CARNOSINE AND ANSERINE CONTENTS OF DIFFERENT PARTS OF NORMAL AND DENERVATED PLANTARIS MUSCLE OF RABBITS

V. I. Telepneva

Department of Animal Biochemistry (Director: Member AMN SSSR S. E. Severin),
M. V. Lomonosov Moscow State University
(Presented by Member AMN SSSR S. E. Severin)
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Different muscles of a single animal differ in their contents of the dipeptides carnosine and anserine, which are specific components of muscle. The contents of these dipeptides vary parallel with the working capacity of the muscles [9], and muscles which carry a large functional load have a higher carnosine content, irrespective of their color or of their percentage content of tonic fibers [8, 9, 12].

Frog sartorius muscle has been found to have a higher content of these dipeptides in its middle, "innervated" segment than in the "nerveless" distal and proximal segments [1, 4].

Only carnosine is present in frog muscles, whereas rabbit muscles contain both of the dipeptides. The object of the present research was to determine the carnosine and anserine contents of different segments of rabbit plantaris muscle, before and after denervation.

EXPERIMENTAL METHODS

Muscles taken from adult male rabbits were used in our experiments. The muscle was denervated by transection of the sciatic nerve at the level of the upper third of the femur. The contralateral nerve of the opposite limb was left intact, and the muscle served as the control. The rabbits were killed 12-14 days after the operation. The muscles were excised from the operated and intact limb immediately after decapitation of the animals, and were plunged into liquid nitrogen. After they had thawed slightly, the muscles were cut transversely into 5 segments of equal length. So as to have sufficient material for analysis, the corresponding segments of denervated and intact muscles of 2-4 rabbits were pooled. Each portion was frozen in liquid nitrogen, ground to a powder in a mortar, weighed, and extracted with 4 volumes of 5% trichloroacetic acid. The extracts were centrifuged, and the supernatants were taken for analysis. The sediments were not washed, and in calculating the content of dipeptides the water content of the portion of muscles was taken into account [5].

TABLE 1. Dipeptide Contents of the Lower and Upper Half of Rabbit Plantar Muscle (Milligrams Percent of Wet Weight)

Date of experiment (1961)	Carnosine		Anserine	
	proximal	distal	proximal	distal
19/XI	98	117	—	—
22/XI	121	161	—	—
23/XI	115	130	—	—
26/XI	134	172	303	360
21/XII	102	131	290	330
Mean . . .	114	142	296	345

The dipeptides were precipitated from the trichloroacetic acid supernatant by adding Hopkins' reagent and alcohol. The mercury salts of the dipeptides were decomposed by treatment with hydrogen sulfide, and the sulfide precipitate was separated by filtration, and washed repeatedly with hot water. The filtrate + washings was dried lyophilically, or on a water bath at 70-80°. The dry residue was dissolved in 10% isopropyl alcohol, so that 1 ml of solution corresponded to 1 g of muscle tissue taken.

Carnosine was determined by the diazo-reaction, both in the trichloroacetic acid filtrate and in the final solution. Anserine was determined after separation of the dipeptides by ascending paper chromatography, using Leningrad Slow paper previously washed with 0.15%

TABLE 2. Carnosine and Anserine Contents of Different Segments of Plantar Muscle (Milligrams Percent of Wet Weight)

Date of experiment (1961)	Carnosine					Anserine				
	Proximal segment, 1st	middle part			Distal segment, 5th	Proximal segment, 1st	middle part			Distal segment, 5th
		2nd	3rd	4th			2nd	3rd	4th	
19/VI	76	85	94	105	114	275	240	302	335	400
29/VI	92	100	112	119	123	255	316	310	263	329
1/VII	107	134	142	161	159	280	316	266	342	330
26/VII	97	100	109	123	129	293	280	298	341	384

TABLE 3. Carnosine and Anserine Contents of Different Segments of Normal and Denervated Muscles of Operated Experimental Animals (Milligrams Percent of Wet Weight)

Conditions of experiment	Date of experiment (1961)	Days after operation	Carnosine					Anserine				
			Proximal segment, 1st	middle part			Distal segment, 5th	Proximal segment, 1st	middle part			Distal segment, 5th
				2nd	3rd	4th			2nd	3rd	4th	
Normal muscle	18/IV	12	63	75	77	72	101	—	—	—	—	—
	16/V	13	80	87	98	113	133	405	378	584	540	538
	10/V	14	47	50	58	60	66	66	—	—	—	—
	6/VI	14	57	50	73	85	102	266	229	288	341	380
	3/VII	13	77	58	76	93	118	320	318	271	268	374
Denervated muscle	18/IV	12	14	16	20	23	30	—	—	—	—	—
	26/IV	13	21	19	19	21	19	419	349	415	462	460
	10/V	14	10	16	16	10	12	—	—	—	—	—
	6/VI	14	30	27	26	21	29	367	359	430	457	415
	3/VII	13	33	30	26	32	30	357	333	399	417	498

8-hydroxyquinoline, and using acid phenol as the solvent. An amount of solution corresponding to 30 mg of tissue was applied to the paper, as well as reference solutions of carnosine and anserine. The chromatograms were sprayed with 0.5% ninhydrin in acetone. The chromatograms were then air-dried, and heated for 7-10 min in an oven at 120°. The anserine content was determined colorimetrically after elution of the spots from the paper [11].

EXPERIMENTAL RESULTS

Carnosine and anserine contents of different parts of normal muscles. We first bisected the plantar muscle, and determined the dipeptide contents of the upper and lower halves, being respectively the proximal half and the half terminating in the Achilles tendon. The results of these experiments are presented in Table 1.

Although the carnosine content of the plantar muscle of different individuals varied fairly widely, it was always greater in the lower than in the upper half of the muscle. Similar differences were also found between the anserine contents of the two halves of the plantar muscle.

The values found by us for the dipeptide contents of the plantar muscle are in agreement with published ones for various leg muscles of rabbits [3, 6, 12]. In our subsequent experiments we divided the muscle into 5 equal parts, numbered sequentially from the proximal to the distal end. The results of these experiments are presented in Table 2.

The results show that carnosine is not uniformly distributed along the muscle. Its content rises progressively from the first, proximal, segment of muscle to the fifth, distal one. To some extent, the same applies to the anserine content: the anserine content of the first segment was always lower than that of the fifth one. There was not, however, the same regular increase in the anserine content of the middle segments as was found for carnosine.

Carnosine and anserine contents of different parts of denervated muscles. The results obtained for the dipeptide contents of plantar muscles of the unoperated limbs of rabbits, serving as controls for the contralateral denervates ones,

gave results concordant with those of Table 2 (see Table 3). The carnosine contents again rose from the proximal to the distal end of the muscle, but the absolute values for each of the five segments were somewhat lower than those found for unoperated animals. The carnosine content of the first segment of the plantar muscle of unoperated rabbits ranged from 76 to 107 mg %, whereas in the muscle contralateral to the denervated one it was 47-80 mg %. The corresponding values for the fifth segment were, respectively, 114-159 and 68-133 mg %.

The carnosine content of the muscle fell to from a third to a quarter of its initial value 12-14 days after denervation. The gradient of carnosine content characteristic of normal muscle was almost totally abolished.

A distinct difference was found in the anserine contents of the first and fifth segments of the control muscle. The absolute amounts of anserine in the various segments of control muscles were close to those found for normal animals. There was practically no change in the anserine contents of the various segments of denervated muscle. While the anserine contents of the 3rd, 4th, and 5th segments of denervated muscle were below those of the controls in the experiment of April 26, the opposite effect was observed in two other experiments. Similar results were reported by N. A. Yudaev for whole rabbit plantar muscle. This author found that the carnosine content had fallen practically to zero by the 20th day after denervation, and that there was a transient rise in anserine content [7, 10].

Our analyses thus show that carnosine is not distributed uniformly along the length of the plantar muscle of rabbits. This was also found to apply to the distribution of anserine. The carnosine content of the muscle fell to from a third to a quarter of the normal value 12-14 days after denervation, and the remaining carnosine was now uniformly distributed along the length of the muscle. The anserine contents of different parts of the muscle changed very little following denervation.

SUMMARY

The carnosine content of normal rabbit plantar muscle rises from 76-107 mg % in its proximal one-fifth to 114-159 mg % in the distal one-fifth of its length. There was a similar, although less well marked gradient in anserine content, which was higher at the distal than the proximal end of the muscle, but which varied irregularly in the middle sections. The carnosine content of the muscle fell to from a third to a quarter of the normal value 12-14 days after denervation, and it was then distributed uniformly along the muscle. Denervation had very little effect on the distribution of anserine in the muscle.

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