Pharmacology of Cyclic ADP-Ribose and NAADP

Synthesis and Properties of Analogs

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INTRODUCTION

Multiple Ca\(^{2+}\) stores are generally present in cells. Principal among them are the mitochondria and the endoplasmic reticulum. It is generally accepted that inositol trisphosphate mobilizes Ca\(^{2+}\) stores in the endoplasmic reticulum [1]. Also present in the organelle is another Ca\(^{2+}\) release channel, the ryanodine receptor. The discovery of two other Ca\(^{2+}\) signaling molecules, cyclic ADP-ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP) [2-4] further highlights the complexity of Ca\(^{2+}\) mobilization as a fundamental signaling mechanism [1].

Pharmacological tools are invaluable in elucidating the multiplicity of Ca\(^{2+}\) stores. In most cases, the use of specific agonists and/or antagonists provides the most straightforward approach for deciphering the intricacy of cellular mechanisms that cells use to distinguish and respond to the myriad of Ca\(^{2+}\) signaling stimuli (cf. the next chapter by Lee in this book). Indeed, pharmacological evidence is among the strongest in establishing the fact that the Ca\(^{2+}\) release mechanism activated by cADPR is independent of that mediated by inositol trisphosphate, but is related to that mediated by the ryanodine receptor [5-7]. A pharmacological approach has also been used to dissect complex Ca\(^{2+}\) signaling associated with multiple agonists in pancreatic acinar cells (see the chapter by Cancela in this book). Thus, it was found that cholecystokinin is shown to signal via the cADPR-pathway while acetylcholine signaling is dependent on inositol trisphosphate [8, 9].

The finding that ADP-ribosyl cyclase can cyclize NAD to produce cADPR [10] has since opened up the first and highly versatile way for synthesizing analogs of cADPR [11, 12]. Remarkably, the cyclase can also synthesize NAADP from NADP and nicotinic acid via a base-exchange
reaction [13], which has since been utilized to synthesize a series of analogs of NAADP as well [14, 15]. These analogs not only have provided insights into structure-activity relationships of cADPR and NAADP, but have also made available many useful research tools such as antagonists, hydrolysis-resistant agonists and fluorescent analogs for investigating the physiological functions of these two novel Ca\(^{2+}\) messengers. This chapter focuses on synthesis and the properties of the analogs that have been shown to have utility in dissecting the role of cADPR or NAADP in Ca\(^{2+}\) signaling.

SYNTHESIS OF cADPR ANALOGS

cADPR is a metabolite of NAD. The structure of cADPR (see Figure 1) is confirmed by X-ray crystallographic analysis [16]. This cyclic nucleotide is unique in nature and features a N-glycosidic linkage between N\(^1\) of adenine and the anomeric carbon of the ribosyl unit originally linked to nicotinamide in NAD.

The synthetic strategy used for most of the cADPR analogs has taken advantage of the broad substrate specificity of *Aplysia* ADP-ribosyl cyclase [10, 12, 17], the enzyme that converts NAD to cADPR. As outlined in Figure 2, the strategy relies on the synthesis of the corresponding NAD analog, followed by enzymatic conversion to the cADPR analog with ADP-ribosyl cyclase. NAD analogs have been successfully produced by one of two routes. The first route is chemical synthesis, which couples corresponding AMP analogs to β-NMN using either carbodiimide [12, 18] or diphenylphosphate [19-21]. Several AMP analogs have been produced by chemical phosphorylation of the corresponding adenosine analog [19-22]. The second route is enzymatic synthesis, which uses NAD pyrophosphorylase [11, 22] to convert the corresponding ATP analogs to the NAD analogs. Some of these ATP analogs can be enzymatically synthesized from the corresponding AMP analogs using the combined actions of adenylate kinase and creatine kinase [22]. The chemical routes of NAD synthesis are applicable to all analogs, while the enzymatic route is restricted to those analogs that can serve as substrates for either NAD-pyrophosphorylase or adenylate kinase/creatine kinase. For instance, 8-substituted AMP derivatives are not substrates for adenylate kinase, while NAD pyrophosphorylase will utilize 8-amino-NAD, but not 8-bromo- or 8-azido-NAD [11].

Recently, the total chemical synthesis of several cADPR analogs has been reported [23-28], which should allow a much wider range of analogs to be synthesized. The properties of some of the chemically synthesized analogs will be discussed below, but the details of the chemical synthesis are beyond the scope of this chapter.