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A Role for Carnosine and Anserine in Histamine Metabolism of the Traumatized Rat[1] (40213)

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Although a variety of biological activities for carnosine (β-alanylhistidine) has been proposed and comprehensively reviewed (1), a definitive metabolic role has not been established. Recently Nagai (2) has reported a wound-repair effect of carnosine in rabbits. In addition to its other functions (3), histamine, a histidine derivative, has been suggested to play an important role in wound-healing. During the course of studies relating muscle and brain carnosine concentrations to level of dietary histidine, we observed in cocks that had developed a *Staphylococcus aureus* infection of the footpad an 80% reduction in pectoral muscle carnosine concentration in comparison with healthy animals suffering no infection.

The purpose of this investigation was to examine muscle carnosine and anserine concentrations in rats and cocks traumatized by fracture of the femur or incisions into the pectoralis major muscle. Rats and cocks were used as experimental animals since considerable information has recently been reported from our laboratory concerning the histidine-containing derivatives in these species (4, 5).

Materials and methods. Twenty-six-week-old, lean, white Leghorn cocks fed a standard poultry ration and weighing between 1670 and 2365 g were anesthetized locally with 2% procaine hydrochloride. Wounds were inflicted into the left side of the pectoralis major muscle by making incisions 80 mm long and 4 mm deep, followed by ten 20-mm crosscuts. The wounds were closed with surgical silk sutures. Four birds were killed by decapitation after 24 hr and another four were killed in the same way after 48 hr. Approximately 1 g of pectoralis major tissue was removed from the wounded area (the left side) and an equal amount from the uninjured area (right side). Four uninjured (control) birds were also killed and tissue removed. Carnosine and anserine were determined on the protein-free extract of muscle homogenate by amino acid analysis according to the procedures previously described (4, 5).

Male, Sprague–Dawley rats were maintained on laboratory rat chow and tap water, *ad libitum.* Animals weighing 125–135 g were divided into six groups of three each. Three groups were anesthetized with ether and their right hind legs (middle of femur) fractured as described by Sitren *et al.* (6). Following its fracture, each rat was immediately (within 10 sec) injected intraperitoneally with 1 ml saline or saline solutions of histidine (0.2 μmoles/ml) or histamine dihydrochloride (0.2 μmoles/ml). The uninjured rats were also etherized prior to ip injections with the same solutions. Twenty-four hours after the injections, tissue (approximately 1 g) was removed from all animals from the gluteus maximus, semitendinosus and the biceps femoris muscles. These samples were weighed, homogenized in 15% sulfosalicylic acid and carnosine, anserine and free-histidine determined according to the procedures described earlier (4, 5).

Results. Twenty-four hours after the incisions had been made in the pectoral muscles of the cocks the concentration of anserine in their tissue was 34% lower than in tissue taken from the same muscle of the uninjured birds (Table I). There was a 26% lower anserine concentration in tissue from the wounded left side as compared to the uninjured right side from the same animal. Similarly, the carno-

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sine concentration in injured tissue was 17% lower in comparison with tissue from uninjured birds and 27% lower in comparison with uninjured tissue from the same animal. After 48 hr there were no significant differences between the anserine and carnosine content of injured versus uninjured muscle tissue.

The data in Table II show muscle concentrations of anserine, carnosine and free-histidine for the three groups of rats with fractures of the femur and their uninjured controls (saline-, histidine- and histamine-injected). After 24 hr, in all but two comparisons the tissues from the injured rats had about 25% lower concentrations of anserine, carnosine and histidine than did tissues from the control (uninjured) animals. The exceptions were the anserine concentration in the histamine-injected group (8% lower) and the free-histidine concentration in the histidine-injected group (12% lower). The injection of either histidine or histamine essentially prevented the lowering in each of the three muscle constituents and, in some cases even increased some of the components slightly above the base line observed in the uninjured animals. The carnosine increase was approximately 50% in the uninjured rats injected with histidine and 29% in histamine-injected, control animals.

TABLE I. ANSERINE AND CARNOSINE CONCENTRATION IN PECTORALIS MAJOR MUSCLE OF COCKS WOUNDED BY INCISION.

Treatment	Anserine	Carnosine
	(nmol/mg wet tissue)	
None (Uninjured controls)	34.7 ± 2.5[a]	14.6 ± 1.4
Wounded		
After 24 hr		
Incision Area (left side)	22.9 ± 3.2[b]	12.1 ± 1.3[c]
Uninjured Area (right side)	30.9 ± 5.0	16.6 ± 1.1
After 48 hr		
Incision Area (left side)	28.6 ± 3.7	13.8 ± 1.7
Uninjured Area (right side)	36.8 ± 3.2	13.6 ± 0.4

[a] Means ± SE for four cocks per treatment group.
[b] Significantly lower than value from uninjured animals ($P < 0.02$).
[c] Significantly lower than value from the uninjured area of the same animals ($P < 0.05$).

Discussion. The results from this study indicate that both carnosine and anserine decreased significantly in wounded pectoralis major muscle of cocks. In muscle tissue of rats injured by fracture of the femur, carnosine and anserine were both 25% lower than in tissue of uninjured animals, but injections of either histidine or histamine prevented the decrease. These observations clearly indicate a metabolic involvement of carnosine in trauma response.

The finding that endogenous carnosine levels change in response to trauma strengthens the recent proposal (2) that exogenous carnosine may enhance wound healing. Furthermore, the reversal of the carnosine and anserine drops induced by ip injection of histamine suggests a metabolic relationship among these imidazole compounds.

An involvement of histamine in wound healing has long been known (3) although its mode of action remains uncertain. Kahlson and Rosengren (3) reported that endogenous histamine mobilization accelerated wound healing as measured by tensile strength of

TABLE II. EFFECT OF FEMUR FRACTURE AND INTRAPERITONEAL INJECTIONS OF HISTIDINE AND HISTAMINE ON LEG MUSCLE ANSERINE, CARNOSINE AND FREE HISTIDINE CONCENTRATIONS.

Treatment				
Fracture*	Injections**	Carnosine	Anserine	Histidine
			*(nmol/mg wet tissue***)*	
−	Saline	2.04 ± 0.27[a]	1.15 ± 0.37[abc]	0.20 ± 0.04[abc]
+	Saline	1.53 ± 0.07[b]	0.87 ± 0.02[c]	0.15 ± 0.02[b]
+	0.2 μmoles histidine	2.27 ± 0.15[a]	1.10 ± 0.03[a]	0.23 ± 0.03[ac]
−	0.2 μmoles histidine	3.00 ± 0.16[c]	1.47 ± 0.09[b]	0.26 ± 0.02[c]
+	0.2 μmoles histamine	1.99 ± 0.02[a]	1.14 ± 0.05[a]	0.19 ± 0.01[ab]
−	0.2 μmoles histamine	2.57 ± 0.16[d]	1.24 ± 0.08[a]	0.27 ± 0.01[c]

* Hind leg fracture.
** Injection volume was 1 ml; histidine and histamine solutions were prepared with saline; all injections were ip.
*** Means ± SE for three rats per treatment group; values within each column that share the same letter superscript are not significantly different at $P \leq 0.05$.

skin in rats. They found, however, no effect on wound healing following ip histamine injection and concluded that exogenous histamine does not participate in the healing process. Our finding that the carnosine decrease could be reversed by ip histamine injection suggests that carnosine may provide a reservoir of histidine for additional histamine synthesis. Therefore animals given exogenous histamine would show no changes in wound healing relative to controls which could draw upon carnosine for any histamine otherwise provided by ip injection. Furthermore, it would be expected that animals given exogenous histamine may not require the histamine they would otherwise have derived from carnosine. The carnosine levels in such animals would then remain unchanged relative to uninjured animals. Apparently the mobilization of carnosine is very rapid since 48 hr following injury its concentration had almost returned to pre-injury levels (Table I) in cocks.

The possibility that carnosine is utilized for histamine synthesis via histidine may explain the effects of histamine or histidine administration (Table II). In this case altered concentrations of the components of the histidine-histamine and histidine–carnosine reactions shift the equilibria in the observed directions. Carnosine may also be converted to histamine by a different route such as direct decarboxylation followed by hydrolysis.

Although our data are consistent with a carnosine to histamine conversion, the possibility remains that carnosine may not be catabolized to histamine. Perhaps it, itself, is active in the trauma response. Furthermore, it is also possible that carnosine is modified to yield a derivative (not histamine) which is the active compound. For example, a decarboxylated carnosine is structurally similar to histamine (with a longer side chain) because of the position of the amine group of beta-alanine. This possibility is interesting in light of carnosine's weak "histamine-like" effect on smooth muscle (1).

Our observation that anserine as well as carnosine is significantly lower following fracture is noteworthy since in earlier studies from this laboratory (5, 7) low-histidine diets induced marked decreases in carnosine, but had no diminishing effect on anserine. It cannot be ascertained here whether the trauma-induced anserine decrease reflects increased catabolism or decreased synthesis. This question is particularly relevant since it has been reported that neither anserine nor 1-methyl histidine are demethylated to yield free histidine (8).

Finally, it can be seen in Table II that the anserine concentration is lower than carnosine in leg muscle of the rats used in this experiment. Pectoral muscle of adult rats contains much higher concentrations of anserine than of carnosine (5, 7). At this point it is not clear whether these differences represent muscle specificities or reflect the younger age of the rats used in this study. However, both the carnosine and free histidine concentrations observed in rat leg muscle (Table II) were of the same order of magnitude as those observed by us in rat pectoral muscle (5).

Summary. Adult cocks were wounded by incisions on the left side of the pectoral muscles and tissues taken 24 and 48 hr later for determination of anserine and carnosine. Both the anserine and carnosine concentrations were lower in the wounded tissue 24 hr after injury in comparison with uninjured controls and tissue from the uninjured area of the same animals. By 48 hr levels of anserine and carnosine were essentially back to normal. Young rats were injured by fracture of the femur and simultaneously injected ip with either saline, histamine or histidine (0.2 μmoles). The leg muscle concentrations of carnosine, anserine and free-histidine were approximately 25% lower in the injured, as compared to the uninjured animals. Histidine or histamine injection prevented the decrease, and in the case of uninjured rats induced an increase, particularly of carnosine.

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