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REVIEW ARTICLE

A Mechanistic Rationale for PDE-4 Inhibitors to Treat Residual Cognitive Deficits in Acquired Brain Injury

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Abstract: Patients with acquired brain injury (ABI) suffer from cognitive deficits that interfere significantly with their daily lives. These deficits are long-lasting and no treatment options are available. A better understanding of the mechanistic basis for these cognitive deficits is needed to develop novel treatments. Intracellular cyclic adenosine monophosphate (cAMP) levels are decreased in ABI. Herein, we focus on augmentation of cAMP by PDE4 inhibitors and the potentially synergistic mechanisms in traumatic brain injury. A major acute pathophysiological event in ABI is the breakdown of the blood-brain-barrier (BBB). Intracellular cAMP pathways are involved in the subsequent emergence of edema, inflammation and hyperexcitability. We propose that PDE4 inhibitors such as roflumilast can improve cognition by modulation of the activity in the cAMP-Phosphokinase A-Ras-related C3 botulinum toxin substrate (RAC1) inflammation pathway. In addition, PDE4 inhibitors can also *directly* enhance network plasticity and attenuate degenerative processes and cognitive dysfunction by increasing activity of the canonical cAMP/phosphokinase-A/cAMP Responsive Element Binding protein (cAMP/PKA/CREB) plasticity pathway. Doublecourtin and microtubule-associated protein 2 are generated following activation of the cAMP/PKA/CREB pathway and are decreased or even absent after injury. Both proteins are involved in neuronal plasticity and may consist of viable markers to track these processes. It is concluded that PDE4 inhibitors may consist of a novel class of drugs for the treatment of residual symptoms in ABI attenuating the pathophysiological consequences of a BBB breakdown by their anti-inflammatory actions *via* the cAMP/PKA/RAC1 pathway and by increasing synaptic plasticity *via* the cAMP/PKA/CREB pathway. Roflumilast improves cognition in young and elderly humans and would be an excellent candidate for a proof of concept study in ABI patients.

Keywords: Blood brain barrier, cell adhesion molecules, cyclic adenosine monophosphate, cytokines, neuroinflammation, traumatic brain injury.

1. INTRODUCTION

Acquired brain injury (ABI) can lead to severe acute effects such as complete one-sided paralysis, dizziness, confusion, incomprehensive speech, difficulty to understand, dysphagia, and very severe headaches. These effects are observed after non-traumatic (nTBI; *e.g.*, stroke) as well as after traumatic (TBI; *e.g.*, accidents, blasts) brain injury (Fig. **1**). Currently, tissue plasminogen activator (tPA) is often the only pharmacological treatment in acute stroke [1]. Recovery from most symptoms after ABI often happens within the first 6 months, provided that correct rehabilitation is given to the patient. The rehabilitation is mainly focussing on recovery of motor functions and other functional adaptations. Despite intensive rehabilitation, most patients still suffer from pervasive cognitive impairments, *i.e.*, impaired attention and memory. These residual symptoms can strongly interfere with daily activities and have a significant impact on the lives of ABI patients. Although the number of patients is high (an estimated 3.6 million people per year in the USA and the EU each), there is no treatment available to improve cognitive performance or daily life activities.

A better understanding of the neuronal mechanisms underlying early and late stage ABI is needed to develop new treatments for the acute and persistent cognitive impairments. Various neuropathological processes have been described for different stages after ABI [2]. The different processes and stages are schematically depicted in Fig. **2**. The mechanisms leading to the acute effects and neuronal damage differ between nTBI and TBI, as described in various reviews [2-10]. The acute effects can be found within a minute and last up to *an hour*; whereas the late stage conse-

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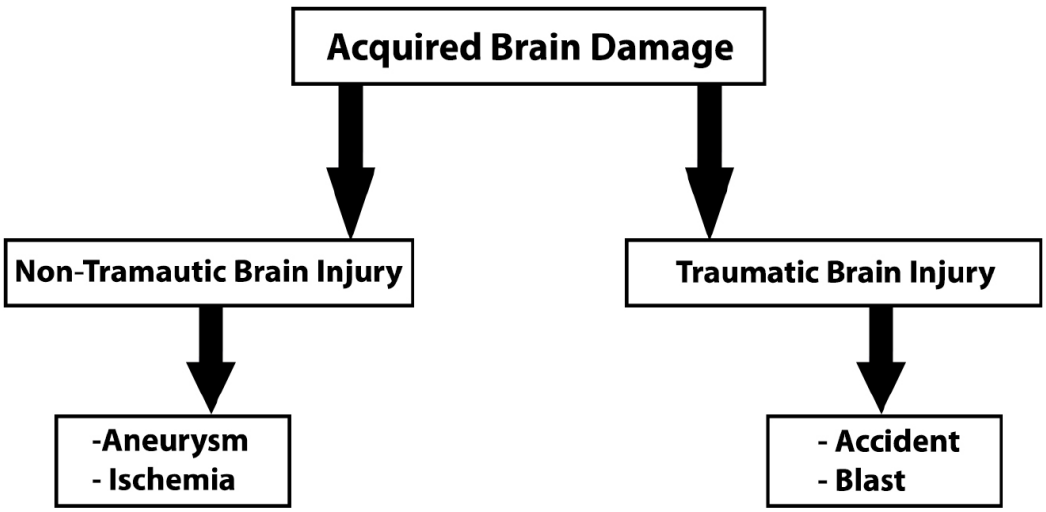

Fig. (1). Schematic overview of different forms of Acquired Brain Injury. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

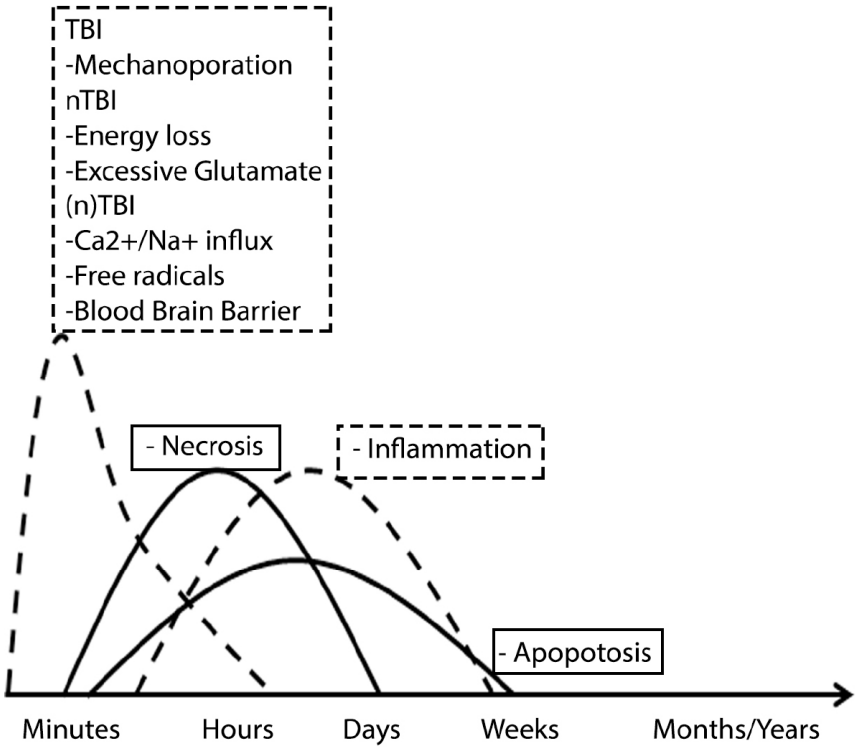

Fig. (2). Time dependent involvement of neuropathological mechanisms (dashed lines and boxes) leading to neuronal damage (solid lines and boxes). The height of the lines indicates the size of the effect on brain damage. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

quences of nTBI and TBI can be found up to *several weeks* after the impact. Compared with the acute effects, the pathophysiological mechanisms underlying late stage effects, such as neuroinflammation, are more comparable for nTBI and TBI.

As mentioned above, the immediate effects of TBI and nTBI differ, which is clearly related to the nature of the impact. In acute TBI, the local and distributed forces caused by a mechanical insult leads to mechanoporation. This is a transient membrane tearing leading to a loss of cellular homeostasis. The resulting influx of Ca^{2+} and Na^{+} ions activates cysteine proteases such as calpains and caspases. This in turn leads to impaired axonal transport and cytoskeletal (CSK) abnormalities [11]. CSK abnormalities contribute to distributed axonal injury which leads to the punctuate swollen axons (a hallmark pathology after TBI), as well as deficits in synaptic plasticity. In acute nTBI the major factor leading to cell death is the reduced availability of blood supply leading to a shortage of oxygen and compromised cell functions due to a loss of energy [2].

The acute effects in ABI are very dynamic and change rapidly during the first minutes and hours after the event. As the most damaging effects to the brain occur during these early phases, treatment should be given during this phase to prevent or minimize the further consequences of ABI. However, it is well known that this requires rapid recognition of brain trauma (especially in nTBI) and transportation to a hospital where treatment can be given immediately after diagnose. This is rather difficult and requires very efficient protocols and rapid actions. It will be very likely that not all ABI patients will receive this optimal treatment during the acute stage (of note, currently only t-PA is available). Therefore, a significant medical need exists for treatments that will counteract late stage neurodegeneration and/or enhance brain plasticity.

Acute effects can lead to subacute and ultimately chronic effects and the blood brain barrier (BBB) plays a role in these (Fig. **4**). Breakdown of the BBB is often found after TBI and nTBI and this can lead to edema, inflammation and hyperexcitability of neuronal tissue [12]. The increase in BBB permeability leads to instable intracellular junctional complexes, such as the CRAC1-AJ-actin skeleton [13]. This contributes to the leakage of inflammatory cells and humoral factors, which in turn further aggravates BBB dysfunction. Leukocytes release pro-inflammatory cytokines and activate local microglia which further contributes to BBB opening. Extended or excessive activation of inflammatory processes may lead to poor clinical outcomes by initiating processes such as cell death. The death of endothelial cells can further augment BBB permeability and lead to neuronal loss, which in turn can lead to functional deficits and impairment of cognitive recovery following ABI. Termination of the apoptotic sequence following ABI and BBB repair can take a long time and might result in chronic degenerative processes. Some ABI patients show long-term BBB disruptions that can last for months to years. It is likely that these, amongst others, are linked to chronic neuroinflammation causing neurodegenerative process [12].

The brain is able to counteract various processes leading to brain damage *via* different endogenous mechanisms. For example, GABA release is enhanced in order to reduce neuronal activity, free radical scavengers come into effect, and nitric oxide synthase activity is reduced [2]. These acute and intermediate protecting effects can however not prevent cell death. After several weeks, the neurochemical and inflammatory processes in the brain normalize, but there is remaining damage. After the brain has recovered from ABI, some mechanisms-such as, angiogenesis, neurogenesis, and synaptogenesis-can lead to the recovery of functions [2]. Treatments that can facilitate these mechanisms could lead to improved function in ABI patients.

In this review, we will discuss the critical role of the cAMP signalling pathways in ABI. Our hypothesis is that these pathways are of particularly interest as they act at multiple points in the pathophysiological processes of ABI. Also, these pathways are involved in processes that could restore brain function at a later stage after ABI. As will be explained in more detail later, phosphodiesterases (PDEs) are well known for regulating intracellular cAMP and cGMP levels. Therefore, PDEs can be considered as interesting targets to modulate the cAMP levels for treating different pathophysiological stages in ABI. In particular, we will focus on the role of the PDE4 isoform since it is selective for cAMP and appears to play an important role in nTBI [14] and TBI [15]. We will first provide an overview of the cAMP signalling pathway and its role in various pathophysiological processes underlying ABI. Subsequently, we will review data showing that PDE4 plays important roles at various stages of ABI which supports our notion that PDE4 can be considered as a relevant target for treating this condition.

2. THE cAMP PATHWAY

In this review, cAMP will be highlighted as a crucial factor for ABI and next an overview of its main signalling pathways and the corresponding intracellular effects will be described. First, it should be noted that cAMP is an intracellular second messenger and can be found in various tissues and cells of the body. In neurons, cAMP plays an important role in two major functions: rapid synaptic transmission and intracellular signaling mediating other processes of synaptic transmission [16]. This latter function is related to metabolism, neurotransmitter synthesis, storage and release, cytoskeletal organization, and neuronal growth and differentiation. This clearly shows that cAMP has an essential role in different neuronal functions. Of note, cAMP also plays an important role in glia cells (microglia and astrocytes) [17-18]. The signaling pathways in these cells are different from those in neurons [19] and offer a mechanistic rationale for potential anti-inflammatory effects that could contribute to efficacy in nTBI (section 4) and act synergistically with neuronal mechanisms.

In neurons, cAMP is mainly activated by adenylyl cyclase that in turn is activated upon stimulation of G protein-coupled receptors (GPCRs) or after influx of Ca^{2+} ions *via* ionotropic receptors. A slower activation of cAMP can be initiated *via* Ca^{2+}/calmodulin-sensitive forms of adenylyl cyclase. For the cAMP pathway, the G_s alpha subunit of GPCRs activates adenylyl cyclase. There are other molecules that can activate cAMP, such as, cholera toxin and forskolin (indirectly *via* activation of adenylyl cyclase). cAMP can also be deactivated, most prominently by PDEs or indirectly *via* activation of the G_i alpha subunit of GPCRs.

Three different cAMP molecular pathways can be distinguished [20]. First, cAMP activates protein kinase A (PKA) which has widespread intracellular effects, including activation of metabolic enzymes and phosphorylation of cAMP-element binding protein (CREB; sections 4.1, 4.2 and 5). The PKA pathway also has some other important functional effects that will be described in more detail below. A second protein that is activated by cAMP is the 'exchange protein directly activated by cAMP' (Epac; *e.g.*, section 3). The cAMP-Epac-Rit pathway can be considered as a PKA-independent pathway that is important for neuronal signaling acting *via* the ERK-pathway [21]. A third effect of cAMP is the activation of cyclic nucleotide-gated ion channels. Activation of these channels leads to depolarization of the membrane *via* an influx of Na^{2+} and Ca^{2+} ions [20].

3. ROLE OF cAMP IN BBB PERMEABILITY

The BBB consists of endothelial and glia cells. The endothelial cells have tight junctions that regulate the transcellular transport but also allows diffusion of some products. The BBB is considered the primary barrier of blood-borne products and circulating immune cells to enter the brain. As mentioned earlier, the breakdown of the BBB occurs fast after ABI.

During and after stroke it seems that the tight junctions stay intact but that the transcellular transport allows more products to enter the brain [22]. This is supported by an animal study showing that a breakdown of the BBB can be explained on the basis of an increase in endothelial vesicles [23]. A disruption of the tight junctions and increase in paracellular permeability has also been reported [24]. Another disturbance after stroke is less reflow of blood to the blood vessels and this leads to edema in brain tissue [25]. In TBI, blood vessels are also injured and animal models have shown that the tight junctions are disrupted and lead to paracellular permeability of molecules and immune cells [26].

The role of cAMP and PDE4 during the post-injury increase in permeability of endothelial cells has been well described [for a review see 13]. cAMP is one of the most potent signaling molecules to stabilize the endothelial barrier, both under resting conditions as well as under challenge of barrier-destabilizing mediators such as inflammatory cytokines. cAMP regulates endothelial permeability by two different signaling pathways. The first involves activation of cAMP dependent PKA and phosphorylation of PKA substrate proteins such as MLCK (Myocin Light-Chain Kinase), ERK1/2 (Extracellular signal-related kinases 1 and 2) and RhoA (Ras homolog gene family member A). The second pathway downstream of cAMP is PKA independent and mediated through its direct binding to exchange protein directly activated by cAMP (Epac), a guanine nucleotide exchange factor (GEF) for the small GTPase Rap1 (Ras-proximate-1). Importantly, the different components involved in these pathways form signaling complexes and the selective anchoring of these complexes to specific subcellular domains – compartmentalization - enables specific actions of a cAMP-mediated signaling event. For example, cAMP elevation in endothelial cells can have opposing effects and, depending on the site of cAMP formation, can either lead to barrier stabilization or breakdown [see also section 4; 13]. Finally, these molecular cAMP pathways affecting endothelial cell permeability have been characterised in *peripheral* cells. There will be differences with the molecular pathways involved in the permeability of endothelial cells in the BBB. The role of cAMP in peripheral endothelial cells may nonetheless translate to its role in BBB endothelial cells is suggested by the finding that elevation of intracellular cAMP induces barrier formation in cultured brain endothelial cells and that PDE4 inhibitors strengthen monolayer integrity [see 27]. It remains to be demonstrated that these beneficial effects of PDE4 inhibitors on the CSK and endothelial barrier will lead to improved BBB integrity in TBI. There are animal studies in experimental autoimmune encephalomyelitis (EAE) indicating that this may be the case [28]. Further, a protective effect of PDE4 inhibition on the BBB has also been shown in an animal model of ischemia [29].

4. NEUROINFLAMMATION IN ABI

As mentioned above, both TBI and nTBI lead to disturbances in the BBB which in turn leads to leakage of inflammatory cells into the brain parenchyma. This in turn leads to cellular stress and activation of apoptotic cascades. These secondary effects can lead to sub-acute effects and chronic effects [30]. Human and animal studies indicate that microglia are chronically activated for weeks to years after TBI [31] and after nTBI [32]. A clinical study using the PET ligand [^{11}C](R)PK11195 to assess chronic microglial activation in patients who sustained moderate to severe TBI months before, demonstrated significantly increased binding bilaterally at sites distant from areas of focal injury. This inflammation marker was correlated with cognitive dysfunction [33]. In an experimental stroke model this PET ligand showed an inflammatory response up to at least 56 days after the lesion [34]. In postmortem studies of TBI patients, chronic upregulation of reactive microglia in the white matter of the corpus callosum and the frontal lobe was found from months to many years after the trauma [35]. Similar white matter lesions have been observed 3 months after ischemia in stroke patients [36]. These effects on the white matter integrity have also been related to inflammatory effects [37]. Thus, in both TBI and stroke neuroinflammation has long-lasting effects on the brain and is related to functional outcome.

4.1. The Role of cAMP in Neuroinflammation and ABI

We will first look at the subacute post-injury effects and the role of cAMP and neuroinflammation pathways. Next, we will describe the role of the canonical neuronal cAMP-PKA-CREB pathway, focusing mostly on chronic post-injury cognitive deficits. This distinction between cAMP-PKA-CREB and chronic effects on the one hand, and neuroinflammation pathways and subacute effects on the other, is not absolute. As we will see, both pathways can contribute at multiple time points through feedback loops to post-injury pathophysiological effects. However, in general the former pathway has been more associated with a role in (longer-term) structural changes, and the latter pathway more with a role in (shorter-term) inflammatory mechanisms.

What are the effects of PDE4 inhibitors on cAMP-PKA pathways and inflammatory cytokines in TBI? In a rat parasagittal fluid-percussion injury (FPI) model for TBI, increased tumor necrosis factor-α (TNF-α) levels were decreased by the PDE4 inhibitor A33 at 3 months [38]. The compound did not alter microglia numbers and no measurable levels of TNF-α were detected in the cortex or hippocampus. In another study using the same model, levels of cAMP were depressed in the ipsilateral parietal cortex and hippocampus, as well as activation of its downstream target, protein kinase A [39]. Rolipram treatment restored cAMP, reduced cortical contusion volume, and improved neuronal cell survival in the parietal cortex and CA3 region of the hippocampus. Traumatic axonal injury was also reduced, as were levels of the pro-inflammatory cytokines, interleukin-

1β (IL-1β) and TNF-α. It was concluded that the cAMP–PKA signaling cascade is downregulated after TBI, and that treatment with a PDE4 inhibitor improves histopathological outcome and decreases inflammation after TBI [39]. Activation of the cAMP– PKA signaling pathway by rolipram is likely, though not the only, mechanism underlying the improved outcome after TBI with rolipram. Rolipram can work through four mechanisms on PDE4 [61]. First, inhibition of cAMP hydrolysis by binding to the cAMP catalytic site (the low affinity rolipram binding site). Second, binding at another region near the PDE 4 catalytic site (the high affinity rolipram binding site) where it does *not* affect cAMP hydrolysis. This mechanism may involve the MAPK pathway. Third, *via* the receptor for activated C kinase 1 and subsequent protein kinase C activation. Fourth, rolipram can inhibit PDE4 hydrolysis of cAMP and increase cAMP levels, but produce anti-inflammatory effects that occur independently of PKA, through Epac1.This is a cAMP-responsive guanine nucleotide exchange factor that activates the Ras family GTPases [39], which leads to stabilization of the endothelial barrier *via* Rac1-mediated enforcement of adherent junctions and strengthening of the cortical actin cytoskeleton [13, 40].

4.2. Anti-inflammatory Effects of PDE4 Inhibitors

PDE inhibitors can target different injury mechanisms throughout the time course of recovery after brain injury: predominantly inflammation and neuronal death at the earlier stages; and predominantly synaptic dysfunction and circuitry repair during the later stages [see section 5; 38, 41].

PDE4 isozymes are the predominant PDEs expressed in lymphocytes and monocytes. Elevation of intracellular cAMP downregulates a wide variety of immune cell functions, including TNF-α, interferon-α (IFN-α), interleukins (IL-2 and IL-12) production, IL-2 receptor (IL-2R) expression in lymphocytes, IgE production, oxidative burst in granulocytes, and nitric oxide (NO) production in macrophages [42]. Accordingly, PDE4 plays a major role in modulating the activity of virtually all cells involved in the inflammatory process. In primary glial cell cultures from C57Bl6 mice, lipopolysaccharide (LPS)-stimulated increases in the pro-inflammatory cytokines TNF–α, IL-1, and IL-6 levels were decreased by rolipram. In contrast, levels of the anti-inflammatory cytokine IL-10 were increased [43].

Several molecular pathways have been associated with the anti-inflammatory effects of PDE-4 inhibitors. cAMP-induced signals interfere with the pro-inflammatory transcription factor Nuclear Factor-κB (NF-κB) which plays a crucial role in switching on the gene expression of a plethora of inflammatory and immune mediators [44]. The PDE4 inhibitor, FCPR03, alleviated LPS-induced neuroinflammation by inhibition of NF-κB and also through activation of the cAMP/PKA/CREB pathway [45]. Also, in stroke PDE4 inhibitors have been found to attenuate the impact of the lesion in animal models. For example, in an ischemic stroke model in rats the PDE4 inhibitor FCPR16 reduced the level of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) [46]. Next to these effects on the inflammatory response, CREB phosphorylation was increased and infarct volume reduced. Neurological function was also improved as measured with a Zea-Longa rating scaling system that assesses motor function on a scale from 1 (failure to extent contralateral forepaw upon whole body lifting by the tail) to 4 (inability to walk spontaneously) [46].

In conclusion, there is good evidence for increased inflammation in patients and in animal disease models for TBI and nTBI. PDE4 inhibitors have a direct effect on pro-inflammatory (increase) and inflammatory (decrease) cytokines. In addition, PDE4 inhibitors have stimulating effects on the cAMP/CREB pathway that may underlie neuroplasticity and recovery of functions in these models.

5. VERSATILE FUNCTION OF CAMP AND PDE4 AND ROLE IN COGNITION

5.1. Role of the cAMP-PKA-CREB Pathway in Neuronal Plasticity

The role of the cAMP/PKA/CREB mediated signalling pathway in neural plasticity has been well described [47] and this pathway is an appealing target for treatments to enhance neuronal plasticity and improve cognitive deficits. Fig. **3** depicts the conventional role of the cAMP/PKA/CREB pathway in neural plasticity and cognition. First, activation of adenylate cyclase (AC) leads to the conversion of adenosine-tri-phosphate (ATP) to cAMP. This often occurs as a consequence of stimulation of neurotransmitter receptors that are positively coupled to AC [48]. Secondly, increased cAMP activation leads to increased activation of PKA, which then phosphorylates CREB to p-CREB. Thirdly, pCREB is a transcription factor that binds to the CREB Responsive Element (CRE) to regulate the expression of genes and synthesis of proteins involved in a multitude of biological functions, including formation of new spines/synapses and new receptors [49]. Other effects of pCREB are the activation of doublecortin (DCX) and microtubule-associated protein 2 (MAP2) which are biomarkers for neurogenesis [50] and neuronal outgrow [51], respectively.

There are multiple molecular targets in the cAMP/PKA/CREB pathway that can be modulated to affect activity of this pathway. One of these are the PDE enzymes that tightly regulate the intracellular cAMP (and also guanine monophosphate, cGMP) levels. Subtype selective PDE inhibitors that increase cyclic nucleotide signalling have been a successful class of medicines [52, 53]. PDEs consist of 11 protein families that are encoded by 20 genes [54]. A subset of PDE families hydrolyse cAMP to 5'-AMP and that leads to the termination of the cAMP-PKA signal. These are PDE1/2/3/4/7/8/10/11 whereby the PDE4/7/8 enzymes only have cAMP as a substrate. Rolipram and roflumilast are selective PDE4 inhibitors that can activate the cAMP-PKA pathway [55].

One active area of research is the improvement of cognitive function by PDE4 inhibitors [55]. The involvement of the cAMP/PKA/CREB pathway in neural plasticity makes PDE4 inhibition an interesting approach for the treatment of cognitive deficits. Understanding the spatio-temporal changes in PDE isoform expression after injury is important for targeting the relevant isoforms as well as for giving treatments within the appropriate therapeutic time window.

Interestingly, recent studies have shown positive effects of PDE4 inhibitors on cognition in humans. The PDE4 in-

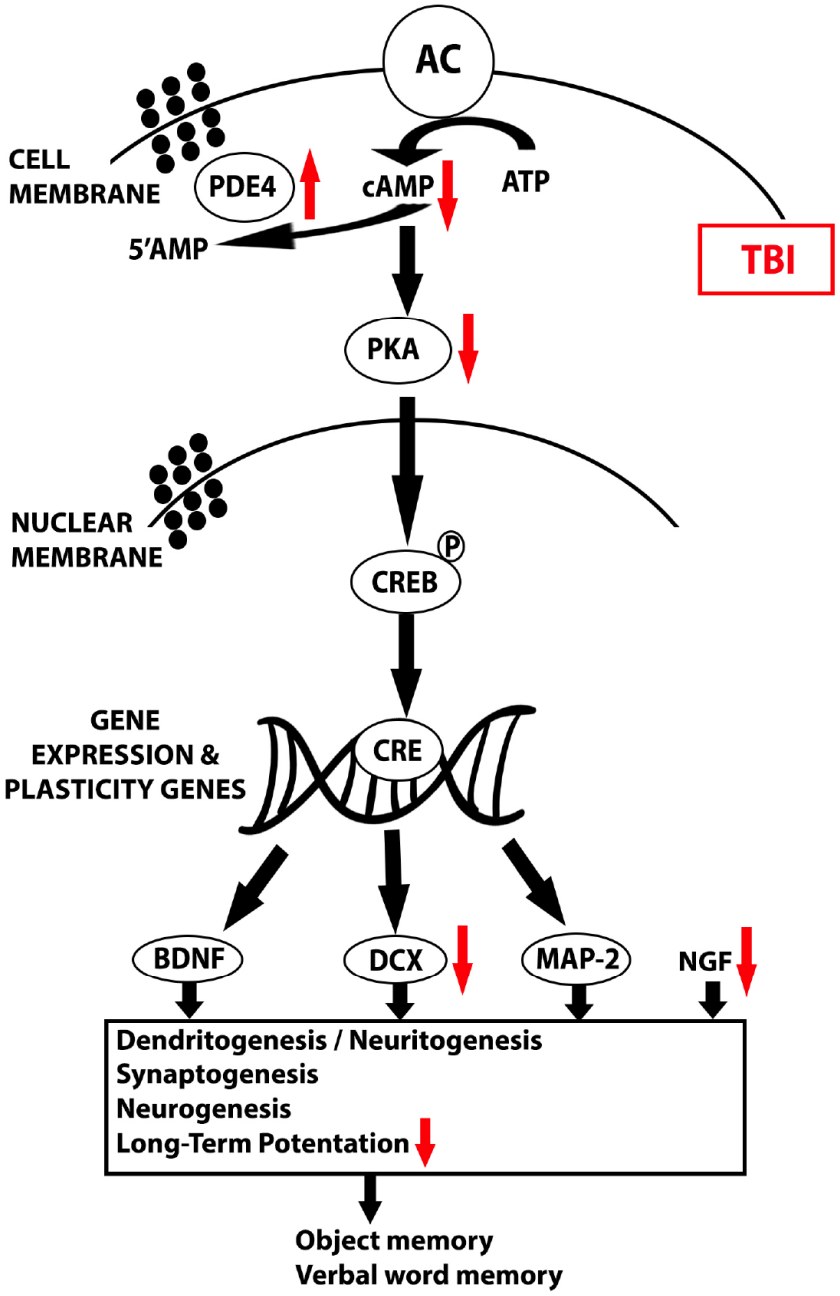

Fig. (3). Diagram describing the possibility for Roflumilast to increase DCX and MAP2 expression. PDE4 can hydrolyse cAMP to 5'-AMP. This cAMP depletion leads to the inactivation of the cAMP-PKA signalling pathway. Roflumilast can inhibit the action of PDE4 and therefore stop the depletion of cAMP which leads to the persistent activity of the cAMP-PKA pathway. Through PKA phosphorylation of CREB, pCREB can upregulate the expression of neurogenesis and neuroplasticity proteins DCX and MAP2. Red arrows depict the (predicted) effects for roflumilast. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

hibitor roflumilast, which is on the market for the treatment of chronic obstructive pulmonary disease (COPD), improved immediate recall of verbal word memory in a 30-word Verbal Learning Task in young healthy subjects [56]. No treatment effects were found on spatial memory performance or on response inhibition and focused attention in a Stroop task. Roflumilast also improved verbal word memory in old healthy subjects [57]. In schizophrenia patients who were treated with roflumilast, verbal memory was improved in a Hopkin verbal learning test-revised task [58]. Working memory was assessed in a spatial span task and not affected by roflumilast [58]. It should be noted that in these studies roflumilast treatment was not associated with adverse side effects at the memory-enhancing dose (100 µg). Although PDE4 appears to be a very interesting target, the development of PDE4 inhibitors, such as rolipram, was hampered by the emetic effects [59]. Roflumilast seems to have a very favourable therapeutic window which makes this drug an excellent tool to investigate the potential of PDE4 inhibition in humans.

5.2. Changes in the cAMP-PKA-CREB Pathway and PDE4 in ABI

Several studies looked at PDE enzyme, cAMP, PKA and (p)CREB levels following brain injury. In a rat moderate parasagittal fluid-percussion brain injury (FPI) model, different PDE4 subtypes were affected in different brain areas [60]. Levels of PDE4B2 and PDE4D2 were upregulated in the ipsilateral cortex, whereas levels of PDE4D4 and PDE4D3 were upregulated in the hippocampus, but not in the cortex. PDE levels seem to be normal 7 days after injury, with the exception of PDE4D3. The increase in PDE4B2 and PDE4D2 correlated with a decrease in cAMP levels and PKA activation in the injured hippocampus in the rat FPI model [61, 62]. A simple mechanistic hypothesis for the decreased cAMP levels under pathophysiological conditions is an increase in PDE activity, as mentioned above. Long-term changes include reductions in pCREB levels at three months post-TBI in the FPI model as measured in hippocampal slices 12 weeks after injury [39]. Other chronic effects included synaptic dysfunction and circuitry remodeling and were associated with a decrease in pCREB [39]. Finally, in an ischemic model an increase in microvascular PDE4D expression was found after ischemia and was associated with increased CA1 damage [63].

5.3. Role of cAMP and PDE4 on Cytoskeletal Function: Effects on Cell Adhesion Molecules

In acute TBI, the local and distributed forces caused by a mechanical insult leads to mechanoporation. This leads to impaired axonal transport and CSK abnormalities [11] that contribute to distributed axonal injury which leads to the punctuate swollen axons and deficits in synaptic plasticity. Improvement of CSK function and reversal of the cascade of maladaptive molecular and (sub)cellular events is expected to ameliorate the deleterious effects of ABI. There is emerging evidence for a role of the cAMP system in CSK function that may have been underappreciated in the PDE4 field. These effects may act synergistically with other beneficial molecular and cellular effects as a consequence of increased cAMP function, such as the reduced inflammation and improved synaptic plasticity and BBB function. We will first look at the selected cell adhesion molecules (CAMs). These play an essential role in brain injury and CSK function, and the role of cAMP in the control of CAM levels and function has been studied.

CAMs span the synaptic cleft and create an interaction and signaling network between the pre- and post-synaptic membranes of neighboring neurons. Following adhesion and signaling, cytoskeletal changes underlie the differentiation of local plasma membrane surfaces into synaptic specializations. In a variety of brain injury models, brain levels of different classes of CAMs are altered [64]. For example, in a model where PDE4 inhibitors were active (Table **1**), the fluid-percussion model, hippocampal levels of neural CAM were increased at 2 and 14 days post-injury.

Effects on CAMs in neurons have been studied in the classical Aplysia gill-withdrawal reflex model [47]. In long-term sensitization, repeated stimulation (for example with pulses of serotonin) causes the levels of cAMP to rise and persist for several minutes. The catalytic subunits can then translocate to the nucleus, and recruit the mitogen-activated protein kinase (MAPK). In the nucleus, PKA and MAPK phosphorylate and activate the CREB transcription factor. Long-term sensitization in this model involves structural changes at the sensory–motor neuron synapse with pulses of 5-HT resulting in an increase in the number of varicosities. Sensory neurons in response to repeated applications of 5-HT show decreased down-regulation of proteins corresponding to apCAMs, Aplysia members of the immunoglobulin class of cell adhesion molecules that includes mammalian NCAM [65]. These effects on apCAM are thought to contribute to the long-term sensitization in Aplysia. That is, *down regulation* of apCAM on the surface of the sensory neuron leads to *defasciculation*. This defasciculation may act in a permissive way, now allowing neurites of the sensory neuron to interact with the motor neuron, leading to the formation of new synaptic connections [65]. It is not clear if PDE4 inhibitors were tested in the Aplysia model. Actually, most studies with PDE4 inhibitors on the effects of CAMs have been performed in peripheral cells, such as leukocytes and endothelial cells, where they inhibit expression of a variety of CAMs [66]. Therefore, we hypothesize that PDE4 inhibitors may likewise downregulate apCAM during long-term sensitization and, possibly, the corresponding CAMs in the CNS of other species during plasticity changes in similar stimulation paradigms.

Integrins are another class of CAMs and one study looked at the effects of PDE4 on integrins and the CSK. Integrin engagement generates cellular signals leading to the recruitment of structural and signaling molecules that, together with rearrangements of the actin cytoskeleton, leads to the formation of focal adhesion complexes. Activation of integrin-mediated signaling pathways – such as the Rho pathway - is a critical step in the formation and regulation of synapse morphology and maturation [11]. The Rho family of small GTPases comprises the principal effectors of cellular actin remodeling, with RhoA, Rac1 (cAMP-Phosphokinase A-Ras-related C3 botulium toxin substrate) and Cdc42 (cell division control protein 42 homolog) inducing distinct and well characterized actin structures [see also section 3; 67]. RhoA activation influences synaptic plasticity [68] potentially by affecting the structural stability of dendritic spines through CSK remodeling. Changes in the shape and size of dendritic spines are correlated with the strength of excitatory synaptic connections and heavily depend on remodeling of its underlying actin cytoskeleton. As a consequence, specific mechanisms of actin regulation, and therefore the Rho family of GTPases, are integral to the formation, maturation, and plasticity of dendritic spines and to learning and memory. Inhibition of ROCK (Rho-associated protein kinase), a downstream effector of Rho-GTPases, reduced the incidence of axonal injury, suggesting that these pathways may be activated in neurons following an acute mechanical stimulus [11]. Injury-induced, integrin-mediated activation of Rho potentiates focal swelling and axonal retraction and downstream Rho signaling pathways may represent a therapeutic opportunity.

What then is precisely the role of cAMP and PDE4 in Rho signaling? One study looked if and how PDE activities

might contribute to the formation of integrin dependent adhesive structures in rat embryo fibroblasts [67]. The transient suppression of RhoA activity by the PDE4 inhibitor rolipram was thought to be a positive effect as it would relieve contractile forces and allow cytoskeletal re-arrangement required for integrin complex assembly. This PDE4 inhibition-RhoA suppression-CSK re-arrangement-rationale is reminiscent of the PDE4 inhibition-CAM inhibition- (predicted) defasciculation rationale [65] and raises the possibility that inhibition of CAMs and the subsequent CSK changes may be a more ubiquitous mechanism involved in restoring and improving cell function. It was concluded from the integrin study that control of cAMP degradation *via* the PDE4 family is required to regulate cellular responses downstream of integrin engagement, including peripheral actin filament assembly and cell migration [67]. It will be interesting to see if PDE4 also regulates downstream cellular responses of integrin in brain cells in a manner that will attenuate or reverse the CSK changes following brain injury.

5.4. Effects of PDE4 Inhibitors on Cognition in ABI Models (Table 1)

Following this marked dysregulation of the cAMP-PKA-CREB pathway and the reduced PDE4 levels following brain injury, the next question is whether these changes will be reversed by PDE4 inhibition and lead to improved cognition. Various studies have shown that this seems indeed to be the case. Rolipram reversed the decrease in cAMP and PKA levels in a rat FPI model [61]. The decrease in pCREB reported in the Atkins, *et al*. [39] study was reversed by the

Table 1. Effects of PDE4 inhibitors on cognitive deficits in models for ischemia.

Task (Memory Process)	Model (Species)	Treatment	Results	Refs.
Morris water maze (spatial, hippocampus dependent)	Microsphere embolism induced ischemia (rat)	Rolipram 3 mg/kg, ip for 10 days after embolism	Attenuation of the acquisition deficit measured at days 7-9	[78]
	Cerebral ischemia by 4-vessel occlusion (rat)	Rolipram 0.3 and 1 mg/kg, ip for 15 days. Start 6 h after onset surgery. Training & testing 30 min after drug.	Escape latency and exploration in target quadrant improved. Neuronal loss in hippocampal CA1 area decreased. Increased in hippocampal PDE4 attenuated	[69]
	Moderate fluid-percussion brain injury (rat)	Rolipram 0,03 mg/kg, ip 30 min before the acquisition and starting 2 weeks after injury	Escape latency and exploration in the target quadrant improved.	[41]
	parasagital fluid-percussion brain injury (rat)	A33 (PDE4B inhibitor) 0.3 mg/kg, ip 30 min before each training trial; 30 min before testing in working memory trial; start treatment 13 weeks after injury	Long-term spatial memory retention and spatial working memory improved.	[38]
	Moderate fluid-percussion brain injury (rat)	D159687 (allosteric PED4D inhibitor) 0.3 and 3 mg/kg ip 30 min before, 3 months after surgery	Escape latency and exploration in the target quadrant improved.	[79]
3-panel runway task (working memory; hippocampal and prefrontal cortex dependent)	Cerebral ischemia by 4-vessel occlusion (rat)	Rolipram 0.032 and 0.1 mg/kg, ip for 15 days. 30 min before 1st trial and immediately after reperfusion.	MED 0.1 mg/kg for decrease in errors	[70]
Passive avoidance (inhibitory avoidance learning, hippocampus and amygdala dependent)	Cerebral ischemia by 4-vessel occlusion (rat)	Rolipram 0.3 and 1 mg/kg, ip for 15 days. Start 6 h after onset surgery. Training & testing 30 min after drug.	Reversal decrease in 24-h latency	[69]
Cue and contextual fear conditioning (hippocampus and amygdala dependent)	Moderate fluid-percussion brain injury (rat)	Rolipram 0,03 mg/kg, ip 30 min before training and 2 weeks after injury	Fear conditioning retention improved. Rescue deficit CREB activity and LTP.	[41]
	Moderate fluid-percussion brain injury (rat)	A33 (PDE4B inhibitor) 0.3 mg/kg, ip 30 min before; treatment 12 wks after injury	Contextual fear conditioning retention and cue fear conditioning improved (24 hrs and 1 month). Rescue LTP deficit.	[38]
	Moderate fluid-percussion brain injury (rat)	D159687 (allosteric PED4D inhibitor) 0.3 and 3 mg/kg ip 30 min before, 3 months after surgery	Contextual fear conditioning retention and cue fear conditioning improved (24 hrs and 1 month). Rescue LTP deficit.	[79]

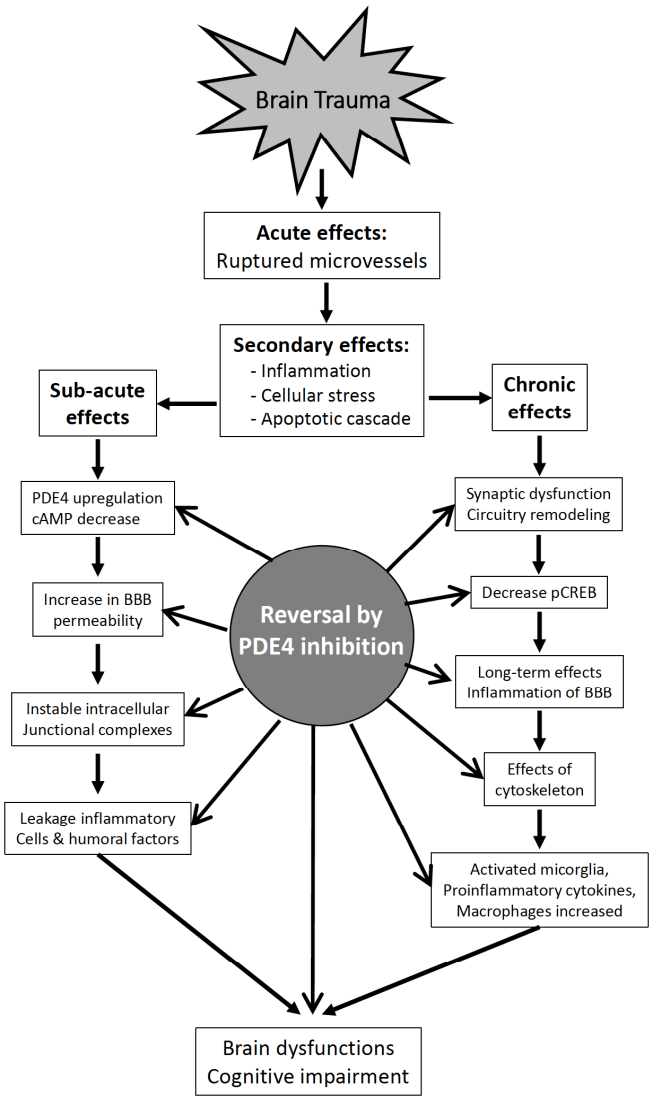

Fig. (4). A mechanistic basis for the treatment of residual symptoms in ABI by PDE4 inhibitors. Acute brain trauma often leads to ruptured microvessels and secondary effects such as inflammation, cellular stress and activation of apoptotic cascades. These can result in a myriad of subacute and chronic effects on molecular, cellular, subcellular and behavioral mechanisms, including: 1) effects on PDE4 enzymes, cAMP, pCREB, anti-inflammatory and pro-inflammatory cytokines; 2) leakage of inflammatory cells through the BBB; 3) effects on intracellular junctional complexes and the CSK, and 4) cognitive dysfunction, respectively. **PDE4, cAMP and pCREB**. In a FPI model, PDE4 enzymes were upregulated [60] and for certain subtypes this correlated with a decrease in cAMP levels and PKA activation in the hippocampus [61, 62]. In a 4-vessel occlusion model, this increase in PDE4 enzyme levels was attenuated by rolipram (Table **1**; [69]). Hippocampal pCREB levels were reduced in the FPI model at 12 weeks after injury and this was associated with synaptic dysfunction and circuitry remodeling [39]. The PDE4B inhibitor, A33, normalised pCREB levels at 13 weeks after TBI [38]. **BBB**. The role of cAMP and PDE4 during the post-injury increase in permeability of endothelial cells [22] has been well described [13]. Data with PDE4 inhibitors in (n)TBI models were not found, but studies were done with other models for brain injury. BBB022A decreased damage to the BBB in a transient middle cerebral artery occlusion (MCAo) model [29]. The sustained increase in BBB permeability and leakage of inflammatory cells and humoral factors aggravate BBB dysfunction [12]. There seems a lack of studies on the role of cAMP and PDE4 on these chronic neuroinflammation processes in ABI. **Intracellular junctional processes and cytoskeletal structure**. These subcellular mechanisms are critically involved in the pathogenesis of ABI [11]. But, again, the role of cAMP and, especially, PDE4, has not been well studied. In an EAE model, BBB022A increased cAMP levels and the electrical resistance of endothelial monolayers by stabilising intercellular junctional complexes [28]. In cell cultures of human brain microvascular endothelial cell monolayers, the increase in permeability by the bradykinin 2 agonist cereport was abolished by rolipram [76]. CAMs such as integrins play an essential role in brain injury and CSK function [64]. Most studies with PDE4 inhibitors were done in peripheral cells where they inhibit expression of a variety of CAMs [66]. The Rho pathway is one of the integrin-mediated signaling pathways. In an assay for the formation of integrin dependent adhesive structures in rat embryo fibroblasts [64], rolipram suppressed RhoA activity, possibly relieving contractile forces and allowing cytoskeletal rearrangement required for integrin complex assembly [67]. **Leakage inflammatory of cells and humoral factors** [28]. In ABI neuroinflammation has long-lasting effects on the brain [77] and is related to functional outcome [33]. PDE4 plays a major role in modulating the activity of virtually all cells involved in the inflammatory process [42]. In a FPI model, increased TNF-α levels were decreased by A33 at 3 months [38]. Rolipram reduced levels of IL-1β and TNF-α [39]. **Cognitive dysfunction**. Table **1** provides an overview of the effects of PDE4 inhibitors on cognitive deficits following (n)TBI. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

PDE4 inhibitor A33 at 3 months [38]. Cerebral ischemia-induced neuronal loss and increased hippocampal PDE4 levels were blocked by rolipram [69]. Now that we know that the cAMP/PKA/CREB pathway is dysregulated in TBI models and that PDE4 inhibitors can potentially normalise this pathway, we can look whether this actually also results in the recovery of functional effects, such as improved cytoskeletal function and cognition.

Most studies used rolipram and tested it a variety of rat cognition tasks and deficit models. In a Morris water maze spatial memory task, rolipram attenuated cognitive deficits by microsphere embolism ischemia, cerebral ischemia induced by 4-vessel occlusion, and moderate fluid percussion brain injury [FPI, 41, 69]. In a 3-panel runway working memory task, rolipram reduced the amount of errors in a 4-vessel occlusion model [70]. In a study using the same occlusion model, rolipram improved impaired inhibitory avoidance learning [69]. Impaired cue and contextual fear conditioning by moderate fluid percussion injury were also improved [41]. The PDE4B inhibitor, A33, improved long-term spatial memory retention and spatial working memory deficits by FPI in a Morris water maze and the impairment in cue and contextual fear conditioning by moderate FPI [38]. These preclinical data are encouraging but do not provide a definite proof of concept (PoC) for the treatment of long-term, residual, cognitive deficits following ischemic injury. It is unclear if the tasks and models used actually result in long-term cognitive deficits. In the rolipram studies, the compound was given before injury and/or shortly thereafter. So even if there were long-term deficits, it remains unclear if these would be affected by drug treatment. Although it is encouraging that A33 still improved cognition 13 weeks after FPI [38]. Even if PDE4 inhibitors improve long-term cognitive deficits in animal models, such a preclinical PoC may not readily translate to a clinical PoC as the predictive validity of cognition and brain injury is low. Therefore, these models may be more useful for establishment of PK-PD relationships and dose selection for human cognition testing. It seems prudent to get a quick clinical PoC instead of investing much effort in further animal model testing.

CONCLUSION

There is a high need for novel treatments against the long-lasting and debilitating residual cognitive deficits in patients with ABI. We argued that PDE4 inhibitors are a promising approach as these drugs can act at two key pathophysiological pathways that disrupt synaptic dysfunction and circuitry remodelling: modulation of the activity in the RAC1 inflammation pathway and increasing activity of the canonical cAMP/PKA/CREB plasticity pathway. Fig. **4** summarizes the mechanistic basis for the treatment of residual symptoms in ABI by PDE4 inhibitors. But – as always - there are some gaps in the preclinical mechanistic rationale that could and should be addressed by translational studies.

The mechanistic rationale is largely built on results with the prototypical PDE4 inhibitor rolipram. No two drugs are the same and some caution is warranted when extrapolating the findings with rolipram to other PDE4 inhibitors such as roflumilast. For example, it seems that rolipram may have more pronounced effects on the cAMP/PKA/CREB plasticity pathway than roflumilast. On the other hand, the latter has a more pronounced anti-inflammatory profile [71]. If these different profiles hold up in future studies, they may have consequences for the efficacy of these drugs in patients. Patient stratification by measurement of inflammation markers and markers for the activity of the cAMP/PKA/CREB pathway might be one way to match clinical pathophysiological profiles with preclinical pharmacological profiles.

Candidate markers for the activity of the cAMP/PKA/CREB pathway include DCX and MAP-2 since these are decreased or even absent after injury. Both proteins are involved in neuritogenesis and the generation of adult new born neurons. In children, levels of nerve growth factor (NGF) and DCX correlated highly with the recovery of TBI symptoms [72] , suggesting that impaired neuroplasticity and neurogenesis may consist of pathological entities for ABI. Subsequent upregulation of these processes could be a promising approach for the development of novel treatments for ABI. Due to its specific presence in the hippocampus, DCX is an excellent choice as a biomarker to study memory related neurogenesis. Degeneration of neurons, growth, plasticity, and differentiation of neurons are highly related to alteration in MAP2 expression [51]. Ischemia and hypoxia induced brain damage resulted in an under expression, or even disappearance of MAP2 in these regions [73]. Furthermore, MAP2 shows its importance in brain tissue damage and neurogenesis in a multitude of regions, not specific to the hippocampus. Upregulation of MAP2 and DCX could lead to a recovery of damaged brain tissue, as a result of (n)TBI. Together, these findings suggest that DCX and MAP2 may consist of candidate biomarkers for human PDE4 inhibitor studies.

In summary, there is substantial evidence that regulation of cAMP *via* PDE4 plays a central role in two processes after TBI and nTBI. Therefore, targeting PDE4 inhibition is considered as a viable approach to treat ABI patients, even after a prolonged time after the injury [15, 74, 75]. Different animal models have shown the effectiveness of PDE4 inhibitors. Therefore, it would be highly relevant testing the clinical potential of roflumilast, a PDE4 inhibitor that has shown cognition-enhancing effects in humans in healthy volunteers.

LIST OF ABBREVIATIONS

ABI	=	Acquired Brain Injury
AC	=	Adenylate Cyclase
apCAM	=	aplysia Cell Adhesion Molecule
ATP	=	Adenosine Tri Phosphate
BBB	=	Blood-Brain-Barrier
BDNF	=	Brain Derived Neurotrophic Factor
CAM	=	Cell Adhesion Molecule
cAMP	=	cyclic Adenosine Monophosphate
CCI	=	Controlled Cortical Impact
Cdc42	=	Cell division control protein 42 homolog

cGMP	=	cyclic Guanylate Mono Phosphate
COPD	=	Chronic Obstructive Pulmonary Disease
CRE	=	cAMP Responsive Element
CREB	=	cAMP Responsive Element Binding protein
CSK	=	Cystoskeletal
DCX	=	Doublecourtin
EAE	=	Experimental Autoimmune Encephalomyelitis
Epac	=	Exchange protein directly activated by cAMP
ERK1/2	=	Extracellular signal-Related Kinases 1/2
FPI	=	parasagittal Fluid-Percussion Injury
GEF	=	Guanine nucleotide Exchange Factor
GPCRs	=	G Protein-Coupled Receptors
IFN-a	=	Interferon-A
IgE	=	Immuglobuline-E
IL-1,1β,2,6,10,12	=	Interleukin-1, 1β, 2, 6, 10, 12
LPS	=	Lipopolysaccharide
MAP-2	=	Microtubule-Associated Protein-2
MCAo	=	Middle Cerebral Arteria occlusion
MHC-II	=	Major Histocompatability Complex-II
MLCK	=	Myocin Light-Chain Kinase
MRI	=	Magnetic Resonance Imaging
MS	=	Multiple Sclerosis
nCAM	=	neuronal Cell Adhesion Molecule
NeuN	=	Neuronal Nuclei
NF-κB	=	Nuclear Factor-κB
NGF	=	Nerve Growth Factor
NO	=	Nitric Oxide
nTBI	=	non-Traumatic Brain Injury
PDE4	=	Phosphodiesterase-4
PKA	=	Phosphokinase-A
PoC	=	Proof of Concept
RAC-1	=	cAMP-Phosphokinase A-Ras-related C3 botulinum toxin substrate
Rap1	=	Ras –proximate-1
RhoA	=	Ras homolog gene family member A
ROCK	=	Rho-associated protein kinase
TBI	=	Traumatic Brain Injury
TNF-a	=	Tumor Necrosis Factor-a
t-PA	=	tissue Plasminogen Activator
VLT	=	Verbal Learning Task

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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