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MAST CELL ORIGIN OF 'MYOFIBRILLAR PROTEASE' OF RAT SKELETAL AND HEART MUSCLE

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Autolysis of a washed heavy particle fraction from rat gastrocnemius muscle was largely prevented when the animals were injected with Compound $^{48}/80$ to degranulate mast cells. The 'myofibrillar protease' activity appears to be due therefore to the proteolytic enzyme from mast cells. Alkaline proteinase activity (casein substrate) in rat heart preparations was also largely depleted by Compound $^{48}/80$. Autolytic activity was similarly affected in the heavy particle fraction but much less so in the homogenate.

Introduction

Homogenates of rat skeletal muscle show proteolytic and autolytic activity in the alkaline pH range. A number of workers have attempted to purify and characterize the enzyme or enzymes involved. It was shown, however [1], that the major alkaline proteinase is similar in many respects to a chymotrypsin-like enzyme found in mast cells and that virtually all of the alkaline proteinase activity (casein substrate) in rat muscle homogenates disappeared if the animals were first treated with Compound $^{48}/80$ (a polymer of *N*-methylhomoanisylamine and formaldehyde which degranulates mast cells). The autolytic activity (pH 8.0) of the homogenates was also drastically reduced by this treatment. This was confirmed by others [2]. Woodbury et al. [3] have subsequently shown that the enzymes obtained from muscle and from peritoneal mast cells have identical physical, chemical and immunologic properties. In preliminary immunofluorescent localization studies they confirmed the mast cell origin of the enzyme in skeletal muscle. Noguchi and Kandatsu [4] and Sanada et al. [5] have purified a proteinase from rat muscle which, in each case, appears to be the mast cell proteinase. The same appears to be true of one of the two alkaline proteinases isolated by Reinauer and Dahlmann [6].

It seems unlikely, therefore, that this enzyme plays any part in muscle protein breakdown in vivo, except perhaps in pathological states involving inflammation and muscle necrosis, where the mast cell enzyme may be released and possibly have access to the muscle proteins. The susceptibility of various muscle proteins to attack by the enzyme [7] is therefore probably not relevant to muscle protein turnover. An increase in alkaline proteolytic or autolytic activity in rat muscle in a variety of muscle-wasting conditions has been reported by many authors. These include vitamin E deficiency [8], starvation [9,10], diabetes [11] and increasing age [12]. Alkaline proteinase activity is also increased in muscle of mice with hereditary muscular dystrophy [13,14]. Clearly, in all such conditions, the increased proteinase activity may be due, at least in part, to an increase in the numbers of mast cells relative to the total muscle protein and may not therefore, as was commonly supposed, be a cause of the loss of muscle protein. Indeed, there is an increased number of mast cells in the muscle of dystrophic mouse [15].

A series of papers by Mayer and his coworkers [16–19] have reported on changes in the activity of a

'myofibrillar protease' in various conditions of muscle wasting. This activity was measured by the rate of autolysis of the washed pellet obtained after centrifuging homogenates of rat gastrocnemius muscle at 5 000 × *g* for 10 min. The activity was increased in muscle wasting resulting from starvation, streptozotocin diabetes, tumours, nephrotic syndrome and treatment with glucocorticoids or triiodothyronine. It was also elevated in muscle from genetically dystrophic mice. The authors have postulated that the enzyme plays an important role in muscle protein catabolism and that its activity adapts to the various abnormal states.

Since the autolytic activity of whole homogenates of rat muscle appears to be due largely to the mast cell proteinase (see above) it seemed likely that the activity measured by Mayer and coworkers was due to the same enzyme: the mast cell proteinase would remain associated with the particle fraction used in their studies. The possibility remained, however, that the major endogenous substrates for the mast cell proteinase are in the cytoplasm, which was not included in their preparation. If such were the case it could be possible that the activities which they have measured were due to another proteinase, possibly normally associated with the myofibrils as they suggest. It was necessary to test this possibility, therefore, by measuring the activity of the 'myofibrillar protease' after removing the mast cell proteinase by treatment with Compound $^{48}/80$. Drabikowski et al. [2] showed that injection of Compound $^{48}/80$ produced a fall in autolysis of a myofibril fraction. However, the injection regime which they used caused the loss of only about one-half of the mast cell proteinase, therefore a more rigorous study was required.

Alkaline proteinase activity has also been studied in cardiac muscle. An enzyme which degrades myofibril protein was purified [20,21] and suggested to be responsible for the initial degradation of cardiac myosin during its turnover. The properties of this enzyme, however, closely resemble those of the mast cell proteinase in skeletal muscle. The proteinase activity of a washed myofibrillar fraction ('myofibrillar alkaline protease') from heart muscle was shown to change during induced conditions of cardiac atrophy and hypertrophy [22]. It is clearly necessary to establish whether the mast cell enzyme is manifest in cardiac muscle and could account for all of these findings.

To attempt to answer these questions we have measured the alkaline proteinase activity in homogenates and washed myofibrillar preparations of rat gastrocnemius and cardiac muscle after a course of injections of Compound $^{48}/80$ to degranulate the mast cells.

Materials and Methods

Male Wistar rats weighing 300–375 g were used. Compound $^{48}/80$ (Sigma) in aqueous solution was injected intraperitoneally daily for 5 days in linearly-increasing doses (100–500 μg/100 g). The rats were killed by cervical dislocation on the 6th day. The gastrocnemius muscles and heart were excised; fat and connective tissue removed as far as possible, and the muscles minced with scissors. The gastrocnemius was homogenized (in ice) in 10 mM potassium phosphate, pH 7.7/50 mM KCl (4 ml/g muscle) using an Ultra-Turrax homogenizer with the thyristor control set at one-eighth of the maximum for 45 s. The homogenate was filtered through gauze. A portion was diluted 6-fold with the buffer, centrifuged (100 000 × *g*, 30 min), and the pellet resuspended in the buffer. A further portion (6 ml) was centrifuged (5 000 × *g*, 15 min), the pellet washed twice with 5 ml buffer and resuspended in 5 ml buffer ('myofibrillar fraction'). Cardiac muscle was treated similarly except that 9 ml buffer/g were used for homogenizing and the myofibrillar pellet was suspended in only 2.5 ml buffer.

Alkaline proteinase assay

The medium (0.3 ml) contained 30 mM glycine-NaOH buffer, pH 10.0/75 mM NaCl/1 mg casein (Hammersten casein previously dissolved using the minimum amount of NaOH and heated at 100°C for 30 min) and 0.1 ml of the diluted muscle homogenate or resuspended 100 000 × *g* pellet. After incubation at 37°C for 2 h, 0.9 ml perchloric acid (6.7%, w/v) was added, the tubes centrifuged and the A_{224} of the supernatant measured. Blanks were run in which the homogenate was added at the end of the incubation, immediately before the perchloric acid.

Measurement of autolysis

This was carried out as described by Mayer et al. [16]; their procedure involves incubation at pH 9.1 in

the presence of 1.2 M KCl, and measurement of the Folin-reactive products not precipitated by trichloroacetic acid. The autolytic activity of both the homogenate (undiluted) and the 'myofibrillar fraction' was measured.

Results and Discussion

Rats treated with Compound $^{48}/80$ lost weight (15–33 g, mean 25 g) during the treatment. The control rats gained weight (11–20 g, mean 15 g) during the same period.

Table I shows the alkaline proteolytic and autolytic activities of the homogenates and particle fractions of gastrocnemius and heart from untreated rats and rats treated with Compound $^{48}/80$. (It should be noted that the proteolytic and autolytic activities cannot be compared directly, since the two activities were measured under different conditions.) It was found also that Compound $^{48}/80$ added to the assay mixtures had no significant effect upon the measured activities. The table shows disappearance from the gastrocnemius homogenate of most of the proteolytic activity and the greater part of the autolytic activity after treatment with the drug, as was observed previously [1]. In the present experiments, the breakdown products of autolysis were measured by the procedure Lowry et al., whereas previously [1] they were measured by absorbance at 224 nm.

Autolysis of the washed particulate fraction from the gastrocnemius (the 'myofibrillar protease') was reduced by Compound $^{48}/80$ to at least the same extent as autolysis in the homogenate. The control values were similar to those obtained by Mayer and coworkers [16–19]. It seems clear, therefore, that the enzyme activity measured by these workers resides largely or wholly in mast cells in the muscle.

Treatment with Compound $^{48}/80$ also drastically reduced the proteolytic activity in heart homogenate and particle fraction although not to quite the same extent as seen in the gastrocnemius. Thus, it appears that most of the alkaline proteinase in rat heart is present in mast cells. In heart muscle, as in gastrocnemius, most or all of the alkaline proteinase was associated with the particle fraction. The autolysis of the heart particle fraction was affected to about the same extent as the alkaline proteinase by Compound $^{48}/80$. However autolysis in the homogenate showed much less response to the drug. This suggests the presence of an additional enzyme in the 5 000 × *g* supernatant, which is not associated with mast cells and which hydrolyses the endogenous proteins more

TABLE I

EFFECT OF TREATMENT WITH COMPOUND $^{48}/80$ UPON PROTEOLYTIC AND AUTOLYTIC ACTIVITIES IN RAT GASTROCNEMIUS AND HEART

Activities are expressed per mg protein in the undiluted coarse-filtered homogenates. Each value is the mean from four or five rats, with the range in parentheses. *P* values for the difference between control and $^{48}/80$-treated rats were obtained using the non-parametric Mann-Whitney U-test.

	Proteolytic activity (A_{224}/2 h)			Autolytic activity (μg tyrosine equivalents/h)		
	Control	$^{48}/80$	*P*	Control	$^{48}/80$	*P*
Gastrocnemius						
Homogenate	1.62 (1.35–1.85)	0.06 (0.00–0.13)	0.004	22.3 (15.3–31.4)	5.2 (0.0–10.0)	0.008
Particle fraction	1.15 (1.04–1.25)	0.10 (0.01–0.22)	0.004	22.9 (17.1–32.9)	2.6 (0.2–9.9)	0.004
Heart						
Homogenate	2.35 (2.01–3.09)	0.49 (0.39–0.63)	0.014	21.3 (18.7–27.1)	13.3 (2.4–25.1)	0.1
Particle fraction	2.44 (1.99–3.17)	0.58 (0.49–1.12)	0.014	28.1 (19.2–40.7)	5.5 (0.8–7.2)	0.014

readily than casein. In gastrocnemius, also, autolysis of the homogenate was affected less by the drug than autolysis of the pellet, but the difference was much less than with heart.

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