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Adenine-based calcium signal pathway messengers: Synthesis and agonistic properties of cyclic ADP-ribose analogs*

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Abstract: A series of cyclic ADP-ribose (cADPR) analogs, in which modifications mainly focused on riboses, was synthesized in order to explore the molecular mechanism of calcium release regulated by cADPR. Biological activities investigated in intact T-lymphocytes showed that the structurally simplified analogs, N1-ethoxymethyl-substituted cyclic inosine diphosphoribose (cIDPRE), N1,N9-diethoxymethyl-substituted cyclic inosine diphosphoribose (cIDPDE), and N1-ethoxymethyl-substituted cyclic adenosine diphosphoribose (cADPRE) in which the northern ribose or both northern and southern riboses were replaced by ether linkages are membrane-permeant and induce calcium release from intracellular stores. This research has provided novel molecules to probe cADPR-mediated calcium signaling and enlarges our knowledge of the structure–activity relationships of cADPR analogs.

Keywords: cADPR analogs; agonists; nucleotides; synthesis; signal messengers.

INTRODUCTION

Calcium signal transduction is one of the most important intracellular signal pathways in cells. Cyclic ADP-ribose **1** (cADPR) (Scheme 1), a metabolite of NAD^+ (nicotinamide adenine dinucleotide) discovered by Lee in 1987, is a signaling molecule to regulate calcium mobilization via ryanodine receptors (RyR) from intracellular stores in a wide variety of biological systems [1,2]. The important biological activities of cADPR have prompted much effort toward the syntheses of structural diverse derivatives, to elucidate structure–activity relationships and to investigate cellular Ca^{2+} signaling [3].

The various syntheses of cADPR analogs can be classified according to enzymatic, chemo-enzymatic, and chemical methodology. Many NAD^+ analogs modified on the adenine, ribose, or pyrophosphate moieties are recognized by ADP-ribosyl cyclase and form cADPR analogs, such as cyclic aristeromycin diphosphoribose (cArisDPR) [4], 3-deaza-cADPR [5], cyclic adenosine triphosphate (cATPR) [6], etc. Hypoxanthine-based analogs such as cyclic inosine diphosphate **2** (cIDPR) and its derivatives can also be synthesized by chemo-enzymatic methods [7]. However, those analogs that can be

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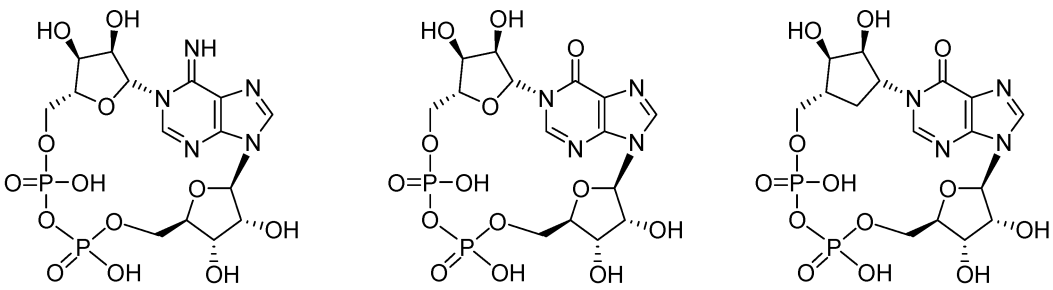
obtained by enzymatic or chemo-enzymatic methods are limited by the substrate specificity of the enzyme. Chemical methods are more amenable to synthesis of a greater variety of structurally diverse cADPR analogs. The main difficulties in chemical synthesis are N1-substitution and intramolecular diphosphate cyclization. The first successful chemical synthesis was achieved by Matsuda and coworkers in 1998, with the synthesis of cyclic IDP-carbocyclic ribose **3** (cIDPcR) [8]. Since then, many cADPR analogs, particularly those with modifications on ribose, have been synthesized.

The pharmacological properties of synthesized cADPR analogs have been investigated in sea urchin eggs, T cells, or other cell systems. The structural diverse cADPR mimics show very different efficacy in regulating calcium signaling, but with the exception of some 8-subsituted cADPR analogs, most of those discovered so far are calcium signaling agonists [3,9]. Some structural modifications on adenine or pyrophosphate moiety of cADPR make the mimic more potent than its parent (e.g., 3-deaza-cADPR is 70-fold more potent than cADPR in see urchin egg homogenates [5] and cATPR is 28-fold more potent than cADPR in inducing calcium release in rat brain microsomes [6]). cArisDPR is the first nonhydrolyzable mimic of cADPR which retains a similar calcium release profile to that of cADPR [4]. 3′-OMe modification on southern ribose turns cADPR into an antagonist [10]. cADPcR, in which the northern ribose of cADPR is replaced by carbocyclic ribose, is a stable cADPR analog which causes a significant calcium release in sea urchin eggs but it acts much weaker in mammalian cells [11,12]. The cell specificity of analogs in regulating calcium release has been disclosed recently by Shuto and coworkers [9], which tells the difference of calcium regulation in different cell systems, and also implies that it is difficult to summarize distinct structure–activity relationships from the data obtained by different groups. For understanding the molecular mechanism of calcium signaling of cADPR/RyR pathway, more structurally varied analogs and systematic biological investigations are expected.



Scheme 1 Structures of cADPR and its analogs.

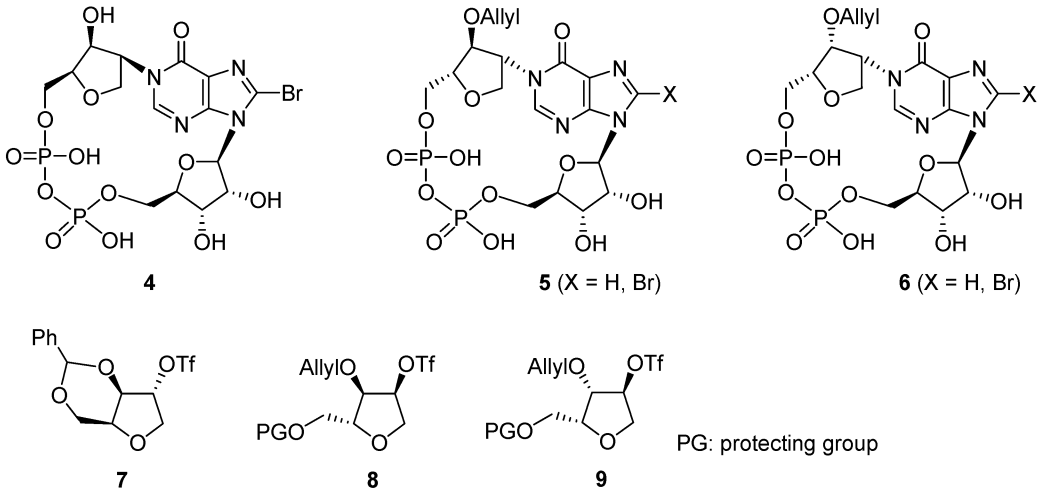
SYNTHESES AND AGONISTIC PROPERTIES OF cADPR ANALOGS

Chemical synthesis is the way to get cADPR mimics with high structural diversity, especially for modifying ribose. In the quest for new analogs to serve as valuable research tools in the elucidation of the mechanism of cADPR action, a series of adenine- or hypoxanthine-based cADPR analogs, with modifications mainly on ribose, has been synthesized by our group in recent years [13–17]. Ca^{2+} signaling behavior of these analogs was examined in living human T cells or other cell systems using fluorescent Ca^{2+} indicators, either in cell suspensions or on the single-cell level.

Modification of northern ribose with glycosyls

Isonucleoside refers to a kind of nucleoside in which the glycoside bond is transferred from native C1' to another position of ribose. Transfer of the C–N bond leads to enhancement of chemical and enzy-

matic stabilities of nucleoside [18]. As an approach to improving the stability of cADPR, various glycosyls with different configurations were introduced to modify the northern ribose of cIDPR **4–6** (Scheme 2), by changing the C1"-N1 glycoside bond to C2"-N1, with the aid of nucleophilic substitution of N1 of hypoxanthine to various triflated glycosyls **7–9** [14]. The formation of intramolecular cyclic pyrophosphate was achieved by using Matsuda's method. The activities of N1-glycosyl-substituted cIDPR analogs were evaluated by induced Ca^{2+} release in rat brain microsomes and HeLa cells. The results indicate that the configuration of the N1-glycosyl moiety in cIDPR is not a critical structural factor for retaining the activity of inducing Ca^{2+} release.



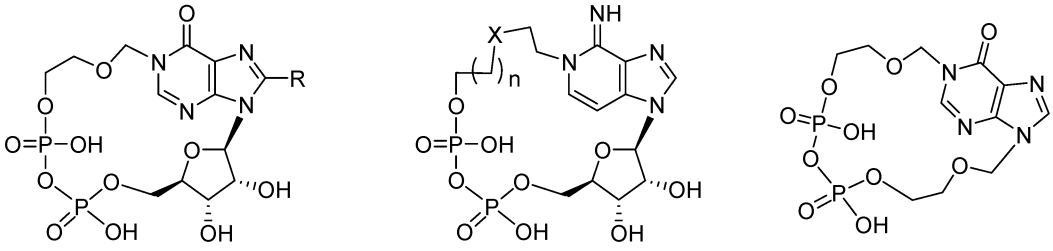
Scheme 2 Structures of some cIDPR analogs with various glycosyl modification on northern ribose **4–6** in which the glycoside bond are transferred from C1" to C2". C2"-N1 bonds are formed by nucleophilic substitution of N1 to triflated glycosyl intermediates **7–9** under the existence of K_2CO_3 and 18-crown-6.

Modification of northern ribose with ether or alkane linkages

More interestingly, when an ether linkage was introduced to replace the northern ribose moiety as in N1-ethoxymethyl-substituted cyclic inosine diphosphoribose **10** (cIDPRE) (Scheme 3), this cADPR mimic exhibited strong activity by inducing Ca^{2+} release in both rat brain microsomes and HeLa cells. A more detailed investigation was performed in intact and permeabilized human Jurkat T-lymphocytes [15,19]. The results indicate that cIDPRE permeates the plasma membrane, releases Ca^{2+} from an intracellular Ca^{2+} store, and subsequently initiates Ca^{2+} entry. The development of global Ca^{2+} signals evoked by cIDPRE was observed by using time-resolved confocal Ca^{2+} imaging at the single-cell level. Enzymatic stability studies showed that cIDPRE antagonizes the hydrolysis of Jurkat T cells and CD38 [20]. In both type 3 RyR-knockdown cells and in control cells microinjected with the RyR antagonist Ruthenium Red, similarly decreased Ca^{2+} signals were observed upon extracellular addition of this membrane-permeant mimic or upon microinjection of cADPR [19]. The result indicates that cIDPRE regulates calcium signaling in a similar way to cADPR.

8-Substituted derivatives **10** of cIDPRE were synthesized started from 8-bromo inosine [15]. Pharmacological evaluation shows that the agonist activities of 8-N_3-cIDPRE and 8-NH_2-cIDPRE are similar to that of cIDPRE, but the halogenated derivatives 8-Br- and 8-Cl-cIDPRE do not significantly elevate calcium release. The results indicate that a hydrogen donor or acceptor will probably be favorable for the binding of cADPR and receptor.

cIDPR and cADPR display similar agonistic calcium signaling activities. If an ether or alkane linkage moiety is used to modify the northern ribose of cADPR, the analogs **11**, designated as



10 R = H (cIDPRE)
R = Cl, Br, N_3, NH_2

11 X = O, n = 1 (cADPRE)
X = CH_2, n = 0-2 (cADPRA)

12 (cIDPDE)

Scheme 3 Structures of cADPR analogs modified with ether or alkane linkages on northern or both northern and southern riboses.

N1-ethoxyethyl-substituted cyclic adenosine diphosphoribose (cADPRE) and N1-alkylenyl-substituted cyclic adenosine diphosphoribose (cADPRA), are obtained [16]. Since N1 substitution on the adenine is very difficult and is complicated by the competing formation of an N6-substituted adenosine as the main product, the synthetic strategy for cIDPRE differs from that for cIDPRE and makes use of an N1-substituted intermediate. Blackburn's method was used to construct the N1-substituted adenosine with the use of imidazole derivative as starting material [21]. The formation of N1 ether- or alkane-substituted adenosines could be achieved regiospecifically by condensation with different amino alcohols. Pharmacological investigation into intact human Jurkat T-lymphocytes reveals that these analogs permeate the plasma membrane and are mild (cADPRE) to weak agonists of the cADPR/RyR signaling system.

Modification of northern and southern riboses with ether linkages

The agonistic properties of cIDPRE and cADPRE indicate that the northern ribose of cADPR tolerates a range of modifications. An oxygen atom in the linkage results essentially in retention of activity. N1,N9-diethoxymethyl-substituted cyclic inosine diphosphoribose **12** (cIDPDE) is a minimal cADPR analog, in which the ether linkage moiety is used to replace both northern and southern riboses [17]. The synthesis of cIDPDE is similar to that of cIDPRE, although the yield of the analogous intramolecular pyrophosphate cyclization product is a little lower, as a consequence of the flexibility of the two ether strands. This analog releases Ca^{2+} from intracellular stores of permeabilized Jurkat T lymphocytes. By using time-resolved confocal Ca^{2+} imaging in single intact T lymphocyte, initial subcellular Ca^{2+} release events, global Ca^{2+} release, and subsequent global Ca^{2+} entry were observed. The response to cIDPDE by increased recruitment of localized Ca^{2+} signals and by global Ca^{2+} waves was observed in freshly prepared cardiac myocytes. The close correspondence of the conformations of cIDPDE and cADPR, as optimized by quantum mechanics calculations, indicates that cIDPRE and cADPR can be expected to interact similarly with the receptor.

CONCLUSIONS

In summary, a series of adenine- or hypoxanthine-based cADPR analogs with modifications mainly on ribose have been synthesized. Pharmacological investigations show that these analogs are membrane-permeant calcium signaling agonists. Their agonistic activities in intact human T cells decrease in the order: cIDPRE ≈ 8-N_3-cIDPRE ≈ 8-NH_2-cIDPRE > cIDPDE ≥ cADPRE > cADPRA (n = 0) > cADPRA (n = 1) > cADPRA (n = 2) > 8-Br-cIDPRE ≈ 8-Cl-cIDPRE. This series of analogs enlarges our knowledge of the structure–activity relationships of cADPR. The results imply that the ribose moi-

ety, especially the northern ribose of cADPR, tolerates structural modification by retaining agonistic activity. Even a replacement of the northern ribose that does not contain polar components is sufficient for retention of (weak) biological activity.

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