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Histidine-containing dipeptides as endogenous regulators of the activity of sarcoplasmic reticulum Ca-release channels

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Abstract

It is shown that histidine-containing dipeptide carnosine (β-alanyl-L-histidine), which is present in skeletal muscles in millimolar concentrations, decreases the rate of Ca^{2+} accumulation by the heavy fraction of sarcoplasmic reticulum from rabbit skeletal muscles. This effect results from the ability of carnosine to induce a rapid Ca^{2+} release from the heavy sarcoplasmic reticulum vesicles via activation of the ruthenium red-sensitive Ca-channels. The effect of carnosine is dose-dependent that indicates the presence of saturable site(s) for carnosine in the molecules of Ca-channels. The $C_{0.5}$ value for carnosine (the concentration that induces the half-maximal Ca^{2+} release) is 8.7 mM. The 1*N*-methylated derivative of carnosine, i.e., anserine, also induces a rapid Ca^{2+} release with the half-maximal effect at 2.7 mM. Conversely, neither histidine nor β-alanine (both separately and in the mixture) cause Ca^{2+} release. In addition, carnosine increases the sensitivity of Ca-channels to their well-known activators (caffeine, AMP, and Ca^{2+}) and decreases inhibitory effect of low concentrations of Mg^{2+}. It is concluded that carnosine as a component of skeletal muscles can be an endogenous regulator of the sarcoplasmic reticulum Ca-channel activity.

Keywords: Sarcoplasmic reticulum; Calcium ion release channel; Carnosine

1. Introduction

Sarcoplasmic reticulum (SR) plays a key role in the regulation of skeletal muscle functional activity. It consists of closed membrane cisterns and tubules that surround myofibrils. The main protein components that provide Ca^{2+} exchange in the course of muscle contraction and relaxation are Ca^{2+}-ATPase of longitudinal tubules and Ca-channels of the junctional face of terminal cisterns. Ca-channels provide a rapid Ca^{2+} release into the cytoplasm resulting in muscle contraction. Ca^{2+}-ATPase performs the ATP-dependent accumulation of Ca^{2+} into SR vesicles during muscle relaxation [1,2]. During the last decade, much progress was made in studying the molecular organization, mechanism of action, and regulation of sarcoplasmic reticulum Ca- channels (for a review, see Refs. [3,4]). Briefly, the Ca-channel protein was isolated and purified [5], several isoforms of Ca-channels were cloned and identified in different tissues [6], the procedure for single channel recording (patch-clump technique) was developed [7,8], and so on. Currently, a great variety of compounds that activate or inhibit sarcoplasmic reticulum Ca-channels was discovered. Among them, the endogenous activators (Ca^{2+} and adenine nucleotides) and inhibitors (Mg^{2+}) are of the particular interest.

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The interaction of Ca-channels with these endogenous compounds and with some endogenous proteins (calmodulin, calsequestrin, triadin, annexin VI, FK506 binding protein, and some others) along with their phosphorylation by a number of protein kinases probably determines the functional state of Ca-channels in vivo [3,4,10–12].

The mechanism of action of different physiologically active compounds was clarified after establishing the role of sarcoplasmic reticulum Ca-channels in the regulation of Ca^{2+} exchange during muscle contraction. For instance, previously it was shown that caffeine increased the sensitivity of skinned muscle fibers to Ca^{2+}, which initiated muscle contraction [13], and itself induced muscle contraction after its addition to muscle preparations [14]. Additionally, caffeine transiently enhances the tension of fatigued muscles [15]. It appeared that all these effects of caffeine were associated with its interaction with sarcoplasmic reticulum Ca^{2+}-release channels.

A very similar physiological effect was shown for histidine-containing dipeptide carnosine (β-alanyl-L-histidine) that, unlike caffeine, is an endogenous component of skeletal muscles. Its content in skeletal muscle tissue is up to 10 mM and higher. Carnosine, like caffeine, increases the sensitivity of skinned muscle fibers to Ca^{2+} [13] and increases the contractility of isolated (especially fatigued) muscle preparations [17,18]. Since the development of muscle fatigue is probably related to the changes in the properties of the sarcoplasmic reticulum Ca^{2+}-release channels [15], the effect of carnosine on the whole muscle may be associated with its influence on the Ca^{2+}-release channels. Our preliminary data indicate that such regulation of the activity of Ca-channels by carnosine is possible [19].

The goal of this study was to investigate in greater detail the regulatory effect of carnosine on the sarcoplasmic reticulum Ca-channels.

2. Materials and methods

Carnosine (97% purity or better) was obtained from St. Petersburg Factory of Medical Drugs; antipyrylazo III was from Fluka (Switzerland); Tris, PIPES, Coomassie brilliant blue G-250 were from Serva (Germany); $CaCl_2$, $MgCl_2$, and caffeine were from Merck (Germany); nucleotides, creatine kinase, and creatine phosphate were from Reanal (Hungary); ruthenium red and EGTA were from Sigma (USA). All other chemicals were of research grade or better.

2.1. Isolation of the heavy fraction of sarcoplasmic reticulum vesicles

The heavy fraction of sarcoplasmic reticulum was obtained according to Morii et al. [20] with some modifications. Briefly, rabbit white skeletal muscles (predominantly of hind legs) were homogenized four times using a Waring-type homogenizer in five volumes of 5 mM Tris-maleate supplemented with 0.2 mM PMSF (pH 7.0) for 30 s with 30-s intervals. The homogenate was centrifuged at $5200 \times g$ for 15 min. The supernatant was filtered through four layers of cheesecloth, and the filtrate was centrifuged again at $10\,000 \times g$ for 30 min. The pellet was washed once in a solution containing 100 mM KCl, 0.2 mM PMSF, and 5 mM Tris-maleate (pH 7.0), and centrifuged under the same conditions. The resulting pellet was resuspended in the solution containing 250 mM sucrose, 0.2 mM PMSF, and 25 mM histidine (pH 7.0), and stored frozen at -70°C for a month. The content of protein was determined by the method of Spector using Coomassie G-250 [21].

2.2. Measurement of Ca^{2+} uptake and release

Accumulation and release of Ca^{2+} by sarcoplasmic reticulum vesicles were monitored using a Ca^{2+} indicator antipyrylazo III [20,22,23]. It does not penetrate across the sarcoplasmic reticulum membrane and forms specific stoichiometric complexes with extravesicular free Ca^{2+}. The measurements were performed on a Hitachi-557 dual-wavelength spectrophotometer (Japan) by monitoring the difference in absorbance between 720 and 790 nm. Accumulation of Ca^{2+} by a suspension of sarcoplasmic reticulum vesicles (1 mg/ml) was started by the addition of a nucleoside triphosphate (0.5 mM) to the reaction medium containing 0.1 M KCl, 0.5 mM $MgCl_2$, 50 mM PIPES-Tris (pH 7.0), and 50 μM antipyrylazo III. When ATP was used as a substrate for the Ca-pump operation, the reaction medium contained an ATP-regenerating system (10 IU creatine kinase and 5 mM creatine phosphate). The regenerat-

ing system was omitted in case of GTP use as a substrate for Ca^{2+} accumulation. Release of Ca^{2+} was induced by addition of 0.2–2 mM caffeine or 5–50 mM carnosine in the case of ATP-dependent accumulation and by addition of 0.025–1 mM AMP when GTP was used as a substrate. In control experiments, PIPES-Tris was used instead of carnosine to mimic the buffer capacity of carnosine. All experiments were performed at 25°C.

2.3. Measurement of $^{45}Ca^{2+}$ release from SR vesicles passively loaded with $^{45}Ca^{2+}$

To measure $^{45}Ca^{2+}$ release, 20 μl of the passively loaded vesicles incubated overnight at 0°C in a solution containing 100 mM KCl, 30 mM PIPES-Tris (pH 7.0), and 5 mM $CaCl_2$ supplemented with $^{45}Ca^{2+}$ ($\approx$ 10,000 cpm/nmol) were diluted 50-fold into a solution containing 100 mM KCl, 30 mM PIPES-Tris or carnosine (pH 7.0), and different combinations of EGTA, Ca^{2+}, and Mg^{2+}. The concentration of free Ca^{2+} was calculated according to Fabiato [26]. At certain intervals after dilution, the reaction was quenched by the addition of a stop solution containing 20 mM $LaCl_3$ and 40 mM $MgCl_2$. The quenched solution was immediately filtered through a 0.45-μm Synpore filter (Czechoslovakia) that was then twice washed with 1 ml of the solution containing 10 mM $LaCl_3$ and 20 mM $MgCl_2$, and dried. The radioactivity of the filters was measured by liquid scintillation counting on a Rackbeta-1214 counter (LKB, Sweden).

3. Results

3.1. Carnosine effect on the rate of Ca^{2+} accumulation by heavy sarcoplasmic reticulum vesicles

Addition of 0.5 mM ATP to the reaction medium containing heavy sarcoplasmic reticulum vesicles and all components necessary for the Ca-pump operation causes a fast decrease of free Ca^{2+} concentration in the medium as a result of its binding to ATP [20,22]. The subsequent accumulation of Ca^{2+} performed by Ca^{2+}-ATPase occurs in two phases. During the first phase, the Ca-channels remain in the open state, and Ca^{2+} accumulation into the vesicles occurs rather slowly. During the second stage, Ca-channels are converted into the close state, that results in a significant increase of the rate of Ca^{2+} accumulation. The fast Ca^{2+} uptake alternates with an equilibrium phase, when the rate of Ca^{2+} leakage from the sarcoplasmic reticulum vesicles is equal to that of Ca^{2+} pumping into the vesicles. As shown in Fig. 1a, the addition of an activator of Ca-channels (for instance, Ca^{2+} or caffeine) at the equilibrium phase causes a rapid release of Ca^{2+} from sarcoplasmic reticulum vesi-

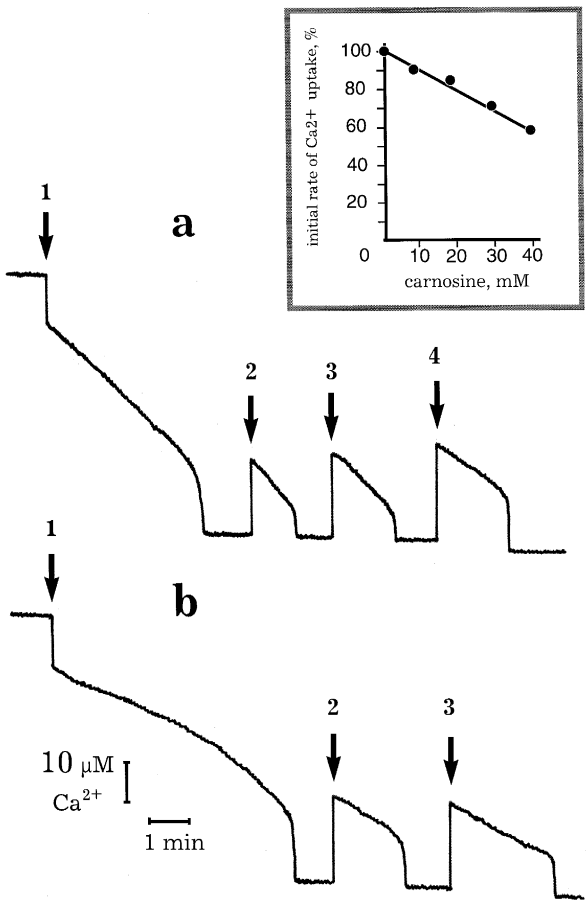

Fig. 1. Time-course of ATP-supported Ca^{2+} accumulation and Ca^{2+} release induced by different ligands in the control (a) and in the presence of 30 mM carnosine (b). The reaction medium contained 100 mM KCl, 0.5 mM $MgCl_2$, 30 mM PIPES-Tris (control) or 30 mM carnosine (pH 7.0), 50 μM antipyrilazo III, 50 μM free Ca^{2+}, 5 mM creatine phosphate, 10 units/ml creatine kinase, and 1 mg/ml sarcoplasmic reticulum protein. The reaction was started by the addition of 0.5 mM ATP (1). Where indicated, 10 μM Ca^{2+} (2), 2 mM caffeine (3), and 30 mM carnosine (4) were added to induce Ca^{2+} release. Inset: Dependence of the initial rate of Ca^{2+} uptake on the concentration of carnosine in the reaction medium.

cles, which is followed by its reaccumulation that occurs also in two stages.

We discovered that the addition of carnosine to the reaction medium at the concentrations corresponding to its content in skeletal muscle tissue resulted in a considerable decrease of the rate of Ca^{2+} uptake at the slow phase of accumulation (Fig. 1b). At the subsequent steady state, the addition of caffeine or Ca^{2+} to the reaction medium also entails a rapid ruthenium red-sensitive Ca^{2+} release from the sarcoplasmic reticulum vesicles, the reaccumulation of the released Ca^{2+} in the presence of carnosine also occurring more slowly. The effect of carnosine is dose-dependent. As the concentration of carnosine increases from 10 to 40 mM, the rate of Ca^{2+} accumulation decreases from 90% to 60% vs. control (Fig. 1, inset).

3.2. Carnosine-induced Ca^{2+} release from heavy sarcoplasmic reticulum vesicles

Carnosine-induced Ca-release was assayed under the usual conditions of ligand-induced Ca^{2+} release. As mentioned above, in the presence of ATP and ATP-regenerating system, all Ca^{2+} present in the reaction medium is accumulated into the sarcoplasmic reticulum vesicles in the course of slow and fast stages of accumulation that is followed by the equilibrium phase. At this step, the addition of a ligand that activates Ca-channels (for instance, caffeine or Ca^{2+}) causes a rapid Ca^{2+} release followed by its reaccumulation (Fig. 1a).

Table 1
Parameters of ligand-induced Ca^{2+} release from heavy fraction of sarcoplasmic reticulum vesicles

Ligand	$C_{0.5}$	A_{max}
Carnosine	8.67 ± 0.15	27.53 ± 2.74
Anserine	2.68 ± 0.11	37.17 ± 4.01
β-Alanine	NE	NE
Histidine	NE	NE
β-Alanine + histidine	NE	NE
Imidazole	NE	NE
Caffeine	0.31 ± 0.01	18.86 ± 0.06
AMP [a]	0.10 ± 0.02	20.03 ± 0.04

$C_{0.5}$, concentration providing half-maximal Ca^{2+} release, mM; A_{max}, the maximal amount of Ca^{2+} released, nmol Ca^{2+} per mg sarcoplasmic reticulum protein. NE, no effect.
[a] The AMP-induced Ca-release was studied with the use of GTP as a substrate for Ca^{2+} accumulation (see Section 2).

Table 2
Values of $C_{0.5}$ (mM) and A_{max} (nmol Ca^{2+} per mg sarcoplasmic reticulum protein) for caffeine- and AMP-induced Ca^{2+} release (using ATP and GTP as substrates, respectively) in the control (30 mM PIPES) and in the presence of 30 mM carnosine

Ligand	30 mM PIPES		30 mM Carnosine	
	$C_{0.5}$	A_{max}	$C_{0.5}$	A_{max}
Caffeine	0.31 ± 0.01	18.86 ± 0.06	0.19 ± 0.01	20.41 ± 0.03
AMP	0.10 ± 0.02	20.03 ± 0.04	0.04 ± 0.01	12.36 ± 0.02

For example, under the conditions used, caffeine induces a Ca^{2+} release in a dose-dependent manner. The dependence of the amount of the released Ca^{2+} is hyperbolic and can be characterized by $C_{0.5}$ and A_{max} values equal to 0.31 ± 0.01 mM and 18,86 ± 0.04 nmol Ca^{2+} per mg of protein, respectively (Table 1).

We discovered that the addition of carnosine to the reaction medium at the equilibrium phase of Ca-pump operation also induces a rapid Ca^{2+} release from sarcoplasmic reticulum vesicles (Fig. 1a). The dependence of the amount of the released Ca^{2+} also depends hyperbolically on the concentration of carnosine with $C_{0.5} = 8.67 \pm 0.15$ mM and $A_{max} = 27.53 \pm 2.74$ nmol Ca^{2+} per mg of protein (Table 1).

We also discovered that the methylated derivative of carnosine, i.e., anserine (1*N*-methyl-L-carnosine), had a similar effect on sarcoplasmic reticulum Ca-channels. It also induces a rapid Ca^{2+} release from the vesicles. Moreover, as shown in Table 1, the value of $C_{0.5}$ for anserine is about 3-fold lower as compared to that for carnosine. Conversely, the amino acids that enter into the composition of carnosine (β-alanine and histidine) added both separately and in the mixture as well as imidazole itself induce no Ca^{2+} release.

3.3. Influence of carnosine on caffeine-, AMP-, and Ca^{2+}-induced Ca^{2+} release from heavy sarcoplasmic reticulum vesicles and on the inhibitory action of Mg^{2+}

As shown in Table 2, the $C_{0.5}$ value for caffeine in the presence of carnosine was about 1.5-times lower as compared to the control, whereas the A_{max} value

was virtually unchanged. The AMP-induced Ca^{2+} release was studied using non-adenine nucleotide GTP as a substrate for Ca^{2+} accumulation into the sarcoplasmic reticulum vesicles. The addition of a non-adenine nucleotide to the medium containing heavy sarcoplasmic reticulum vesicles and all components necessary for Ca^{2+} uptake entails a fast decrease in free Ca^{2+} concentration due to its accumulation into the vesicles and then an equilibrium phase. However, in this case, neither caffeine nor Ca^{2+} induce Ca^{2+} release from the vesicles, since, under these conditions, Ca-channels are blocked by Mg^{2+} [9,24]. Conversely, adenine nucleotides (ATP, ADP, AMP, or β,γ-methylene-ATP) cause a dose-dependent Ca^{2+} release [9,24,25]. We discovered that, in the presence of 30 mM carnosine, the value of $C_{0.5}$ for AMP decreased in 2.5-times vs. the control. However, in this case, the amount of Ca^{2+} released decreased in the presence of carnosine (Table 2). Thus, as in the case of caffeine, carnosine increases the affinity of binding sites in Ca-channels for the activating ligand.

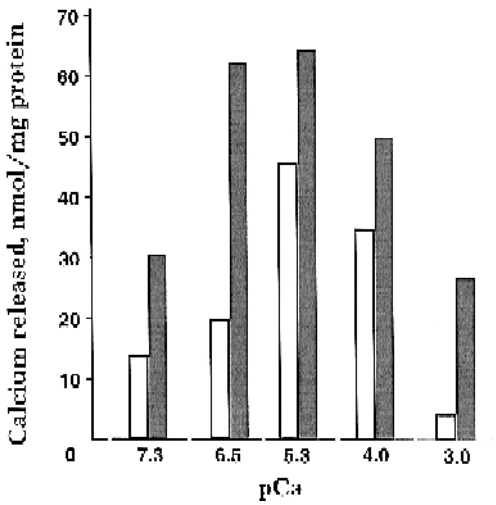

Fig. 2. Dependence of the amount of Ca^{2+} released from heavy sarcoplasmic reticulum vesicles on the concentration of free Ca^{2+} in the control (unpainted rectangles) and in the presence of 30 mM carnosine (black rectangles). The amount of Ca^{2+} released was determined using passive loading of sarcoplasmic reticulum vesicles with $^{45}Ca^{2+}$ as described in Section 2 by 15 s of the reaction. After dilution, the reaction medium contained 10 μg/ml protein, 100 mM KCl, 30 mM PIPES (control) or 30 mM carnosine (pH 7.0), and different amounts of $CaCl_2$ and EGTA. Indicated concentrations of free Ca^{2+} calculated according to Ref. [26] were obtained by varying the proportion of $CaCl_2$ and EGTA in the reaction mixture.

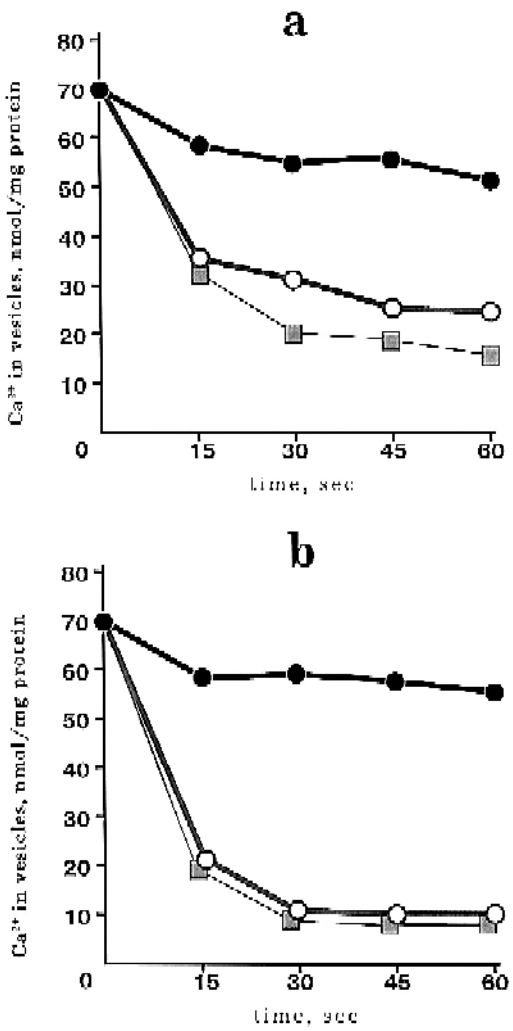

Fig. 3. Time-course of the content of Ca^{2+} in heavy sarcolasmic reticulum vesicles passively loaded with $^{45}Ca^{2+}$ in the control (a) and in the presence of 30 mM carnosine (b) in dependence of the concentration of Mg^{2+}. The content of Ca^{2+} was determined as described in Section 2. The effect of Mg^{2+} was evaluated at pCa = 5.3. After dilution, the reaction medium contained 10 μg/ml sarcoplasmic reticulum protein, 100 mM KCl, 30 mM PIPES (control) or 30 mM carnosine (pH 7.0), 50 μM or 1 mM $MgCl_2$, 0.5 mM EGTA, and 0.4 mM $CaCl_2$. Symbols: ●, 1 mM Mg^{2+}; ○, 50 μM Mg^{2+}; ■, control (without additions).

The influence of carnosine on the rate of Ca^{2+}-induced Ca^{2+} release was studied using passive loading of sarcoplasmic reticulum vesicles with $^{45}Ca^{2+}$. With regard for bell-shaped dependence of the rate of Ca^{2+}

efflux on its concentration, we studied Ca^{2+} release in a wide range of Ca^{2+} concentrations, from subactivating (pCa = 7.3) to inhibitory (pCa = 3.0). Fig. 2 illustrates that, over the all range of Ca^{2+} concentrations used, the rate of Ca^{2+} efflux from sarcoplasmic reticulum vesicles in the presence of carnosine is significantly higher than that in the control. The activating effect of carnosine is the most pronounced in case the lowest and the highest concentrations of Ca^{2+} are used.

To study the effect of carnosine on the inhibitory action of Mg^{2+} on Ca-channels, we also passively loaded sarcoplasmic reticulum vesicles with $^{45}Ca^{2+}$. As shown in Fig. 3, Mg^{2+} in the concentration of 50 μM and 1 mM considerably decreases the initial rate of Ca^{2+} efflux from preliminary loaded vesicles. In the control and in the presence of 50 μM Mg^{2+}, carnosine increases the rate of Ca^{2+} release. Moreover, in the presence of carnosine, the inhibitory action of 50 μM Mg^{2+} is completely removed, whereas in the presence of 1 mM Mg^{2+} the effect of carnosine is not manifested.

4. Discussion

We discovered that carnosine considerably affects the rate of Ca^{2+} uptake by the vesicles of heavy fraction of sarcoplasmic reticulum. Under the conditions used, the rate of accumulation of Ca^{2+} depends on two processes, i.e., the functioning of Ca-pump that removes Ca^{2+} from the external medium into the vesicles, and the state of Ca-channels, through which Ca^{2+} efflux from the vesicles occurs. Both mentioned processes may be the targets of carnosine action. In addition, carnosine may have a non-specific influence on the permeability of sarcoplasmic reticulum membranes for Ca^{2+}. This hinders the interpretation of the obtained results. However, the data published in the literature indicate that carnosine not only does not inhibit Ca^{2+}-ATPase, but even improves the efficiency of Ca-pump operation by increasing the Ca/ATP coefficient [27]. Thus, the decrease of the rate of Ca^{2+} uptake in the presence of carnosine in our experiments cannot be explained by the inhibition of Ca-pump. Therefore, we suggested that Ca-channels of the heavy fraction of sarcoplasmic reticulum were the most probable target for carnosine action.

We showed that carnosine and anserine themselves induce rapid Ca^{2+} release from heavy sarcoplasmic reticulum vesicles. In both cases, especially for anserine, the amount of released Ca^{2+} is considerably higher compared to caffeine and AMP. We suggest that carnosine increases either the mean open time or open probability or, perhaps, affects both these parameters more efficiently than caffeine and AMP. The fact that effect of carnosine and anserine is characterized by saturation seems to indicate that Ca-channels contain specific site(s) for the binding of these dipeptides.

The absence of activatory effect of amino acids that enter the composition of carnosine implies that the effect of carnosine is provided by its molecule on the whole rather than by any functional group of the molecule. We suggest that the certain stereospecific relative position of these groups in the molecule of carnosine is likely to play a crucial role in its interaction with the binding site(s) in the molecule of Ca-channel.

Although carnosine and related compounds were found in the muscle tissue of a great number of vertebrates [16], there is little information on its content in different cell compartments and on the changes in its concentration in the course of muscle contraction. It is unlikely that the concentration of carnosine can change rapidly enough in the course of a single cycle of muscle contraction and relaxation. Given this reason, the fact that carnosine may modulate the effect of other endogenous regulators of Ca-channels, both activators and inhibitors, is of much more interest than the carnosine-induced Ca^{2+} release itself. According to the data published in the literature, the total content of carnosine and anserine in rabbit skeletal muscles is about 20 mmol/kg of wet tissue [16]. We studied the possible influence of carnosine on the interaction of well-known modulators with Ca- channels in the presence of 30 mM carnosine, believing this value to be sufficiently close to the real concentration of these dipeptides in vivo.

When studying the effect of carnosine on the interaction of Ca-channels with their other activators, we discovered that carnosine not only does not compete with caffeine and AMP for the binding sites, but even increases their affinity for these ligands, potentiating thus their effect. The finding that the value of AMP-induced Ca^{2+} release in the presence of carno-

sine is considerably lower compared to the control can be explained with regard to the following data. It is known that the amount of Ca^{2+} released from sarcoplasmic reticulum vesicles depends, to a great extent, on the level of Ca^{2+} loading of vesicles [28] and is controlled by the interaction of Ca-release channels with Ca^{2+}-binding protein calsequestrin located on the inner surface of sarcoplasmic reticulum membrane [29]. We suggest that the difference in A_{max} in our experiments may reflect some changes in this interaction in the presence of carnosine.

It appeared that carnosine also increases the rate of Ca^{2+} efflux from the passively loaded with $^{45}Ca^{2+}$ heavy sarcoplasmic reticulum vesicles, the effect of carnosine being the most pronounced at low (subactivating) and high (inhibitory) concentrations of Ca^{2+}. Since carnosine considerably broadens the range of activating Ca^{2+} concentrations, we can conclude that carnosine both increases the affinity for Ca^{2+} of the high-affinity (activating) and decreases that of the low-affinity (inhibitory) Ca^{2+}-binding sites.

According to the literature, half-maximal inhibition of Ca^{2+} efflux is observed in the presence of 70 μM Mg^{2+} [9]. Hence, our finding that carnosine removes the inhibitory effect of 50 μM Mg^{2+} allows to assume that carnosine decreases the affinity for Mg^{2+} of those sites, the binding to which provides the inhibitory effect of Mg^{2+}.

Currently, the exact target for carnosine action on Ca-channels is unknown. The fact that Ca-channels contain, probably, saturable binding site(s) for carnosine indicates that carnosine can interact with Ca-channel itself. However, heavy sarcoplasmic reticulum contains a great number of other proteins that bind to Ca-channels and can modulate their activity. These are glyceraldehyde 3-phosphate dehydrogenase, aldolase, annexin VI, 170-kDa low-density lipoprotein binding protein, S-100 protein, calmodulin, calsequestrin, FK506 binding protein, 106-kDa protein, and triadin. Carnosine may bind to some of these proteins or to alter their interaction with Ca-channels, modulating thus the activity of Ca-channels in vivo. For instance, it was shown that carnosine activated glyceraldehyde 3-phosphate dehydrogenase [30]. Additionally, it was shown recently that the sensitivity of Ca-channels to caffeine depends on the interaction of Ca-channels with FK506-binding protein that was tightly bound to Ca-channels. The lesion of their interaction renders Ca-channels more sensitive to caffeine and decreases the rate of Ca^{2+} accumulation by sarcoplasmic reticulum vesicles [31]. Since we discovered that carnosine increased the sensitivity of Ca-channels to caffeine and decreased the initial rate of Ca^{2+} uptake, it can be assumed that carnosine may somehow disrupt the interaction between Ca-channels and FK506-binding protein.

Thus, using different approaches, we showed that carnosine, first, itself activated Ca-channels, and, second, potentiated the effect of other activators of Ca-channels and decreased the influence of inhibitors. We suggest carnosine-induced Ca^{2+} release in vivo to be unlikely, because its concentration in skeletal muscle cell is relatively high and comparable to $C_{0.5}$ for carnosine. For this reason, the second aspect of carnosine action seems to be much more important. Probably, it can account for some previously observed clinical phenomena related to the presence of carnosine in the muscle cell. For example, it is known that muscle pathology associated with astenia and anemia, i.e., Duchenne myodystrophy, is developed along with the decrease of the amount of carnosine and anserine in muscle fibers [32]. Obviously, the decrease in the content of carnosine results in the decrease of its potentiating effect, and Ca-channels become insensitive to the usual concentrations of ligands that induce muscle contraction. In addition, the influence of carnosine should be taken into account when performing experiments in vitro, because the parameters characterizing the activity of Ca-channels in vivo evidently will be different from those obtained as a result of standard isolation of sarcoplasmic reticulum preparations. Thus, to obtain conditions that most closely reflect the natural environment of sarcoplasmic reticulum Ca-channels in muscle cell, we suggest it is worthwhile to carry out the experiments on measuring the parameters of Ca^{2+} exchange in the presence of physiological concentrations of carnosine.

There is another hypothesis on the activating effect of carnosine and anserine. It was reported recently that carnosine was likely to form cyclized forms as a result of oxidation of the terminal NH_2-group [33]. Based on the computer simulation of the molecular structure of carnosine (the DeskTop Molecular Modelling Program, Oxford Electronic Publ., 1987, UK), the possible tautomeric structures of carnosine mini-

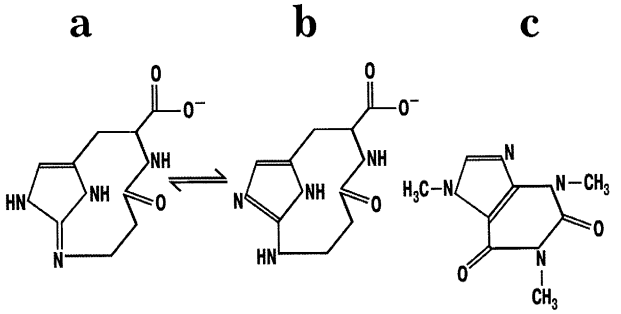

Fig. 4. Structures of tautomeric forms of carnosine (a, b) and caffeine (c).

mized in free energy were calculated. It was shown that, in the minimized structure, the amino terminal of carnosine was close to the imidazole ring (the distance between N and C was 4.61 Å). Since the length of a coordinate bond is 3–4 Å, the most probable structure that can be formed is a ring tautomer [33]. Tautomeric carnosine was proposed to exhibit pronounced biological activity [34], and the product of its oxidation may be even more efficient [33]. We suggest that heterocyclic structures of caffeine and cyclized forms of carnosine are somewhat similar (Fig. 4). Moreover, based on this hypothesis, the greater Ca^{2+}-releasing effect of anserine vs. carnosine can be explained by the fact that the cyclized structure of anserine, which contains methylated imidazole ring, resembles to a greater extent the structure of caffeine (1,3,7-trimethyl-2,6-dioxopurine). Possibly, it is the methyl group that is essential for binding to the receptor on the molecule of Ca-channel. Cyclic forms of carnosine may be produced in the course of metabolism of carnosine in the muscles and in solutions due to different oxidative processes. If this hypothesis is true, even small portions of percent of cyclic forms among the total amount of carnosine and anserine in muscles can provide the activating effect on Ca-channels, and the real $C_{0.5}$ values for carnosine and anserine might be significantly lower (judging by that for caffeine).

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