Central plausible that racial differences in publications did not link data from 10,500 patients and 10,102 control subjects from 17 arguments: 1) the results of a recent meta-analysis including position. Such negative reports present three seemingly solid as a risk factor as does the most recent literature. However, publications from Asia and North America support interaction, others have not Table 1 [2-51]. Most of the clinical while the majority of publications have supported such an association with ischemic stroke. Originally, the Vulnerability to stroke. Originally, the gene was proposed as a factor in ischemic stroke, particularly carotid and cardiogenic stroke, the types most often associated with atherosclerosis [1]. Several studies have suggested: a correlation between cognitive dysfunction and carotid endarterectomy [2]; a link between the severity of atherosclerotic stenosis in stroke and pde4d SNPs [13]; an association between interleukin 1, alpha (IL1A) or PDE4D and stroke [36]; and an increase in PDE4D expression in peripheral blood mononuclear cells in acute ischemic stroke patients [31]. While these clinical studies do not establish a causative factor for PDE4D expression in the occurrence of stroke, understanding the role—if any—of PDE4D in the pathological processes associated with stroke will be important.

The generally accepted criteria for identifying genes involved with complex traits include four steps: 1) genome-wide evidence that demonstrates a statistically significant linkage or association of the gene with the trait; 2) fine-mapping to reduce the critical interval; 3) sequence analysis in the critical interval to identify candidate nucleotide variants; and 4) functional tests of candidate genes [53]. Statistically significant genome-wide evidence of a linkage of pde4d SNPs with stroke is insufficient by itself to resolve the dispute of whether pde4d is a risk factor for stroke. Thus, it would be helpful to directly address the fourth step in the process and conduct functional assessments of PDE4D in stroke. The present review attempts to connect the PDE4D signaling pathway to the pathophysiological cascade observed in ischemic

stroke, exploring how PDE4D may be linked to the occurrence of ischemic stroke and how clinical association studies may seem controversial given the presumed biological function of PDE4D.

**Genetic approaches to modeling stroke**

Ischemic stroke is defined as a complex disorder resulting from a combination of genetic and environmental factors [54]. Genetics may certainly influence the development of stroke with Mendelian traits [55,56]. On the other hand common, non-mendelian ischemic stroke is complex, polygenic, and multifactorial [57]. While the role of PDE4D in stroke remains largely unknown, a genetic approach can lead to “naturally” modeling cerebral ischemia. The best evidence of genetic influence would result from replacing a suspect causative variant nucleotide and determining if such a change led to an alteration from the “disease” phenotype to the “control” phenotype [58]. The use of transgenic “knock-in” technology [59] or a combination of gene targeting to produce deficiencies followed by transgenic complementation in rodents in vivo seems feasible. In species where in vivo functional assessments are not possible, other lines of experimentation may provide sufficient evidence to support gene discovery [60]. Such approaches might include in vitro complementation tests or reporter gene assays to assess gene expression.

Nevertheless, determining whether PDE4D is functionally relevant to stroke may best be demonstrated using in vivo approaches in experimental animals.

**Pathogenic role of PDE4D in stroke**

1. Hypothesis linking PDE4D to ischemic stroke. Ischemic stroke can be classified into three categories: 1) large vessel occlusive disease characterized by atherosclerosis; 2) small vessel occlusive disease characterized by nonatherosclerotic narrowing of small end-arteries in the brain; and 3) cardiogenic stroke mainly attributed to emboli derived from heart disease. Hypothetically, PDE4D may act inappropriately and lead to the occurrence of one or some combined form of these types of stroke.

2. Fundamental knowledge of PDE4D. In general, phosphodiesterases (PDEs) regulate the local concentration of 3',5'-cyclic adenosine monophosphate (cAMP) and 3',5'-cyclic guanosine monophosphate (cGMP) that serve as second messengers controlling many essential biological functions within cells. The human PDE superfamily includes 11 families that involve 21 genes [61-63]. pde4d encodes nine variants (pde4d1-9) having identical catalytic domains and carboxyl termini and unique amino termini essential to their subcellular localization [64,65]. It is well accepted that cAMP signaling responses are compartmentalized [66] and that subcellular localization of the PDEs is critical for their normal function. For example, in pde4d deficient mice, PDE4D3 deficiency in the ryanodine-receptor complex promotes heart failure and arrhythmias, even though global cAMP signaling is normal [67]. Thus, an alteration in the level or an inappropriate localization of PDE4D may lead to PDE4D malfunction.

<table>
<thead>
<tr>
<th>Publication Year</th>
<th>Approaches</th>
<th>Continents</th>
<th>Outcome</th>
<th>Negative</th>
<th>Positive</th>
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<td>Asia, North America</td>
<td>−</td>
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<td>Europe</td>
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</table>

Table 1: Assorted publications linking PDE4D to Stroke.
Since cAMP mediates multiple cellular biological functions via the cAMP-dependent pathway, PDE4D may regulate those functions by controlling cAMP levels and location. For example, PDE4 controls intermediary metabolism and cellular proliferation, motility or contractility/relaxation [68]. It is plausible that PDE4D dysfunction may lead to large vessel sclerosis and/or small vessel occlusive disease due to dysregulation of cAMP (changes in concentration and/or compartmentalization) which could lead to alterations in vascular regeneration/proliferation as well as contractility/relaxation. It is also possible that PDE4D dysfunction may alter the survival capacity of ischemic parenchyma after stroke onset, which may indirectly affect the incidence of stroke.

3. Evidence regarding PDE4D in stroke-pathogenic mechanisms. There is no direct evidence in the literature demonstrating a causative role for PDE4D dysfunction in the induction of clinical stroke. Experimentally, pde4d deficient mice were created in 1999 [69] and, as of yet, no spontaneous strokes have been reported in these animals. On the other hand, pde4d deficient mice display age-related cardiomyopathy characterized by heart dilatation in association with reductions in pumping capability and cardiac contractility [67]. Furthermore, myocardial infarcts in these animals have been shown to accelerate heart failure. Thus, in spite of the lack of direct evidence of increased stroke incidence in pde4d deficient mice, the heart disorders exhibited by these animals make a strong argument for the potential role of PDE4D in cardiogenic stroke. There are genetically altered rodent models that exhibit spontaneous stroke [55]; but the involvement of PDE4D in these models has yet to be assessed.

4. cAMP-PDE4D pathways and pathogenic mechanisms underlying cerebral ischemia.

Activation of many hormone and neurotransmitter receptors leads to the conversion of ATP to cAMP. cAMP in turn activates a variety of cAMP-dependent protein kinases which then elicit a wide array of biological processes via enzyme-mediated phosphorylation. PDEs also catalyze the hydrolysis of cAMP to adenosine-5'-monophosphate (5'-AMP), thus, contributing to the control of the dynamic changes in intracellular cAMP concentrations and the elicited biological processes as well. PDE4 is highly selective for cAMP and is the high affinity PDE present in most cell types. Thus, an increase in PDE4 activity results in decreases in cAMP while inhibition of PDE4 activity leads to increases in cAMP.

PDE4D and cerebral vasculature

The question arises as to whether PDE4D dysfunction occurs in the brain, thus possibly contributing to the occurrence of two subtypes of ischemic stroke, large and small vessel occlusions. The activities of PDE4D have been examined in vitro in cultured vascular smooth muscle and endothelial cells and in vivo in other major arteries, including the aorta and femoral artery of rats [70-72]. Those reports provide some general observations about the potential role of PDE4D in the vasculature: 1) inhibition of PDE4D results in increases in intracellular cAMP; 2) administration of agents that increase cAMP levels results in an increase in the activities of PDE3A, PDE3B and PDE4D; 3) PDE3 and PDE4 are the dominant cAMP PDEs in the aorta and femoral arteries (redundant PDEs that participate in the control of cellular cAMP will likely increase the difficulty of identifying the role of PDE4D in stroke); 4) local inhibition of transcription or translation attenuates the increases in PDE expression induced by compounds that increase levels of cAMP; and 5) PDE4 inhibitors do not exhibit a vasorelaxant effect [73]. Our recent study demonstrated that microvessels in the rodent cerebral cortex conventionally express pde4d [74], at both the transcriptional and translational levels, suggesting a role for PDE4D in the physiological control of these microvessels. Currently, there are no data on how chronically increased PDE4D activity might cause large or small brain vessel degeneration leading to stroke. On the other hand, PDE4D does appear to affect the acute pathological processes associated with cerebral ischemia: increased expression of microvascular PDE4D is associated with reduced tissue density and increased parenchymal albumin immunoreactivity in the perimicrovascular space indicating increased blood-brain-barrier (BBB) permeability, as well as neuronal death in the hippocampal CA1 region [75]. Notably, PDE4 inhibition improved BBB function and ameliorated ischemic brain damage [76,77].

PDE4D and neuronal survival following cerebral ischemia

Neurons, especially in the hippocampal CA1 region, express pde4d. Hypothetically, PDE4D activity may affect the sensitivity or vulnerability of the parenchyma in a cerebral ischemic lesion. Currently, there are no quantitative data available on PDE4D expression in parenchymal tissue of ischemic regions. Nevertheless, a possible link between PDE4D and cerebral ischemia might be inferred from the pathological effects attributed to nitric oxide (NO) generated by inducible nitric oxide synthase 2 (NOS2), an enzyme catalyzing the synthesis of the free radical NO. cAMP activates intracellular signaling by regulating protein kinase A, calcium influx, and Rap guanine nucleotide exchange factor 3 (RAPPGEF3). cAMP also inhibits cytokine-induced expression of NOS2 [78]. NOS2-derived NO is strongly involved in neuronal death, inflammatory processes, and the dysregulation of cerebral blood flow following cerebral ischemia. cAMP-elevating PDE inhibitors can influence NOS2 activation in different cell types in vitro and their potent anti-inflammatory effects in experimental disease models and clinical studies have been shown to be frequently accompanied by profound modulation of NO production [79]. In support of these observations, treatment with the PDE4 inhibitor, 1C486051, was shown to ameliorate traumatic spinal cord damage by inhibiting free radical generation and reducing NOS2 expression [80]. Another way in which PDE4D may be involved in ischemic neuronal death could stem from its interaction with the N-methyl-D-aspartate (NMDA) receptor-signaling pathway. NMDA-mediated excitatory neuronal death plays a pivotal role in the damage that occurs following cerebral ischemia. Stimulation of neuronal NMDA receptors activates both cAMP and cGMP signaling pathways and, as mentioned earlier, PDE4 critically downregulates cAMP [81]. A dual role for the NMDA receptor as a mediator of both excitotoxic cell death and activity-dependent cell survival has been proposed [82].
Cellular and molecular signaling events are influenced by both excitotoxic and homeostatic levels of NMDA receptor stimulation, indicating that activation of the cAMP response element-binding protein (CREB)/cAMP Response Element (CRE) is essential to that dual nature. Via phosphatidyl inositol 3-kinase and ERK signaling pathways, oxidative stress can activate PDE4D through multisite phosphorylation [83], indicating a potential role for PDE4D in the pathogenic mechanisms exhibited in ischemia reperfusion models. cAMP controls mTORC1 through regulation of PDE4D activity [84]. Disruption of some isoforms of Akt (a member of the AGC kinase family) is correlated with neuronal apoptosis with a tendency toward increased active caspase 3 and decreased phosphorylation of some elements of the mTORC1 pathway [85]. Nevertheless, the pathogenic mechanisms by which PDE4D is involved in NMDA-related pathways remain unclear.

CONCLUDING REMARKS

Importantly, PDE4D deficiency promotes heart failure and arrhythmias, thus, suggesting the importance of its compartmentalization [67]. Knocking out pde4d, thus, could hypothetically increase risk of cardiogenic stroke. On the other hand, enhanced PDE4D expression correlates with BBB dysfunction in association with neuronal death following the onset of cerebral ischemia [75]. Furthermore, PDE4 inhibition improves the outcome of cerebral ischemia [76,77]. Accordingly, PDE4D activity may be dose/location dependently relevant to ischemic pathogenic processes. Because clinical genomic studies have yet to demonstrate if and/or how PDE4D contributes to the risk of cerebral ischemia, other experimental approaches are critically needed. Fortunately, in vivo experiments with pde4d knock-out rodents (both mice and rats are commercially available) could directly address the issue of whether enhanced PDE4D expression postcerebral ischemia exacerbates stroke outcome. Such studies may help to determine if PDE4D is a risk factor for stroke. More importantly, they will provide opportunity for exploring new avenues in the development of stroke therapies.

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Author Contributions

L.C. and Z.H. designed and wrote the manuscript. S.A.F. and M.G.P. advised L.C. and Z.H. and revised the manuscript.

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REFERENCES


Suvanna NU, O’Donnell JM. Hydrolysis of N-methyl-D-aspartate receptor-stimulated cAMP and cGMP by PDE4 and PDE2 phosphodiesterases in primary neuronal cultures of rat cerebral cortex and hippocampus. J Pharmacol Exp Ther. 2002; 302: 249-256.


Bender AT, Beavo JA. Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. Pharmacol Rev. 2006; 58: 489-520.


