and CAF inhibitor activity in the skeletal muscle of rats were measured, and their activity changes in response to fasting were studied.

Male Fischer strain rats (7 weeks old) were obtained from a commercial source.* They were kept in individual cages with wire bottoms, and fed with a commercial diet (CE-2, Clea Japan, Inc.) and water *ad libitum* prior to

fasting. After a week of preliminary feeding, they were divided into several groups. The fasting rats had unrestricted access to water.

Crude CAF and CAF inhibitor preparations were obtained from rat longissimus muscle of back. One gram of minced muscle was homogenized in 6 ml of 4 mM EDTA (pH 7.2) and centrifuged at 44,000 × *g* for 20 min. Isoelectric precipitation was made by adjusting the pH of the supernatant to 4.9 in order to separate the CAF from the CAF inhibitor. The CAF preparation was obtained from the precipitate of isoelectric precipitation using the method described in the previous paper.¹⁾ The CAF inhibitor preparation was obtained from the supernatant of isoelectric precipitation after heating at 80°C for 5 min, a partially modified method of Takahashi-Nakamura *et al.*³⁾

CAF activity was measured by caseinolytic activity, using the method described in the previous paper.¹⁾ For the determination of CAF inhibitor activity, test samples were pre-incubated with the reference CAF preparation at 25°C for 20 min, and the decrease in proteolytic activity was measured. CAF activity was calculated by the difference in absorbance according to the presence or absence of Ca²⁺. One unit of CAF activity is defined as the change in a 0.1 absorbance unit at 280 nm for 30 min. One unit of CAF inhibitor activity was shown to completely inhibit one unit of CAF.

DNA contents were measured by surface fluorometry according to Karsten *et al.*⁵⁾ CAF and CAF inhibitor activities are expressed in units per 30 min per mg DNA. Student's *t*-test was used to evaluate the significant difference between each pre-arranged fed and fasted rat.

Changes in CAF and CAF inhibitor activity in the skeletal muscle of fed and fasted rats are shown in Figs. 1(a) and (b). CAF activity increased remarkably in response to fasting, as reported in the previous paper¹⁾ (Fig. 1(a)). On the 2nd day of fasting, the CAF activity of fasted rats increased about 1.4 fold over that of the initial control rats, and increased further

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FIG 1. Effect of Fasting on CAF Activity (a) and CAF Inhibitor Activity (b) in Rat Longissimus Muscle of Back. Each symbol represents mean $\pm$ SD of 8 rats. Activity is expressed in units per 30 min per mg of DNA. * $p < 0.01$ vs. fed rats; $\square$, CAF activity; $\circ$, CAF inhibitor activity.

to about 2.2 fold on the 4th day of fasting. On the other hand, the CAF inhibitor activity showed almost same values in both fed and fasted rats (Fig. 1(b)). These results show that CAF inhibitor activity might not be affected by fasting, while CAF activity was increased in response to fasting as the result of an increase in either the amount of CAF or the specific activity of this CAF. It is therefore presumed that CAF activity in fasted rats might suggest a higher possibility of degradation in the myofibrillar proteins than that of fed rats.

In the skeletal muscle, it has been described that there are few enzymes which show specific changes in activity during protein deficiency or fasting.⁶⁾ However, in this experiment as well as in our previous paper, the results show that CAF activity increased significantly in response to fasting. This confirms the report of Seperich *et al.* in which there was a remarkable increase of CAF activity in the skeletal muscle of fasted rabbits.⁷⁾ These results suggest that CAF might take an important role in myofibrillar protein metabolism.

In this experiment, we have discussed the

effect of fasting on the "total" CAF and CAF inhibitor activities. It is presumed, however, that a large amount of the inactive form of CAF and a considerable amount of CAF inhibitor not bound to CAF might be contained in the living cells. It is not yet clear whether both CAF and CAF inhibitor were actually localized in the same compartment of a cell. Moreover, two types of CAF, *i.e.* the high calcium-required form (H-CAF) and low calcium-required form (L-CAF), which require about 10^{-3} M and $10^{-5} \sim 10^{-6}$ M of Ca^{2+} for activation respectively, have been found in several animal tissues.⁸⁻¹³⁾ Suzuki *et al.* have described that L-CAF was produced from H-CAF by the specific autolysis of H-CAF *in vitro*,¹⁴⁾ but the mechanism for transforming H-CAF into L-CAF in the living cells was not established. Considering these points, we will study further the role of CAF and CAF inhibitor concerning myofibrillar protein degradation in response to fasting.

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