Biochemical and Biophysical Research Communications **279,** 219–222 (2000)
doi:10.1006/bbrc.2000.3906, available online at http://www.idealibrary.com on IDEAL®

Callipeltin A, a Cyclic Depsipeptide Inhibitor of the Cardiac Sodium–Calcium Exchanger and Positive Inotropic Agent

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Received October 31, 2000

Callipeltin A, a cyclic depsipeptide from the New Caledonian Lithistida sponge *Callipelta* sp., is a macrocyclic lactone containing four amino acids in the L configuration, Ala, Leu, Thr (2 residues); one (Arg) in the D configuration; two *N*-methyl amino acids, N-MeAla and N-MeGln; a methoxy tyrosine, a 3,4-dimethyl-L-glutamine; and a 4-amino-7-guanidino-2,3 dihydroxypentanoic acid (AGDHE), formally derived from L-Arg. In cardiac sarcolemmal vesicles Callipeltin A induces a powerful (IC_{50} = 0.85 μM) and selective inhibition of the Na^+/Ca^{2+} exchanger. In electrically driven guinea-pig atria, at concentrations ranging between 0.7 and 2.5 μM, Callipeltin A induces a positive inotropic effect, which at the highest concentrations is accompanied by a rise in resting tension. It is suggested that the positive inotropic effect is linked to the inhibition of the Na^+/Ca^{2+} exchanger and that Callipeltin A may be an useful tool to study the role of the cardiac Na^+/Ca^{2+} exchanger in physiological and pathological conditions.

Key Words: **Callipeltin A; cardiac Na^+/Ca^{2+} exchanger; guinea pig atria; positive inotropic effect.**

The sarcolemmal Na^+/Ca^{2+} exchanger, catalyzes the countertransport of three Na^+ for one Ca^{2+} and is a major Ca^{2+} extrusion mechanism in the heart cell thus contributing to the regulation of intracellular Ca^{2+} concentration and therefore cardiac contractility. The cardiac Na^+/Ca^{2+} exchanger has been cloned (1) and its molecular biology and functional roles in heart and several other tissues have been established and recently reviewed by Blaustein and Lederer (2). However the studies on the physiological and pathological role of the cardiac Na^+/Ca^{2+} exchanger have been hampered by the lack of a specific inhibitor (3). Starting from the observation that a synthetic peptide, exchange inhibitory peptide (XIP), with an amino acid sequence corresponding to a cationic region of the Na^+/Ca^{2+} exchanger, is a relatively potent and specific inhibitor of the Na^+/Ca^{2+} exchange activity (4) Khananshvily *et al.* (5) synthesized a series of positively charged cyclic peptides and found that conformationally constrained cyclic peptides with two arginine exhibit high inhibitory potency of the sarcolemmal Na^+/Ca^{2+} exchanger. Recently it has been reported that cyclic peptides and depsipeptides produced by microorganisms induce positive inotropic and negative chronotropic effects in rat heart (6). The positive inotropic effect of those cyclic peptides and depsipeptides could be attributed to none of the known molecular mechanisms inducing an increase of the force of contraction, that is receptor activation, inhibition of Na^+, K^+-ATPase, increase of cAMP level, increase of Na^+, K^+, Ca^{2+}, Mg^{2+} transport (6). The effect of those peptides on Na^+/Ca^{2+} exchange activity was not tested. It is our hypothesis that the inhibition of the exchanger may explain the positive inotropic effect elicited by those peptides. Therefore we have considered worth to investigate the effect of Callipeltin A, a cyclic depsipeptide from the New Caledonian Lithistida sponge *Callipelta* sp., whose isolation and structure (Fig. 1) has been recently reported by Zampella *et al.* (7). The effect of Callipeltin A has been studied on Na^+/Ca^{2+} exchange activity in cardiac sarcolemmal vesicles and on the force of contraction of electrically driven guinea-pig left atria.

Here we show that Callipeltin A is a potent (IC_{50} = 0.85 μM) and selective inhibitor of the Na^+/Ca^{2+} exchanger in cardiac sarcolemmal vesicles and induces a positive inotropic effect in guinea pig atria.

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0006-291X/00 $35.00

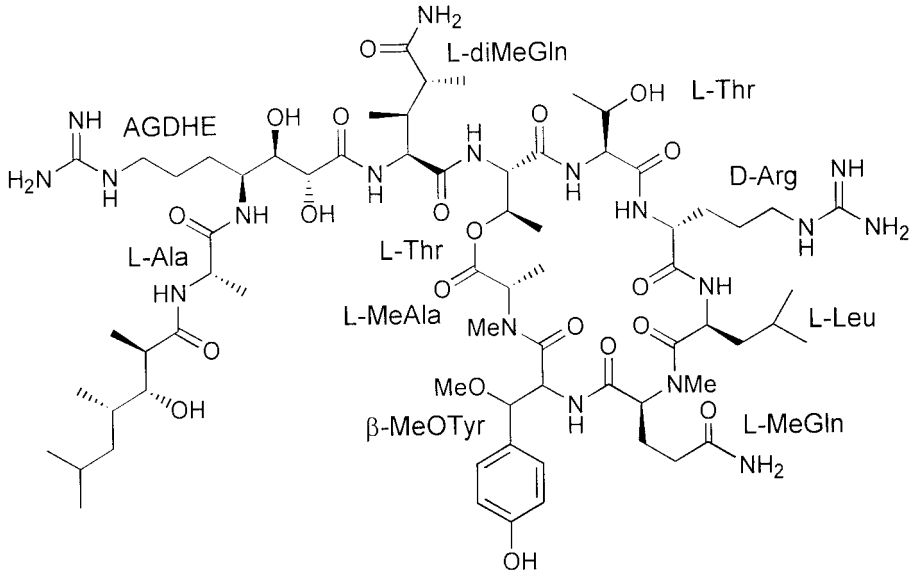

FIG. 1. Structure of Callipeltin A.

METHODS

Na^+/Ca^{2+} exchange assay. Na^+/Ca^{2+} exchange activity was measured as Na_i^+-dependent $^{45}Ca^{2+}$ uptake in cardiac sarcolemmal vesicles prepared from bovine ventricular tissue as described previously (8).

Cardiac contraction experiments. Left atria from hearts of guinea pig were placed vertically in an organ bath containing 20 ml of carbogen saturated PSS and stimulated electrically at 1 Hz. Isometric tension was recorded as detailed previously (9). The PSS was of the following composition (in mM): NaCl 137, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1, NaH_2PO_4 0.4, $NaHCO_3$ 19 and glucose 5.4 (37° and pH 7.4). Separate sets of atria were used for each concentration of Callipeltin A.

Other assays. Na^+, K^+-ATPase activity was measured in cardiac sarcolemmal vesicles as detailed previously (8). Type III phosphodiesterase was measured in bovine heart as described (10). Passive Ca^{2+} binding was measured by incubating K^+ (160 mM)-loaded vesicles in 160 mM KCl 10 mM $^{45}CaCl_2$ 20 mM 4-morpholinepropanesulphonic acid (MOPS) for 10 min at room temperature followed by millipore filtration of the vesicles and washing the filters with 2 × 2.5 ml of H_2O.

Skinned fibers preparation. Right ventricular papillary muscles were chemically skinned as previously described (11, 12). pCa/tension curves were obtained by exposing the bundles sequentially to solutions with increasing free calcium concentrations (from pCa 7.0 to 5.0). The various pCa solutions used throughout the experiments were divided into two equal parts, one containing dimethylsulfoxide (DMSO) and the other 5 μM Callipeltin A and used as control or treated cardiac muscle bundles, respectively.

Callipeltin A was dissolved in DMSO. At the concentrations reached in the medium DMSO had no effect either on the enzymatic assays or the isometric tension.

RESULTS

The cyclic depsipeptide Callipeltin A at concentrations ranging between 0.1 and 4 μM inhibits cardiac sarcolemmal Na^+/Ca^{2+} exchange activity measured as Na_i^+-dependent Ca^{2+} uptake at 10 μM Ca^{2+} (Fig. 1).

The maximal inhibition was about 95% and IC_{50} 0.85 μM. The inhibition was independent of Ca^{2+} concentrations in the range of 10 to 80 μM. In order to evaluate the selectivity of Callipeltin A, the effects of this cyclic depsipeptide was studied on sarcolemmal Ca^{2+} binding, Na^+, K^+-ATPase, Ca^{2+}-ATPase and on cardiac phosphodiestrase III. As shown in Table 1, Callipeltin A had negligible effect on these assays. In addition, Callipeltin A did not show Ca^{2+} ionophore action as this peptide did not induce Ca^{2+} entrance into sarcolemmal vesicles in the absence of sodium gradient (not shown).

At concentrations ranging between 0.7 and 2.5 μM, Callipeltin A induced a positive inotropic effect in electrically driven guinea-pig atria (Fig. 3A). The increase in force of contraction was twofold at 0.7 μM and about

TABLE 1

Specificity of Callipeltin A (2 μM)

	Control	Treated	% change
Na^+/Ca^{2+} exchange (nmol Ca^{2+}/mg prot/10 s)	8 ± 2.1	0.6 ± 0.1	−92
Ca^{2+} binding (nmol Ca^{2+}/mg prot/10 min)	2.23 ± 0.17	1.9 ± 0.1	−15
Na^+, K^+-ATPase (nmol ATP/mg prot/min)	470 ± 73	533 ± 65	+13
Ca^{2+}-ATPase (nmol ATP/mg prot/min)	120 ± 50	110 ± 30	−8
Phosphodiesterase III (nmol cAMP/mg prot/min)	0.44 ± 0.02	0.41 ± 0.02	−7

Note. Data are means ± SE of at least three independent experiments.

fivefold at 2.5 μM with respect to control value (Fig. 3B). A significant rise in the resting tension was observed 30 min after addition of the highest concentration (2.5 μM) of this peptide to the incubation medium (Fig. 3B). After longer periods of incubation (120 min) the increase of resting tension was observed also at lower concentrations of Callipeltin A. The increase of developed tension linked to an increase of myofibrillar proteins Ca^{2+} sensitivity (13) was also studied. The myofibrillar protein Ca^{2+} sensitivity was not modified by the addition of 5 μM Callipeltin A: the pCa_{50} value (pCa corresponding to 50% of maximum tension) was 6.09 ± 0.05 and 6.12 ± 0.04 and the Hill coefficient (an estimate of cooperative interactions between myofibrillar elements) was 3.28 ± 0.35 and 3.35 ± 0.33 in control and treated cardiac muscle bundles, respectively.

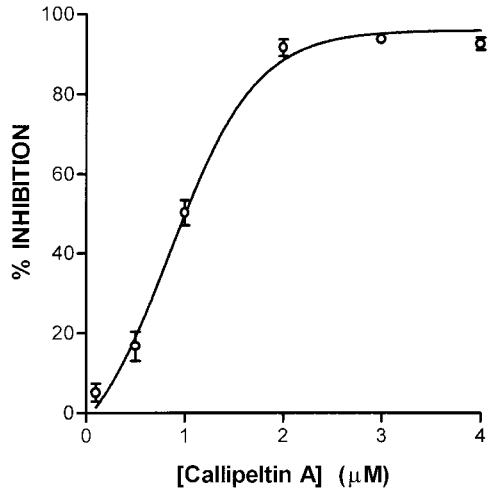

FIG. 2. Effect of Callipeltin A on Na^+/Ca^{2+} exchange activity in cardiac sarcolemmal vesicles. Na_i^+-dependent Ca^{2+} uptake was measured at 10 s. $[Ca^{2+}]$ was 10 μM. 100% activity was 8 ± 2.1 nmoles/mg protein/10 s. Data are means $\pm$ SE of six independent experiments.

DISCUSSION

The cyclic depsipetide Callipeltin A is a macrocyclic lactone (Fig. 1) containing four amino acids in the L configuration, Ala, Leu, Thr (2 residues); one (Arg) in the D configuration; two *N*-methyl amino acids, N-MeAla and N-MeGln; a methoxy tyrosine, a 3,4-dimethyl-L-glutamine; and a 4-amino-7-guanidino-2,3 dihydroxypentanoic acid (AGDHE), formally derived from L-Arg (7). The macrolactone ring closure involves a linkage between the C-terminus with a Thr residue, whereas the N-terminus forms an amide bond with a hydroxy acid. In their extensive study of conformationally constrained synthetic cyclic peptides as selective inhibitors of Na^+/Ca^{2+} exchanger in cardiac sarcolemma, Khananshvili *et al.* (5) showed that the presence of two arginine residues confers to these peptides an high inhibitory effect which is further increased when one arginine is in D configuration. The natural cyclic depsipeptide Callipeltin A shows some unique structural features that makes it a promising lead compound. In fact it contains two guanidinium amino acids: D-Arg and AGDHE. On the other hand, the presence of two N-alkylated (L-MeAla and L-MeGln) and two hydrophobic amino acids (L-Ala and L-Leu) and the lack of N- and C-termini make this peptide highly membrane permeable and suitable to interact with the negatively charged domains of the Na^+/Ca^{2+} exchanger at the cytosolic side of the membrane. Furthermore the cyclic structure and the presence of non-ribosomal amino acids, including D-series, a N-alkylated versions of the natural ones as well as strongly modified residues cause an improved stability to enzymatic degradation. The structural features of Callipeltin A, including lipophilicity and resistance to proteolitic degradation, may explain: (a) the high inhibitory potency ($IC_{50} = 0.85$ μM) of this peptide on the Na^+/Ca^{2+} exchanger of cardiac sarcolemmal vesicles and (b) the observation that Callipeltin A-induced inhibition, like that of the cyclic hexapeptides (5), but in contrast to XIP (4) or phosphorylated glycerophosphoinositols (8), comes to completion (Fig. 2).

As to the positive inotropic effect in guinea-pig atria the results presented in this study indicate that the increase in contractile force induced by Callipetin A is not supported by the inhibition of Na^+, K^+-ATPase, neither by the inhibition of phosphodiesterase III. In addition, the myofibrillar protein Ca^{2+} sensitivity (13) was unaffected by Callipeltin A. Since the positive inotropic effect is evident in the same range of concentrations as those inhibiting the cardiac Na^+/Ca^{2+} exchanger it can be proposed that the inhibition of the exchanger, by raising $[Ca^{2+}]_i$ can explain the increase in force of contraction of guinea pig atria induced by Callipeltin A. It is widely accepted that Na^+/Ca^{2+} exchanger might be responsible for the pharmacological effect of cardiac glycosides (14) and that the inhibitors of the exchanger induce a positive inotropic effect in guinea-pig atria (15). The increase of resting tension, which is an expression of Ca^{2+} overload in the cardiac myocytes, can be explained by the powerful effect of Callipeltin A on the cardiac Na^+/Ca^{2+} exchanger, reaching a complete inhibition (95%) at the highest concentrations of this peptide.

The inhibitory potency of the natural peptide Callipeltin A is significantly higher and more selective than that of the known synthetic compounds such as amiloride and its derivatives (16) but comparable to that of the most potent synthetic cyclic hexapeptides (5). The lipophilic cyclic depsipeptide Callipeltin A, similarly to the conformationally constrained and positively charged synthetic peptides (5), may interact with the negatively charged intracellular domains of the ex-

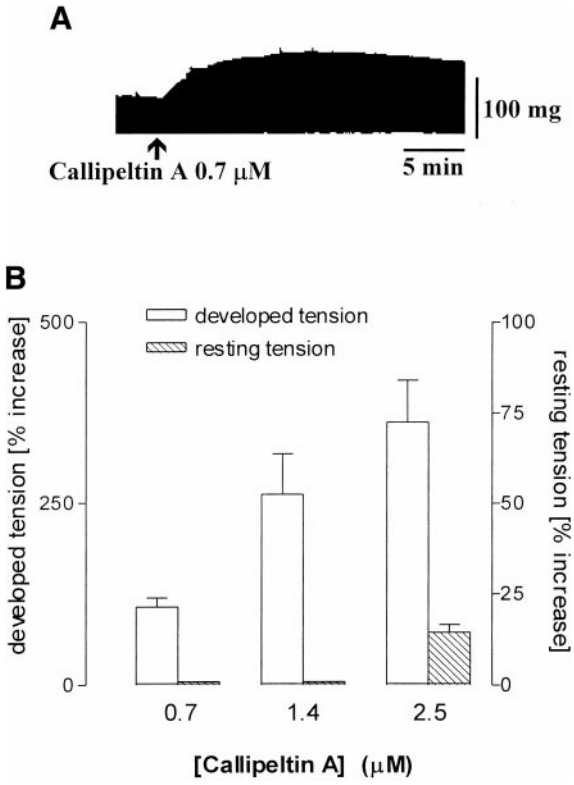

FIG. 3. (A) Original record of an experiment showing the inotropic effect of Callipeltin A on electrically driven guinea pig left atrium. (B) Effect of different concentrations of Callipeltin A on developed tension (open) and resting tension (dashed) in electrically driven guinea pig left atria. Data are means ± SE of three independent experiments.

changer protein, thus downregulating the exchange process. Although the effects of Callipeltin A on Na^+/Ca^{2+} exchanger and other major ion channel currents of the cardiac cell together with the characteristics of the positive inotropic effect on the whole heart remain to be studied, this natural peptide may be of value in studies to investigate the pathophysiology of the cardiac Na^+/Ca^{2+} exchanger.

ACKNOWLEDGMENTS

This work was supported by grants from Ministero Università e Ricerca Scientifica, Cofin. 9906104773-007 (to S.L.).

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