Physiological Implications of Carnosine on the Inflammation
—With Reference to Its Inhibitory Action of Allergy
and the Vital Defense Mechanism—

By

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1. Introduction

Such peptides as carnosine and anserine are abundantly attested to in the muscles and, quite naturally, their physiological implications have been studied in their relation to the muscular system [1, 2].

Homocarnosine is another peptide discovered in the bovine brains by Pisanois[3] and there is available a fair amount of published literature on its relation to the central nervous system. Among recent research efforts, there are known an anti-convulsive action of homocarnosine by Mori[4], its efficacy in the treatment of epilepsy by Kosaka et al.[5], its role as a chemical mediator in the limbic system by Hayashi[6], prophylactic effects of carnosine and homocarnosine on experimental Straphylococcal infections by Tanaka[7], Mukkada[8] and Tsuchiya et al.[9].

Despite a spate of research findings, however, from physiological, biochemical and pharmacological points of view as above, there seems to be lacking consensus of scholarly opinions as to the exact physiological implications of these substances. The author and his associates [10, 11, 12, 13, 14, 15] have studied actions of various \( \alpha \)-amino acids on the inflammation both in the oral and systemic realms. As a result, \( \alpha \)-amino 3-hydroxy butyric acid and 4-hydroxy 3-amino butyric acid are found to be possessed of a potent anti-inflammatory action.

The present paper attempts to review some of the salient conclusions from our previous studies for the purpose of placing carnosine in its exact physiological role.

2. Effect of Carnosine on the Inflammation and Allergy

1. Anti-edematous action of carnosine and homocarnosine [16, 17].

Edemata were artificially caused in rats by striking them on the hind legs by a hammer which was dropped from a prescribed height (Photo 1).

Chronological changes in the anti-traumatic edemata are given in Figs. 1 and 2. 0.1 \% carrageenine injected into the rat toes was not very helpful as an anti-inflammatory agent. Its effect was judged to be within a range of 10mg/Kg to 20mg/Kg (Table 1).

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2. Effect of carnosine and homocarnosine on the adrenalectomized animals [16, 17].

As shown by Table 2, it is decidedly clear that both carnosine and homocarnosine work beneficially on the edemata induced in animals which had been adrenalectomized.

Two questions may be asked. The first question: do carnosine and homocarnosine promote the adrenal cortex function in secreting corticoid and thus bring about an anti-edematous action? In other words, is this anti-edematous action lost when the adrenal bodies are removed? The second question: will an anti-edematous action of carnosine or homocarnosine become activated in the presence of an adrenal hormone such as cortisone? Then, it will be naturally assumed that the anti-edematous action will disappear in the absence of cortisone.

Table 1. Anti-edematous actions of carnosine and homocarnosine on carrageenine induced edema

<table>
<thead>
<tr>
<th>Dose</th>
<th>Carnosine</th>
<th>Homocarnosine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SIP</td>
<td>SIP</td>
</tr>
<tr>
<td>5 mg/kg</td>
<td>2.3</td>
<td>4.2</td>
</tr>
<tr>
<td>10</td>
<td>25.5*</td>
<td>26.4*</td>
</tr>
<tr>
<td>20</td>
<td>9.5</td>
<td>10.3</td>
</tr>
<tr>
<td>30</td>
<td>23.8*</td>
<td>18.3</td>
</tr>
<tr>
<td>40</td>
<td>10.5</td>
<td>20.5*</td>
</tr>
<tr>
<td>50</td>
<td>30.3*</td>
<td>29.4*</td>
</tr>
<tr>
<td>60</td>
<td>28.4*</td>
<td>30.8*</td>
</tr>
</tbody>
</table>

* Significant effect (α=0.05 n=10)

A: Weight of the soles of control animals
B: Weight of the soles of experimental animals
\[
\frac{A-B}{A} \times 100 = \text{SIP}
\]

SIP: Swelling index in percent

Table 2. Loss of anti-edematous action in adrenalectomized animals by carrageenine and its re-activation by cortisone

<table>
<thead>
<tr>
<th>Dose</th>
<th>SIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carnosine</td>
<td>30 mg/kg ip.</td>
</tr>
<tr>
<td>Homocarnosine</td>
<td>30 mg/kg ip.</td>
</tr>
<tr>
<td>Cortisone</td>
<td>16.7 mg/kg im.</td>
</tr>
<tr>
<td>Carnosine+</td>
<td>30 mg/kg ip.</td>
</tr>
<tr>
<td>Cortisone</td>
<td>16.7 mg/kg im.</td>
</tr>
<tr>
<td>Homocarnosine+</td>
<td>30 mg/kg ip.</td>
</tr>
<tr>
<td>Cortisone</td>
<td>16.7 mg/kg im.</td>
</tr>
</tbody>
</table>

* Significant effect (n=10, α=0.05)

With the adrenalectomized animals, carnosine, homocarnosine or cortisone will have no anti-edematous action when used singly but, when one combines with another, a potent anti-edematous effect will be produced. From this phenomenon, it can be safely inferred that the anti-edematous effect which lies latent in carnosine or homo-
carnosine will come to the surface in the presence of cortisone. This action on the part of carnosine or homocarnosine belongs to the category two. ACTH can be substituted for cortisone for the activation of either carnosine or homocarnosine. As shown by Table 3, when used in combination with ACTH, carnosine or homocarnosine will possess a greater anti-edematous action than it is used singly.

3. Effect of carnosine and homocarnosine on the granulation process [16, 17].

Unlike other so-called anti-edematous substances which interfere with the formation of granules, carnosine and homocarnosine promote the granulation and are capable of encapsulating a foreign matter such as a formalin filter-paper placed inside the skin.

Here again, this effect of encapsulation will become much pronounced when used in combination with cortisone.


Carnosine is capable of inhibiting an inflammation owing to the Arthus phenomenon in rabbits that had been sensitized by the antigen horse blood-serum and rabbit blood-serum (Table 5, Photos 2, 3).

Here a total amount of carnosine administration is 300–600mg/Kg by way of an intravenous injection. The inhibitory effect of carnosine is seen from the edemata to the blood necrosis. As shown by Table 6, it either inhibits a shock due to the serum anaphylaxy or prolongs the length of survival. A local anaphylaxy induced in the intestinal tracts of guinea pigs by the sensitization through horse blood-serum can be inhibited by $10^{-4}$/mol (Fig. 3). An anti-allergic effect of homocarnosine is appreciably weaker than that of carnosine.
Photo 1  Traumatic method
(Hammer weight 230g dropped from 15cm)

Fig. 1  Anti-edematous behavior of carnosine on traumatically induced edemata
(10 male rats weighing 150g to 170g)
Table 4. Synergistic effect of carnosine (or homocarnosine) and cortisone on the granulation process (7-day embedding of formalin filter-paper)

<table>
<thead>
<tr>
<th>Dose</th>
<th>No. of granules</th>
<th>Mean weight in mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>163.1</td>
</tr>
<tr>
<td>Carnosine 1.67 mg/kg im × 6 day</td>
<td>20</td>
<td>205.1*</td>
</tr>
<tr>
<td>Carnosine + 25 mg/kg ip × 6 day</td>
<td>20</td>
<td>221.0*</td>
</tr>
<tr>
<td>Cortisone 1.67 mg/kg im × 6 day</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
<td>193.1</td>
</tr>
<tr>
<td>Homocarnosine 25 mg/kg ip × 6 day</td>
<td>20</td>
<td>235.0*</td>
</tr>
<tr>
<td>Homocarnosine + 25 mg/kg ip × 6 day</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Cortisone 1.67 mg/kg im × 6 day</td>
<td>20</td>
<td>241.8*</td>
</tr>
<tr>
<td>Cortisone 1.67 mg/kg im × 6 day</td>
<td>20</td>
<td>96.0</td>
</tr>
</tbody>
</table>

* Significant effect (n=20 α=0.05)

Fig. 2 Anti-edematous behavior of homocarnosine on traumatically induced edemata (10 male rats. The left legs were experimentally traumatized and the right ones as controls)
Table 5. The inhibitory effect of carnosine on the Arthus phenomenon (Animals were sensitized by the rabbit serum having horse serum antibody. Doses indicated were given 6 times every 2 hrs. and 4 times every 4 hrs. Skins were removed in 48 hrs.)

<table>
<thead>
<tr>
<th>Exp. No</th>
<th>after 6 hrs</th>
<th>after 24 hrs</th>
<th>after 48 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>+ +</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>+ + +</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Carnosine 30 mg/kg iv</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Carnosine 60 mg/kg iv</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

(-) No observable change  (+) Edema  (++) Edema and scarlet spots  (+++) Hemorrhage  (++++) Necrosis

Table 6. Effect of carnosine on blood serum shock induced by 0.5 mol horse blood-serum (10 male guinea pigs, 250g to 350g).

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Dose</th>
<th>Time passage till death</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Control</td>
<td>3 min. 47 sec.</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>Carnosine 100 mg/kg iv</td>
<td>14 min. 50 sec.</td>
<td>60</td>
</tr>
<tr>
<td>10</td>
<td>Carnosine 200 mg/kg iv</td>
<td>8 min. 54 sec.</td>
<td>80</td>
</tr>
</tbody>
</table>

Fig. 3 An inhibitory effect of carnosine on local anaphylactic conditions (sensitized guinea pig tract)
Photo 2  Carnosine group (300mg/Kg in total, given in 10 installments intravenously).
This shows autopsy findings after 48 hours. Note the mere presence of scarlet spots and no necrotic degeneration is evinced.

Photo 3  Control group.
Note the presence of marked necrotic changes.
3. Physiological Considerations concerning the Vital Defense Mechanism

It is widely known that, though the anti-edematous effect of carnosine or homo-
carnosine is very unstable by itself, the effect will become quite pronounced when it is
administered conjointly with cortisone or ACTH.

When the minimum effective dosage of either chemical is assumed to be 10mg/Kg
and is not enzymically decomposed, about 1/40 of carnosine usually found in the
musculature is enough to have the anti-edematous effect[19]. When other factors are
taken into account, however, such as the lag time from the time of administration and
enzymic actions, the amount of effective carnosine will be regarded as being much
smaller than this 1/40. It is in this respect that a physiological significance is to be
sought for carnosine and its analogous peptides.

Carnosine in 300–600mg/Kg is known to inhibit the Arthus phenomenon to a
marked degree. But since a dosage of 30–60mg/Kg is given every 2 hours, the effective
concentration of carnosine may be assumed to be quite low. An effective concentra-
tion to a local anaphylaxis is $10^{-4}$ mol, which is considered to be close to that concen-
tration of carnosine which inhibits a local inflammation (Fig. 3). Carnosine is much
stronger than homocarnosine in these effects. As a chemical mediator, either carnosine
or homocarnosine works antagonistically against 50Å bradykinine but not against
histamine, Ach or serotonin. Carnosine inhibits the promotion of the blood vessel
infiltration due to bradykinine (Tab. 7, Figs. 4, 5, Photos 4, 5). At the present state of
knowledge, bradykinine is not considered to play a role of principal chemical mediator
for all the inflammatory and anaphylactic phenomena. According to SUGAYA[20],
carnosine causes the hyperpolarization in the excitatory film and thus works antago-
nistically against carnitine which induces the hypopolarization.

Along a similar line of thinking, the author is inclined to the view that carnosine
and analogous peptides are participatory in the maintenance control in stabilizing the
body cells. This maintenance control function forms an important part of the anti-
inflammatory or anti-anaphylactic action of carnosine. As has been pointed out, the
anti-edematous effect of carnosine or homocarnosine becomes evident in the present
of cortisone. In other words, cortisone is furnished with the anti-edematous action by
carnosine or homocarnosine. This synergetic effect of cortisone and carnosine or
homocarnosine promotes the absorption of a foreign matter and activates the granula-
tion process to encapsulate a formalin-filter paper inserted under the skin. This effect is
related to such defensive reactions as demarcatio, remotio, organizatio and absorptio.
This effect of carnosine or homocarnosine is unlike that of steroid substances, which
absorb edemata but rather interfere with the granulation.

The author explicates the synenergetic action of carnosine with cortisone as
follows.

Today there is a general agreement, beyond any doubt, that cortisone works as
a vital defender in the hypophysial suprarenal cortex system. On the other hand, cor-
tisone has a two-sided action: in its administration to infected animals it rather en-
hances the mortality rate and, occasionally, it works beneficially. This problem of two-
sided effect cannot be solved by the fact whether cortisone is administered in excess or
not. To the author's way of thinking, cortisone requires some co-factor(s) before it can
work as a vital defender. This co-factor is called the protective response inducing substance (PRIS). Therefore, cortisone will give rise to its life-defensive properties in the presence of some PRIS but, in the absence of it, it will work adversely against a vital body to which it is administered (Fig. 5). In his previous studies dealing with 4-hydroxy 3-amino butyric acid and 4-amino 3-hydroxy butyric acid, the author proposed the afore-mentioned hypothesis and considered these substances as “typical models” of co-factors in favor of cortisone[14]. When cortisone fails to promote the granulation process, it will be attributed to the absence of this kind of co-factor. To recapitulate, physiological contributions of carnosine and homocarnosine are found in their role as forming co-factors with cortisone.

Table 7. Inhibitory action of carnosine and homocarnosine against bradykinine, histamine, serotonin and Ach. (Accensor method)

<table>
<thead>
<tr>
<th>Carnosine</th>
<th>Homocarnosine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Concentration of chemical mediator</strong></td>
<td><strong>Concentration of chemical mediator</strong></td>
</tr>
<tr>
<td>Bradykinine</td>
<td>Histamine</td>
</tr>
<tr>
<td>50γ</td>
<td>10⁻⁴mol</td>
</tr>
<tr>
<td>Histamine</td>
<td>Serotonin</td>
</tr>
<tr>
<td>10⁻⁴mol</td>
<td>10⁻³mol</td>
</tr>
<tr>
<td>Serotonin</td>
<td>Ach</td>
</tr>
<tr>
<td>10⁻⁴mol</td>
<td>10⁻³mol</td>
</tr>
</tbody>
</table>

Fig. 4 Anti-bradykinine effect of carnosine (guinea pig intestinal ducts)
The research findings by Tsuchiya and his associates[7, 8, 9] have important bearings on the investigation of the author here. They established the prophylactic effect of carnosine and homocarnosine on experimentally induced Staphylococcal infections in the brain and concluded that these substances might be involved in some aspect of host defense mechanism. The author likes to find the defensive reaction of cortisone and carnosine (or homocarnosine) in their manifestation as an inhibitor of the inflammation and anaphylactic conditions. The effect of these substances in the reticulo-endothelial system will also need to be studied.

Fig. 5 Anti-bradykinine effect of homocarnosine (guinea pig intestinal ducts)

The part in which antiprotective effect of cortisone is still more enhanced to have an unfavorable by-effect. Infectious diseases.

The part in which cortisone acts supporting physiological protective response.

The part in which cortisone is expected to exert anti-granulomatous effect (anti-protective effect) as a symptomatic treatment. A part of rheumatic therapy.

Fig. 6 Nagai’s hypothesis for the life-defense action of cortisone
Photo 4  Blood vessel permeability test
Control animal (Male mouse 23g.)
0.5γ/0.1ml bradykinine injected under the skin
and skin removed 30 minutes afterward.

Photo 5  Blood vessel permeability test
Experimental animal (Male mouse 23g)
10^{-4} mol/1ml iv carnosine was injected
5 minutes prior to 0.5γ/0.1mol bradykinine
injected under the skin and skin removed
30 minutes afterward.
Conclusion

In the presence of cortisone, carnosine and homocarnosine in 10–50mg/Kg possess the inhibitory effect on carrageenine- and traumatic edemata.

Similarly, beneficial effect is observed in the promotion of granulation process. These substances also inhibit the Arthus phenomenon, blood-serum anaphylactic shocks and local anaphylactic conditions.

They do not antagonize histamine, serotonin and Ach in the flat muscles but are in antagonism with bradykinine. The phenomenon of absorbing edemata and promoting the granulation is considered to be in keeping with the defense mechanism of a vital body. It is concluded that carnosine and homocarnosine form important cofactors in helping cortisone to do justice to its life-defensive properties.

Acknowledgement

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References


