Cyclic ADP-ribose (cADPR) is an endogenous Ca^{2+}-mobilizing second messenger in many cell types and organisms. Although the biological activity of several modified analogues of cADPR has been analyzed, most of these structures were still very similar to the original molecule. Recently, we have introduced simplified analogues in which the northern ribose (N^1-linked ribose) was replaced by an ether strand (Gu, X., Yang, Z., Zhang, L., Kunerth, S., Fliegert, R., Weber, K., Guse, A. H., and Zhang, L. (2004) J. Med. Chem. 47, 5674–5682). Here we also demonstrate that the southern ribose (N^9-linked ribose) can be replaced by an ether strand resulting in N^9-[(phosphoryl-O-ethoxy)-methyl]-N^1-[(phosphoryl-O-ethoxy)-methyl]-hypoxanthine-cyclic pyrophosphate (cIDP-DE). This minimal structural analogue of cyclic ADP-ribose released Ca^{2+} from intracellular stores of permeabilized Jurkat T lymphocytes. In intact T lymphocytes initial subcellular Ca^{2+} release events, global Ca^{2+} release, and subsequent global Ca^{2+} entry were observed. Cardiac myocytes freshly prepared from mice responded to cIDP-DE by increased recruitment of localized Ca^{2+} signals and by global Ca^{2+} waves.

Cyclic ADP-ribose (cADPR)\(^1\) (compound 1; see Fig. 1), a metabolite of NAD\(^+\) discovered by Lee and co-workers in 1987 (1), is a signaling molecule that regulates calcium mobilization via ryanodine receptors from intracellular stores in a wide variety of biological systems such as sea urchin eggs, pancreatic \(\beta\) cells, smooth and cardiac muscle, T lymphocytes, and cerebellar neurons (reviewed in Refs. 2–5). Because of the important biological activities of cADPR, much effort has been focused on the syntheses of structural derivatives to elucidate the structure-activity relationship and to supply tools for investigating cellular Ca^{2+} signaling (reviewed in Refs. 6–8). However, the differences in pharmacological properties between the various modified cADPR analogues are still difficult to explain in terms of the structure-activity relationship.

Recently, cyclic IDP-ribose (cIDPR) analogues have been described as agonists of the cADPR/Ca^{2+} signaling system. 8-Br-N^1-cIDPR, a cyclic metabolite obtained by cyclization of 8-Br-nicotinamide hypoxanthine dinucleotide, induced Ca^{2+} signaling in intact Jurkat T lymphocytes (9). This finding was unexpected, because 8-substituted cADPR analogues have been known as potent antagonists of cADPR (reviewed in Refs. 6–8). A series of N^1-glycosyl-substituted cIDPR derivatives with different configurations at the N^1-glycosyl moiety also retained agonistic Ca^{2+}-releasing activities (10). More interestingly, a simplified molecule in which the northern ribose was replaced by an ether strand, the N^1-ethoxymethyl-cIDPR (cIDPDE) analogue, exhibited a strong potency for inducing Ca^{2+} release in intact and permeabilized human Jurkat T lymphocytes (11). The 8-substituted cIDPDE analogues N^2,N^1-cIDPDE and 8-NH\(_2\)-cIDPDE showed similar biological activities as compared with cIDPDE, whereas the halogenated derivatives 8-Br-cIDPDE and 8-Cl-cIDPDE did not significantly elevate [Ca^{2+}] (11). Thus, the N^1-riboyl moiety (northern ribose) and the 6-amino group in cADPR are not, per se, critical structural factors for biological activity, whereas different substituents at the 8-position of the base moiety obviously influence strongly the overall electron affinity and sterical conformation of the analogue, thereby greatly influencing its biological activity.

Concerning the southern ribose moiety of cADPR, it was found that the 3'-hydroxy group was essential for Ca^{2+} release in the sea urchin egg system, whereas the 2'-hydroxy was not (12). O-Methylation of the 3'-hydroxy group generated an antagonist (12). The stable carbocyclic derivative cyclic aristeromycin diphosphoribose was an agonist in both sea urchin eggs and T lymphocytes (13, 14). To further explore the structure-activity relationship of cADPR in mammalian cells, we replaced both the southern and northern ribose moieties of cIDPR with ether strands. The resulting molecule, called N^1-[(phosphoryl-O-ethoxy)-methyl]-N^2-[(phosphoryl-O-ethoxy)-methyl]-hypoxanthine-cyclic pyrophosphate (cIDP-DE, compound 2) (Fig. 1), is a minimal structural analogue of cADPR that retained biological activity. Here we report on the chemical and biological characterization of cIDP-DE.

**EXPERIMENTAL PROCEDURES**

**Materials**—Fura2/AM and ionomycin were purchased from Calbiochem. Cyclic ADP-ribose, 8-Br-cADPR, and BDM were obtained from Sigma-Aldrich. Solvents were dried by reflux over CaH\(_2\) and distilled...
before use. Chemical reagents were supplied by Acros or Aldrich. Laminin was purchased from Roche Applied Science, culture medium reagents were from Sigma-Aldrich and Invitrogen, and HEPES was from Invitrogen.

Chemistry—UV spectra were recorded on a Varian DMS200 UV-visible spectrophotometer. Steady-state emission spectra were recorded on a PerkinElmer Life Sciences LS50B fluorescence spectrophotometer. Time-of-flight high-resolution mass spectrometry was performed with Bruker BIFLEX III. Elemental analysis was determined by a PE-240C analyzer. 1H NMR and 13C NMR were recorded with a Bruker Avance 300 spectrometer (121.42 MHz). Orthophosphoric acid (85%) was used as an external standard. Compounds 2 and 13 were purified on an Alltech preparative C18 reversed phase column (2.2 x 25 cm) with Gilson high performance liquid chromatography by two different buffer systems, acetonitrile/triethylammonium acetate (0.1 M, pH 7.0) and acetonitrile/triethylammonium bicarbonate (0.1 M, pH 7.5).

Molecular Modeling—The conformations of cADPR and cIDP-DE were minimized using the density functional theory method. Equilibrium geometries of all molecules were fully optimised at the B3LYP/6-31G(d) level of theory. Vibrational frequencies, each calculated at the same level, were used to determine the nature of the stationary points and to give the zero point vibrational energies. Minima were characterised with zero imaginary frequency. All calculations were performed using the Gaussian 98 program package. Superimposition was performed by the Accelrys Insight II package.

Jurkat T Lymphocyte Cell Culture—Jurkat T lymphocytes (clone J9-13T2) were cultured as described previously (15).

Cardiac Myocyte Isolation and Cell Culture—Wild-type C57/Black Swiss mice (5–7 months old, 3 males and 2 females) were anesthetized with CO2. The heart was quickly removed and retrogradely perfused for 5 min (flow rate of 3 ml/min at 37 °C) with a perfusion buffer containing 113 mM NaCl, 4.7 mM KCl, 0.6 mM KH2PO4, 0.6 mM Na2HPO4, 1.2 mM MgSO4, 12 mM NaHCO3, 10 mM KHCO3, 10 mM HEPES (pH 7.46), 30 mM taurine, 20 mM glucose, and 15 mM BDM. The heart was then digested with perfusion buffer containing 0.1 mg/ml Blendzyme 3 (Roche Applied Science) and 12.5 μM CaCl2 for 9 min. After digestion, the heart was removed, placed in a dish, and cut gently into small pieces. Digestion was stopped with the perfusion buffer containing 12.5 μM CaCl2 and 5% bovine serum albumin. CaCl2 was slowly added back to a final concentration of 1 mM. A total of 211,275 ± 62,203 rod-shaped cells were obtained per heart. Cells were plated in laminin-coated Labtek chambers (0.01 mg of laminin per milliliter; Roche Applied Science) at a density of 20,000 rod-shaped myocytes per milliliter in the

![Fig. 1. Structures of cADPR and cIDP-DE.](image)

![Fig. 2. Preparation of N\(^{\alpha}\)-(TBDMS-O-ethoxy)-methyl-hypoxanthine (compound 7). Synthesis was completed as described in the supplemental data (available in the on-line version of this article), and the structure of compound 7 was identified by 1H NMR and elemental analysis. Reagents and conditions: a, benzylic chloride, pyridine, room temperature, 2 h; b, CICH\(\_\)CH\(\_\)CH\(\_\)OAC, bovine serum albumin, 80 °C, 1.5 h; c, NH\(\_\)H2/OH, room temperature, 24 h; d, NaNO\(\_\)2, pyridine, room temperature, 24 h; e, tert-butylimethyletheryl chloride, imidazole, N,N-dimethylformamide, room temperature, 3 h.

![Fig. 3. Synthesis of cIDP-DE (2) from N\(^{\alpha}\)-(TBDMS-O-ethoxy)-methyl-hypoxanthine (compound 7). Syntheses were completed as described in the supplemental data (available in the on-line version of this article), and the structures of synthesized compounds were identified by 1H NMR and elemental analysis or high-resolution mass spectrometry (time-of-flight, positive). Compound 2 was purified twice by high performance liquid chromatography using a C18 reversed phase column and developed by a linear gradient of 0–40% CH3CN in water/acetonitrile/triethylammonium acetate and triethylammonium bicarbonate buffers (0.1 M, pH 7.0 and 0.1 M, pH 7.5, respectively). Reagents and conditions: a, 1,8-diazabicyclo[5.4.0]undec-7-ene, CICH\(\_\)CH\(\_\)CH\(\_\)OAC, CH\(\_\)Cl2, room temperature, 0.5 h; b, tetraethylammonium fluoride, tetrahydrofuran, room temperature; 2 h; c, (PhNH\(\_\))2POCl, tetrazole, pyridine, room temperature, 48 h; d, CH3ONa, CH3OH, room temperature, 2 h; e, cyclohexylammonium S,S-diphenyl phosphorodithioate, triisopropylbenzenesulfonil chloride, tetrazole, pyridine, room temperature, 24 h; f, (i) isosomyl nitrite, pyridine/ACOH/AC\(\_\)O (2:1:1), room temperature, 8 h, (ii) H3PO\(\_\)4, Et3N, pyridine, room temperature; g, I3, 3AMS, pyridine, room temperature, 24 h.)
permeabilized medium containing minimum essential medium with Hank's salts and l-glutamine (Invitrogen, catalog number 21575), 5% bovine calf serum, 100 units/ml penicillin-streptomycin, and 10 mM BDM and incubated for 1 h at 37 °C in a 5% CO₂ incubator.

**Ca²⁺ Measurements in Intact and Permeabilized Jurkat T Cell Suspensions**—Intact Jurkat T lymphocytes were loaded with fura2/AM as described (15). Ratiometric determination of [Ca²⁺], was carried out in cell suspension in a Hitachi F2000 fluorometer at room temperature at excitation wavelengths of 340 and 380 nm (alternating) and an emission wavelength of 495 nm. Each experiment was calibrated by the addition of Triton X-100 (10% v/v final concentration) to obtain the maximal ratio and subsequent addition of 4 mM EGTA and 40 mM Tris-base to obtain the minimal ratio.

Permeabilized Jurkat T cells were prepared as detailed previously (14). In brief, cells were transferred into an intracellular medium (nominally Ca²⁺-free, pH 7.2), permeabilized with 55 mg/ml saponin for 20 min, and afterward washed three times to remove all saponin. Then, the cells were left on ice for ~2 h to allow for the rescaling of intracellular Ca²⁺ stores. Finally, ~1.5 × 10⁷ permeabilized cells were transferred into an quartz cuvette and placed into an F2000 fluorometer (Hitachi Instruments). The cell suspension was warmed to 37 °C and stirred slightly. Fura2/AM (1 μM final concentration) was added, and the free Ca²⁺ concentration was monitored at 340 and 380 nm as alternating excitation wavelengths and 495 nm as the emission wavelength. Experiments were started by the addition of creatine kinase (20 units/ml) and creatine phosphate (20 mM), followed by the addition of 1 mM ATP to allow loading of the intracellular Ca²⁺ pools by endoplasmic/sarcoplasmic Ca²⁺ ATPases.

Chelex resin was added generally to solutions of compounds to be tested for Ca²⁺ release to avoid any Ca²⁺ contamination. The quality of the permeabilized cell suspension was checked each day by its responsiveness to cADPR and D-myo-inositol 1,4,5-trisphosphate.

**Confocal Calcium Imaging of T Cells and Cardiac Myocytes**—Jurkat T lymphocytes were loaded with fura2/AM as described (15). Imaging experiments were carried out on thin glass coverslips (0.1 mm) coated with bovine serum albumin (5 mg/ml) and poly-l-lysine (0.1 mg/ml). Silicon grease was used to seal small chambers that consisted of a rubber O-ring on the glass coverslips. Then, 60 μl of buffer A containing 140 mM NaCl, 5 mM KCl, 1 mM MgSO₄, 1 mM CaCl₂, 1 mM NaH₂PO₄, 5.5 mM glucose, and 20 mM HEPES (pH 7.4) along with 40 μl of cell suspension (2 × 10⁷ cells/ml) in the same buffer were added into the small chamber (16). The coverslip with cells slightly attached to the bovine serum albumin/poly-l-lysine coating was mounted on the stage of a fluorescence microscope (Leica DM IRE2).

Cardiac myocytes attached to laminin-coated chamber slides were loaded with fura2/AM (4 μM final concentration) for 45 min at 37 °C in plating medium (see above for the composition). Then, the medium was removed by gentle aspiration and replaced by buffer A. This step was repeated one or two times to completely remove fura2/AM as well as rounded or non-attached myocytes.

Ratiometric Ca²⁺ imaging was performed as described recently (16–18). In brief, we used an Improvement imaging system (Heidelberg, Germany) built around the Leica microscope at 100-fold magnification. Illumination at 340 and 380 nm was carried out using a monochromator system (Polychromator IV, TILL Photonics, Graefelfing, Germany). Images were taken with a gray scale charge-coupled device camera (type C4742–95-12ER; Hamamatsu, Enfield, United Kingdom) operated in 8-bit mode. The spatial resolution was 512 × 640 pixels at either 40-fold or 100-fold magnification for cardiac myocytes or 100-fold magnification for T cells. The acquisition rate was approximately one ratio per 160 ms. Raw data images were stored on hard disk. Confocal Ca²⁺ images were obtained by off-line no neighbor deconvolution using the volume deconvolution
because the strategy of intramolecular cyclization requires different protecting groups on the \( N^1 \) and \( N^9 \)-substituted hypoxanthines, respectively, it would be difficult to use hypoxanthine as a starting material. Therefore, \( N^9 \)-benzoyl-\( N^1 \)-substituted adenine 4 was obtained from \( N^9 \)-benzoyl-adenine 3 by the known method (19, 20) (Fig. 2). After deprotection and oxidation of compound 4, \( N^9 \)-substituted hypoxanthine 6 was synthesized (Fig. 2), and \( N^1 \), \( N^9 \)-disubstituted hypoxanthine 8 with different protecting groups was provided from 4 conveniently. Following the procedure for the synthesis of cIDPRE (11), the intermediate 13 was obtained in very good yield (Fig. 3). It was very interesting to find that the intramolecular cyclization of compound 13 was completed in 61% yield, although the more flexible \( N^1 \) and \( N^9 \) ether strands in compound 13 resulted in a larger spatial distance of the two phosphate groups (Fig. 3).

The photo-physical properties of cIDP-DE were analyzed. The UV spectrum of cIDP-DE in water exhibited intense absorption at 250 nm (\( \lambda_{max} \)) with an extinction coefficient (\( \epsilon \)) of 6212.66 mol\(^{-1}\) dm\(^3\) cm\(^{-1}\) (data not shown). A moderately intense shoulder band at 278 nm (\( \epsilon = 2478.95 \) mol\(^{-1}\) dm\(^3\) cm\(^{-1}\)) was observed. The absorption spectral properties were found to follow Beer’s Law at concentrations below \( 1 \times 10^{-3} \) mol/liter. Interestingly, cIDP-DE displayed moderate fluorescence in water at room temperature, making it suitable for intracellular imaging applications.

**RESULTS**

*Chemistry*—The key steps for the synthesis of cIDP-DE (compound 2; Fig. 1) include the synthesis of the \( N^1 \), \( N^9 \)-disubstituted hypoxanthine 8 and the intramolecular cyclization of the intermediate 13 (Fig. 3). It is well known that hypoxanthine exists in the 6-oxo form in solution and can be converted to \( N^1 \), \( N^9 \)-disubstituted hypoxanthine after alkylation. However,
perature (Fig. 4). The excitation spectrum showed two prominent peaks at 223 and at 278 nm (Fig. 4, top). When excited at 278 nm, an emission spectrum with a single peak at 355 nm was observed (Fig. 4, bottom). By using single photon counting, the fluorescence lifetime was estimated to be 6.2 ns (data not shown). In contrast, no fluorescence was observed in cIDPR and cIDPRE (data not shown). Thus, it is likely that the more flexible N1 and N9 ether strands in cIDP-DE made the electronic transition in the purine ring easier.

**Biochemistry and Pharmacology**—Because cIDPRE and some of its analogues were recently identified as membrane-permeant Ca2+-mobilizing agonists of the cADPR/Ca2+ signaling system (11), we intended to also analyze the biological activities of cIDP-DE. However, due to the unexpected fluorescence properties of cIDP-DE, any interference with the Ca2+ indicator fura2 had to be ruled out first. Thus, [Ca2+]i was determined in Jurkat T cells in the absence of extracellular Ca2+ (Fig. 5). Intracellular Ca2+ pools were completely depleted by the addition of ionomycin; a second addition of ionomycin did not release any further Ca2+ (Fig. 5A). Under these conditions cIDP-DE did not change the fura2 ratio (Fig. 5A). In contrast, when the intracellular Ca2+ pools were filled, cIDP-DE induced a transient Ca2+ release (Fig. 5B) after a short delay. Together with the control data (Fig. 5A), this indicates that cIDP-DE is a membrane-permeant analogue of cADPR with agonistic properties. In addition, Fig. 5B shows that Ca2+ store depletion by cIDP-DE activates Ca2+ entry upon the re-addition of extracellular Ca2+.

In saponin-permeabilized Jurkat T cells, cIDP-DE did indeed release Ca2+ from intracellular stores (Fig. 6A). However, comparison of the concentration-response curves for cADPR versus cIDP-DE indicates that cIDP-DE is an ~3-fold weaker agonist (Fig. 6B). The specificity of Ca2+ release evoked by cIDP-DE was demonstrated by its sensitivity to 8-Br-cADPR and ruthenium red; in contrast, Ca2+ release by d-myo-inositol 1,4,5-trisphosphate was almost unaffected (Fig. 6C).

Regardless of its weak activity in permeabilized cells, a pronounced effect of cIDP-DE on Ca2+ signaling was observed in intact Jurkat T cells when the extracellular Ca2+ concentration was adjusted to 1 mM during the whole experiment (Fig. 7). Both 500 µM and 1 mM cIDP-DE-activated biphasic Ca2+ signaling consisting of a dose-dependent initial peak (Fig. 7B) and...
a less dose-dependent sustained elevation of $[\text{Ca}^{2+}]_i$, (Fig. 7A). cIDP-DE, added at 1 mM extracellular concentration, induced $\text{Ca}^{2+}$ signaling in a manner comparable with that of the anti-CD3 monoclonal antibody OKT3 (Fig. 7A). At prolonged incubation periods with cIDP-DE, $[\text{Ca}^{2+}]_i$ did not return to baseline values but remained slightly elevated. This indicates that, in addition to $\text{Ca}^{2+}$ release, $\text{Ca}^{2+}$ entry is also activated and, thus, confirms the data obtained with the $\text{Ca}^{2+}$-free/Ca$^{2+}$ reintroduction protocol shown in Fig. 5B. The $\text{Ca}^{2+}$-mobilizing activity of cIDP-DE was also observed in $\text{Ca}^{2+}$ imaging experiments. Although the addition of extracellular buffer did not significantly change $[\text{Ca}^{2+}]_i$, an increase was observed upon the addition of cIDP-DE (Fig. 8). The increase was not immediate but delayed. In the phase between the cIDP-DE addition and the onset of the global signal, subcellular hot spots of $\text{Ca}^{2+}$ signaling were visible (Fig. 8A, arrowhead, time point 96.826 s).

The $\text{Ca}^{2+}$-mobilizing activity of cIDP-DE was also tested in cardiac myocytes freshly prepared from murine hearts. In these cells, the addition of cIDP-DE induced global $\text{Ca}^{2+}$ signaling with a very slow onset (Fig. 9) that was obviously due to a slower uptake into the cell as compared with the Jurkat T cells (Fig. 8A). Upon the addition of cIDP-DE, the global $[\text{Ca}^{2+}]_i$, either increased continuously (Fig. 9B) or in an oscillatory manner (Fig. 9C). Small increases in $[\text{Ca}^{2+}]_i$ were also observed in control myocytes (Fig. 9A), probably due to the extended exposure to UV light (21). Preincubation of cardiac myocytes with the antagonist 8-Br-cADPR resulted in a similar weak increase of $[\text{Ca}^{2+}]_i$, as compared with buffer additions (Fig. 9D).

In confocal $\text{Ca}^{2+}$ imaging experiments, local spots with oscillating $[\text{Ca}^{2+}]_i$ signals were observed upon the addition of cIDP-DE (Fig. 10, A and B, arrowheads). Over time, in the neighborhood of these relatively small subcellular $\text{Ca}^{2+}$ signals further $\text{Ca}^{2+}$ microdomains were recruited (Fig. 10B). At later time points global $\text{Ca}^{2+}$ waves were observed (Fig. 10, A and C). These waves often started at one end of the cell, as shown in the example displayed in Fig. 10C on the right-hand side. The surface plots show the directed movement of the wave over a distance of $\sim 40 \mu\text{m}$ in $-1.2$ s (Fig. 10C). Along with the $\text{Ca}^{2+}$ wave, a wave of weak local contraction of the cell was visible.

**DISCUSSION**

In this report we demonstrate chemical synthesis and characterization, fluorescence properties, molecular modeling, and biological activity of the novel cADPR analogue cIDP-DE, a minimal structural analogue with replacement of both the northern and southern riboses by ether strands. The structure of cADPR in solution was investigated by Sekine and co-workers using NMR techniques (22). They reported a predominant C2′-endo conformation for the southern ribose unit and a flat northern ribose conformation in the cADPR structure in solution. These structural characteristics in solution are very similar to those revealed by x-ray crystallographic analysis (23).

However, in the structure of cIDP-DE two more flexible ether strands substitute for both the northern and southern riboses of cADPR. Nevertheless, cIDP-DE is still active as a $\text{Ca}^{2+}$-mobilizing compound in both T lymphocytes and cardiac myocytes. Although the identity or the structure of the receptor for cADPR is not clear at this time, the data reported here may indicate some clues to the structural interaction between ligand and receptor. First, the binding pocket may recognize, relatively specifically, the nucleobase on the one side and the diphospho-bridge on the other side of the ligand. Secondly, the two parts of the binding pocket located close to the northern and southern ribose moieties may be sufficiently variable; e.g., one electronegative atom such as oxygen and two spacers of the right length appear to be sufficient for the binding of the ligand and for a weak biological activity. To model the structure of cIDP-DE, its conformation was minimized by a quantum mechanics calculation (Fig. 11). The different ways of superimposing the energetically minimized conformations of cIDP-DE (Fig. 11, pink) and cADPR (yellow) clearly show that both compounds are characterized by very similar three-dimen-
The conformations of cADPR (Fig. 11A). The optimal superimposition of the two whole molecules in addition suggests that the two ether groups in cIDP-DE almost perfectly replace the corresponding C1-O-C4-units of the two riboses (Fig. 11C). Because of the flexible ether strands used as linkers in cIDP-DE, it is also imaginable that the binding pocket of the cADPR receptor induces the correct “binding conformation” during the process of ligand binding. However, as long as the binding protein for cADPR has not been identified, this remains speculative.

The biological activity of cIDP-DE in Jurkat T cells was quite similar to that of cIDPRE in the following ways: (i) Ca\(^{2+}\) release activity in permeabilized cells; (ii) induction of both Ca\(^{2+}\) release and Ca\(^{2+}\) entry in the Ca\(^{2+}\)-free/Ca\(^{2+}\) reinduction protocol; and (iii) stimulation of Ca\(^{2+}\) signaling (11). This similarity of cIDP-DE and cIDPRE, especially in the induction of Ca\(^{2+}\) signaling in intact T cells, indicates that the further replacement of the southern ribose by the second ether bridge in cIDP-DE did not significantly influence its biological activity. Compounds of the type cIDP-DE or cIDPRE may thus both be used as membrane-permeant full agonists of the cADPR/Ca\(^{2+}\) signaling system in intact cells. Because both phases of anti-CD3-mediated Ca\(^{2+}\) signaling can be mimicked by cIDP-DE (this study) or cIDPRE (11), it will be extremely interesting to stimulate primary T cells by cIDP-DE or cIDPRE (and perhaps an additional stimulus) and to analyze the downstream effects of T cell activation such as transcription, proliferation, or cytokine release.

The role of cADPR in cardiac myocytes has been discussed somewhat controversially in the past. Chu and co-workers were the first to provide evidence that Ca\(^{2+}\) release via type 2 ryanodine receptor is modulated by cADPR (24). In contrast, a study carried out in rat ventricular myocytes using caged cADPR suggests that cADPR does not regulate sarcoplasmic reticulum Ca\(^{2+}\) release (25). However, work from several groups strongly suggests the following possibilities for cardiac myocytes: (i) that the enzymatic machinery for synthesis of cADPR is expressed (26, 27); (ii) that cADPR increases both the amplitude of whole cell Ca\(^{2+}\) transients and the frequency of Ca\(^{2+}\) sparks in rat and guinea pig isolated myocytes (28); and (iii) that Ca\(^{2+}\) signaling and contractions in electrically driven guinea pig or rat cardiac myocytes are sensitive to the specific cADPR antagonists 8-NH\(_2\)-cADPR and 8-Br-cADPR (29, 30).

In accordance with these findings, we demonstrate here that cIDP-DE evoked an increase of global [Ca\(^{2+}\)], and induced the recruitment of local Ca\(^{2+}\) microdomains and the development of global Ca\(^{2+}\) waves. Taken together, the data obtained in cardiac myocytes suggest a central role of cADPR in modulating Ca\(^{2+}\) release via the ryanodine receptor, a mechanism that now may be activated without touching the cells simply by incubation with the membrane-permeant agonist cIDP-DE. In summary, evidence is provided here indicating that replacement of the two ribose moieties of cADPR by flexible ether strands retains significant albeit lower biological activity and additionally creates a membrane-permeant agonistic molecule.

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