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CYCLIC AMP-SPECIFIC PDEs: A PROMISING THERAPEUTIC TARGET FOR CNS REPAIR

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Abstract

Research to date has indicated that cAMPspecific PDEs, particularly the members of PDE4 family, play a crucial role in the pathogenesis of CNS injury and neurodegeneration by downregulating intracellular levels of cAMP in various cell types. Reduced cAMP signaling results in immune cell activation, inflammation, secondary tissue damage, scar formation and axon growth failure, ultimately leading to an exacerbation of injury, the prevention of endogenous repair and limited functional recovery. Although inhibition of cAMPspecific-PDE activity through the use of drugs like Rolipram has been shown to reverse these deficiencies and mediate neurorepair, an inability to develop selective agents and/or reduce dose-limiting side-effects associated with PDE4 inhibition has hampered their clinical translation. Recent work with more selective pharmacological inhibitors of cAMP-specific PDEs and molecular targeting approaches, along with improved understanding of the basic biology and role of PDEs in pathological processes may enable this promising therapeutic approach to advance clinically and have a similar impact on CNS injury and disease as PDE5 inhibitors have had on the treatment of sexual dysfunction.

Keywords

Phosphodiesterase • cyclic AMP • Rolipram • CNS repair • SCI • TBI

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1. Introduction

The second messenger cyclic adenosine monophosphate (cAMP), first discovered in 1957 by Sutherland and colleagues [1], is an important second messenger involved in intracellular signaling for a diverse range of physiological events from cell proliferation and survival to differentiation and plasticity. Cellular cAMP concentrations are determined by synthesis (adenylyl cyclases) and hydrolysis (cAMP-specific phosphodiesterases). PDEs are the only known negative regulators of cAMP, participating in the fine tuning of cyclic nucleotide mediated signaling by controlling the rate of degradation of cAMP and cyclic guanosine monophosphate (cGMP) [2]. The association of PDEs with specific anchoring proteins, A kinase anchoring proteins (AKAP) and protein kinase A (PKA), within distinct subcellular domains, allows compartmentalized cyclic nucleotide signaling [3-4] in response to specific extracellular stimuli. From 11 different PDE families that have been characterized to date, which vary in cyclic nucleotide specificity, affinity, regulatory control and subcellular compartmentalization, there are eight PDE gene families that are capable of hydrolyzing cAMP, some of which hydrolyze cAMP exclusively and others both cAMP and cGMP [2,5]. In the central nervous system (CNS), under normal physiological conditions, basal levels of cAMP degrading PDE4 have been shown within various regions of the brain and spinal cord, such as in the cerebral cortex, hippocampus, hypothalamus, striatum and the substantia nigra, both in neurons and glia; 6-8. For the other cAMP-specific PDEs; PDE7 and 8, high mRNA expression of both *PDE7A* and *PDE7B* are seen in the rat brain. *PDE7A* is found in the olfactory bulb and tubercle, the hippocampus, particularly the granule cells of the dentate, and several brainstem nuclei [9] while the highest amount of *PDE7B* mRNA expression is observed in the cerebellum, striatal complex and the dentate gyrus of the hippocampus [10-11]. The production of the PDE7s' respective proteins has not yet been reported. *PDE8B* mRNA has been shown to be present in the hippocampus and is elevated in patients with late-stage Alzheimer's disease [12]. However, as both PDE7 and PDE8 are cAMP-degrading PDEs, exhibiting 10- and 40-fold higher affinity than PDE4 respectively, it has led to speculation that these enzymes are largely involved in the regulation of basal (or low concentrations) versus stimulated levels of cAMP. Further studies are needed to examine basal protein levels of PDE7 and 8 isoforms in the CNS and to determine how they are altered after CNS injury and disease [13].

In the acute phase of CNS injury, subsequent to mechanical trauma, various degenerative processes including neuron and oligodendrocyte cell death, axon axotomy and demyelination as well as the formation of cellular reactivity at the injury site contribute to loss of neurological function and abortive endogenous repair [14]. Tissue levels of cAMP have been shown to be reduced in

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experimental animal models of both traumatic brain [15-16] and spinal cord injury (TBI or SCI; 16). These levels can be largely restored and the detrimental effects of injury partially reversed when the PDE4-specific inhibitor Rolipram is given. [15-16]. Members of the PDE4 family have been found to play a key role in initiating neuroinflammation [15-17], accelerating secondary tissue damage [18] and restricting the intrinsic ability of injured neurons to regenerate [16,19] and/or be remyelinated [16]. The involvement of cAMP-PDEs in these deleterious events makes them a promising therapeutic target for neurorepair and restoration of function. The current review describes the regulation of cAMP-specific PDEs following traumatic injury to the CNS and surveys the use of cAMP-PDE specific inhibitors as a therapeutic approach to prevent tissue damage, promote neuroregeneration and enhance functional outcome.

1.1. Regulation of cAMP-specific PDEs in the CNS following injury

Since cAMP-specific PDE activity in CNS tissue was first identified several decades ago, high isozyme expression levels for PDEs 1, 2, 4, and 10 have been reported in specific areas of the brain and the spinal cord following injury [7]. The activity of these PDE's may be responsible for lowering intracellular levels of cyclic nucleotides, stimulating in turn the migration and activation of various immune cell populations; neutrophils, macrophages, and microglia [20-23], impairing blood brain barrier function and/or reducing neuronal activity and suppressing survival programs important for preventing cell death [21].

Though neurons express multiple PDEs, members of the PDE4 family have been found to be the cAMP-specific PDE most abundantly expressed in CNS under pathological conditions, whether triggered by an injury or as a result of a neurodegenerative condition or aberrant neurophysiological functioning, such as depression, memory deficits and Alzheimer's or Parkinson's disease [16, 24-28]. Furthermore, therapeutic approaches using Rolipram have demonstrated that locomotor and/or cognitive deficits associated with CNS injury or neurodegenerative disorders can be ameliorated if PDE4 activity is antagonized [15-16, 18].

There are four genes that encode different PDE4 enzymes, of which PDE4A, B and D, but not C, are expressed within the CNS [7]. Although there are a number of PDE4 gene families and long and short isoforms, generated through alternative splicing, methods for antagonizing PDE4 activity have been non-specific as the majority of experimental studies have used the general PDE4 inhibitor, Rolipram, or pharmacological agents with a similar profile. Thus it has been difficult to identify PDE4 gene and isoform-specific roles in CNS pathophysiology from these investigations. Therefore, in the ensuing review of the therapeutic potential of PDE4 inhibition for CNS injury protection and repair, the exact PDE4 gene that is targeted is lacking, without the availability of more specific pharmacological agents or molecular approaches for PDE4 inhibition.

1.2. cAMP specific PDE inhibition for cytoprotection

Central to the cytoplasmic effects of cAMP–specific PDE inhibitors is the potent anti-inflammatory action of cAMP signaling. Rolipram and other PDE4 inhibitors have been demonstrated to block neutrophil and macrophage recruitment to the site of injury [29-30], to reduce mononuclear phagocyte activation [31-32] and to decrease production of proinflammatory cytokines, such as TNF-α, IL-1β and IL-6 [15-16] while enhancing expression of suppressor cytokines, like IL-10 [31]. These actions are desirable in the acutely injured CNS as immune cell activation and ensuing inflammation is a primary mediator of secondary tissue damage following the initial mechanical insult [21-22, 33]. Recent work by Beaumont and colleagues [34] has shown that there is an elevation of PDE4A, B and D in oligodendrocytes and PDE4B in microglia following SCI; inhibition of PDE4 with 2 weeks of continuous Rolipram administration reduces immune cell activation [31] and can in turn attenuate acute oligodendrocyte death following contusive SCI [16,34]. PDE4 inhibition can also increase the number of preserved neuronal cells within the penumbra of the injury after SCI [31]. Supporting evidence for the role of PDE4 in apoptotic signaling, an important mechanism of secondary injury post-SCI, also comes from studies that have examined cellular injury in vitro using primary neuronal cultures. The aberrant stimulation of cell cycle proteins, such as cyclin D1, and the activation of death-signaling caspase 3 after cellular injury, could be attenuated by treatment with specific PDE inhibitors such as vinpocetine (PDE1 inhibitor), trequinsin (PDE3 inhibitor), and rolipram (PDE4 inhibitor) when delivered in a concentration-dependent manner [35]. From these *in vivo* and *in vitro* investigations it is evident that targeting PDEs produces potent anti-inflammatory and neuroprotective effects within the injured CNS.

1.3. PDE4 inhibition for neurogeneration

Previous work with Rolipram from our group and others [15-16] provides strong evidence for a negative role of PDE4 isozymes in restricting endogenous neuroregeneration following CNS injury. The reversal of injury-induced reductions in cAMP levels in the brain and spinal cord after SCI with the combinatory administration of Rolipram and a cAMP analog can promote significant supraspinal axon growth across the injured spinal cord [16]. Studies have demonstrated that an elevation of cAMP in vitro allows axons to grow over inhibitory substrates [36] and is important for axon guidance and turning behaviors [37]; conversely, decreases in cAMP levels are thought to occur during development, reducing intrinsic neuronal growth capacity and restricting plasticity in the adult organism. Gao and colleagues [38] have implicated PDEs in axon growth arrest in response to inhibitory substrates by showing that neurotrophins, which can prime neurons and act similarly to cAMP in allowing them to extend axons on inhibitory myelin, reduce PDE activity by ERK-mediated phosphorylation, thus maintaining high intracellular cAMP and allowing them to grow. Although these studies have identified a role for PDE4 isoforms in axon growth failure, whether other cAMP-specific PDEs may be involved or how various inhibitory signals alter PDE activity is not known.

1.4. Limitations of currently available cAMP-specific PDE inhibitors for CNS injury repair

For the treatment of a pathological condition within the CNS it is important that the therapeutically applied compound targeting selective PDE members be readily able to cross the blood–brain barrier (BBB) following systemic delivery. The small molecule Rolipram is one such PDE inhibitor that can cross the BBB; being a well studied cAMP specific–PDE4 inhibitor, Rolipram was originally developed as an anti-depressant drug and later gained significant attention as a cognitive enhancer [25] and as an anti-inflammatory drug [39]. In recent years, Rolipram has shown efficacy in a number of CNS injury and neurodegenerative disorders, including SCI [16, 18, 26, 40], TBI [15] as well as Huntington's [41], Alzheimer's [42] and Parkinson's diseases [43], highlighting the importance of PDE4 in the CNS.

Non-selective PDE inhibitors, such as theophylline and papaverine, have been used therapeutically for over 70 years for a range of human diseases [39]. Only in the last decade has greater understanding of PDEs at the molecular level enabled the development of more selective PDE inhibitors. The successful translation, widespread use and clinical safety of the PDE5 inhibitor, Sidenafil, for erectile dysfunction have shown that PDE inhibitors possess a safety profile that is amenable to clinical use. This first PDE targeted therapy success story is now spurring significant investment into the further development of PDE inhibitors for a variety of human health conditions. In contrast to PDE5 inhibitors, although numerous PDE4 targeted drugs have been developed, their clinical use is still limited due to an unfavorable side effect profile, which includes adverse drug reactions such as nausea, emesis, gastric discomfort and arthritis [39, 44-46]. This may be due to the widespread tissue expression and functions of PDE4 and the inability of currently available PDE4 inhibitors to selectively block a single gene product, either A, B or D. One side effect of PDE4 inhibitors, nausea-emesis, has been correlated through gene knockout approaches to the antagonism of PDE4D [47]. Therefore, the design of PDE4 inhibitors with higher and more restricted specificity to PDE4A and/or B isoforms may reduce this adverse effect and thus exhibit a more tolerable dose to efficacy profile. For cAMP-specific PDE's clinical translation has solely focused on PDE4 to date, particularly in the area of respiratory disorders, such as chronic obstructive pulmonary disease (COPD) and asthma, rather than neurological injury or diseases. A large number of PDE4 inhibitors have been developed from various structural classes, though they have been plagued with dose-limiting side-effects. PDE4 inhibitors, including filaminast, lirimilast, piclamilast, tofimilast, AWD-12-281 (GSK 842470), CDP840, CI-1018, D-4418, IC485, L-826,141, SCH 351391 and V11294A have all been discontinued either due to lack of efficacy or a low therapeutic ratio due to adverse effects severely limiting their dose and potential effectiveness clinically [48]. Despite the large number of PDE4 inhibitors that have been manufactured to date, the best clinical progress to date has been achieved with the oral, non-isoform selective PDE4 inhibitors cilomast and roflumilast [49], which have been recently approved by the FDA for COPD and asthma.

1.5. Future clinical use of cAMP-specific PDE inhibitors

The development of more specific PDE4 inhibitors with favorable side-effect profiles at therapeutic doses is a major area of interest due to the problems associated with earlier generation drugs that have lacked PDE4 gene product specificity. Several PDE4 inhibitors which are either in their final stages of their clinical trials or have been approved by FDA and have been marketed, (Cilomilast; GlaxoSmithKline, Roflumilast; ALTANA Pharma) continues to exhibit dose limiting side effects of nausea, diarrhoea and headache limiting their clinical advancement. Recently, new PDE4 inhibitors with low emetogenic potential that are currently in development, such as oglemilast, (Glenmark Pharmaceuticals, 2005) or IPL512602 (Inflazyme pharmaceuticals, 2005), is expected to exhibit improved therapeutic dosing and tolerability as well as clinical efficacy. Development of non-emetic and sub-type selective PDE4 inhibitors and their effective mode or route of delivery or the use of mixed inhibitors for associated proteins that can target them to microcellular domains suggests an alternative approach to provide improved therapeutic dosing and efficacy is in progress. One way that more selective PDE4 targeting may be achieved could be through molecular approaches, such as targeted gene knockout [20, 50-53] Antisense [54], interference RNA (siRNA) [39] or dominant–negative mediated disruption of enzyme intracellular targeting [55] and currently these strategies are already beginning to show promising results at the bench thus raising hope for their effective clinical application in the future.

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