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Neuroprotective Strategies of Blood-Brain Barrier Penetrant “Forskolin” (AC/cAMP/PK_A/CREB Activator) to Ameliorate Mitochondrial Dysfunctioning in Neurotoxic Experimental Model of Autism

Sidharth Mehan, Himanshi Khera and Ramit Sharma

Additional information is available at the end of the chapter

Abstract

New developments in the study of brain are among the most exciting frontiers of contemporary neuroscientific research for the clinical practitioner. Increasing knowledge of neurocomplications and of their discrete localization in the various regions of brain permits new modes of pharmacological management of some major neurological disorders like autism. The research work reported in this scheme is undertaken with an objective to explore the potential molecular targets (AC/cAMP/PK_A/CREB) for the development of newer therapeutics strategies (forskolin) for the management of neurological disorders and associated symptoms. Studies aimed at addressing these questions have fallen into two main categories: in-vivo behavioral paradigms and in-vitro differentiation biochemical, morphological and histopathological analysis. Therefore, first time, we aim to gather the propensity of mitochondrial cofactors, neuropathological mechanisms and various diagnostic methods to explore the clinical therapeutic strategies to ameliorate the neurodevelopmental disorder autism.

Keywords: neurodegeneration, autism, mitochondrial dysfunction, adenylyl cyclase, forskolin

1. Introduction

Neurological disorders are a heterogeneous group of diseases of the nervous system having different etiologies. They represent illnesses of the selective regions of the brain and nervous

tissues which control vital physiological functions such as learning and memory, posture and coordination of movements of nerves/muscles [1]. A variety of CNS disorders including Alzheimer's disease (AD), Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS), autism spectrum disorders, brain abscess, multiple sclerosis, spinal cord injury, and cerebral stroke, traumatic brain injury are characterized primarily by neurodegeneration and neuroinflammation [2].

Intracellular molecules also known as secondary messengers such as cyclic nucleotides i.e. cAMP and cGMP play a critical role in neuronal signaling and synaptic plasticity by activation of several pathways like cAMP/PKA/CREB, cGMP/PKG/CREB and factors like brain-derived neurotrophic factor (BDNF) [3], semaphorins [4], netrin-1&16 [5], nerve growth factor (NGF) [6], Neurotrophins 3,4,5-inhibitory factors associated with myelin and myelin associated glycoprotein [MAG] [7]. These pathways and factors are well known to help in neuronal survival, neurogenesis and protect neurons from injury [8].

Elevation of cAMP causes both short- and long-term increase in synaptic strength [9] and stimulates cholinergic neuronal cells to release acetylcholine [10]. But, the levels of cAMP and cGMP are reported to be decreased in neuropathological conditions including cerebral stroke and AD [11].

It has been reported that cerebral ischemia-induced energy failure also leads to reduction in the levels of key signaling molecules such as cAMP and cGMP and results in disruption of cAMP/PKA/CREB [11] and cGMP/PKG/CREB signaling pathways [12]. On the other hand it had been reported to impair hippocampal long-term potentiation (LTP), a neurophysiological correlate of memory [13], by inhibiting the activation of both cAMP/PKA/CREB [14] as well as cGMP/PKG/CREB pathways in ICH pathology [15]. The pyramidal CA1 neurons of hippocampus, involved in learning and memory become vulnerable target in cerebral stroke [16]. Further, cAMP or cGMP dependent CREB phosphorylation has too been reported to induce long term memory (LTP) [17] and inhibit apoptotic and necrotic cell death [18].

CREB is a transcriptional factor responsible for synthesis of proteins which are important for the growth and development of synaptic connections and increase in synaptic strength [19]. Thus, agents that enhance cAMP/PKA/CREB & cGMP/PKG/CREB pathways have potential for the treatment of stroke [73], AD and other neurological diseases [20]. cAMP and cGMP mediate signaling of several neurotransmitters including serotonin, acetylcholine, glutamate and dopamine, which play important role in cognitive functioning [21]. The activation of the cAMP-dependent protein kinase [PKA] significantly inhibits TNF- α [22] and inducible nitric oxide synthase [iNOS] in astrocytes and macrophages [23] which are implicated in neuroinflammation [22] and oxidative stress, respectively [24]. cAMP system is closely involved in the regulation of BDNF expression too [25] which play important role in neuronal survival [3], synaptic plasticity [26], learning and memory [27]. Further elevation of cAMP and cGMP levels is known to restore the energy levels [28], reduce excitotoxic damage [29], prevent A β -mediated neurotoxicity [14], enhance biosynthesis and release of neurotransmitters [22], inhibit apoptotic and necrotic cell death [30] leading to improvement in cognitive functioning [31]. Central administration of cAMP and cGMP has been reported to

enhance neuronal survival [32] and memory performance [31]. In view of the above, the enhancement and prolongation of cAMP and cGMP signaling can thus be helpful in dealing with neurodegenerative disorders including ICH. This can be accomplished by activating the adenylyl cyclase enzyme, which metabolizes these cyclic nucleotides. Forskolin a major diterpenoid isolated from the roots of *Coleus forskohlii* directly activates the enzyme adenylyl cyclase, thereby increasing the intracellular level of cAMP and leading to various physiological effects.

Despite substantial research into neuroprotection, treatment options are still limited to supportive care and the management of complications. Currently available drugs provide symptomatic relief but do not stop progression of disease. Thus, the development of new therapeutic strategies remains an unmet medical need. Failure of current drug therapy may be due to their action at only one of the many neurotransmitters involved [33] or their inability to up regulate signaling messengers reported to have important role in neuronal excitability [34], neurotransmitter biosynthesis and release [35], neuronal growth and differentiation [30], synaptic plasticity and cognitive functioning [36].

2. Experimental animal model of PPA-induced neurotoxicity

Administration of PPA to rodents, results in CNS lesions that selectively target right lateral ventricle associated within striatum, cortex, cerebellum, hippocampus, amygdala recapitulating the regional and neuronal specificity of pathologic events especially in autism [37]. The mitochondrial toxin PPA interferes with the conversion of succinate to fumarate in TCA cycle, responsible for the generation of FADH₂ utilized in the complex-II in mitochondrial electron transport chain (ETC) by which it direct inhibits the activity of the mitochondrial metabolic enzyme succinate dehydrogenase and reduced the definite amount of NADH where it consumed in complex-I with the help of an enzyme complex-I (NADPH oxidase) as well as involve in the dysregulation of complex-IV (cytochrome c oxidase), is the final protein complex in the ETC helping to establish a transmembrane difference of proton electrochemical potential that the complex-V (ATP synthase) then uses to synthesize ATP [38]. PPA has now become an experimental tool to study neuronal susceptibility and motor phenotypes that are characteristic of autism (**Figure 1**) [39].

In rats, PPA-induced lesions in brain region that are associated with elevated lactate levels resulted in increased NMDA-receptor binding. PPA toxicity arises from secondary excitotoxic mechanisms, whereby energy depletion within vulnerable neurons facilitates abnormal activation of NMDA receptors and subsequent Ca²⁺ influx [40]. Stimulating energy generation by administering creatine markedly attenuates PPA toxicity and ameliorates lesion volume, lactate production and ATP depletion in PPA-treated rats [41]. Numerous reports assert that PPA toxicity is associated with increased oxidative damage within the CNS. The involvement of impairments in intrinsic anti-oxidant protection pathways after PPA administration is further supported by observations of reduced glutathione (GSH) levels in autistic brain [42].

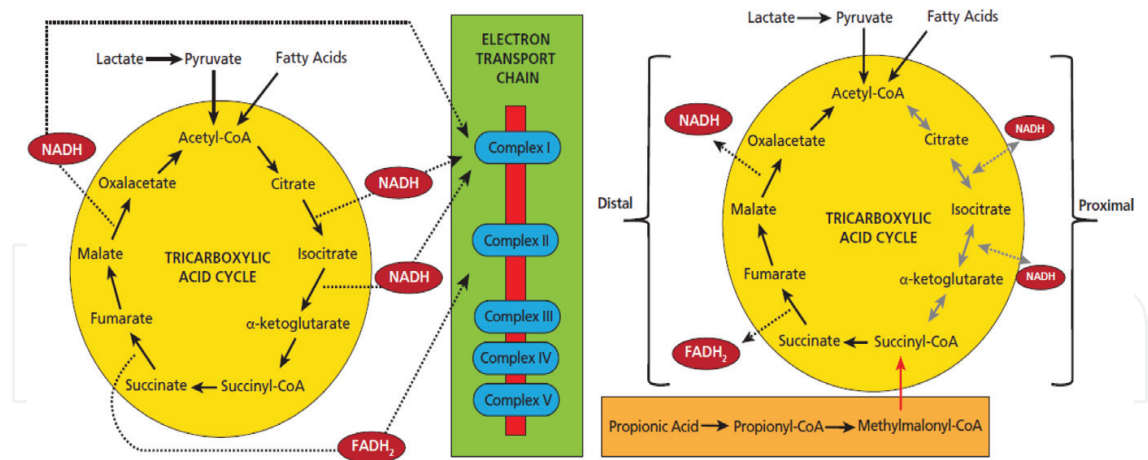


Figure 1. PPA-induced neurotoxicity; selective mitochondrial inhibition.

2.1. Propionic acid and autism

Propionic acid (PPA) is a short chain fatty acid formed endogenously in the human body as an intermediate of fatty acid metabolism and a metabolic end product of enteric gut micro biota such as clostridia and propionic bacteria [43–46]. MacFabe et al. and Shultz et al. have demonstrated that PPA intraventricularly infused to rats provides a suitable animal model to study autism. Being a weak organic acid, PPA exists in ionized and nonionized forms at physiological pH allowing it to readily cross lipid membranes, including the gut-blood and blood-brain barriers. PPA and other short-chain fatty acids (i.e., butyrate and acetate), affect diverse physiological processes such as cell signaling, neurotransmitter synthesis and release, mitochondrial

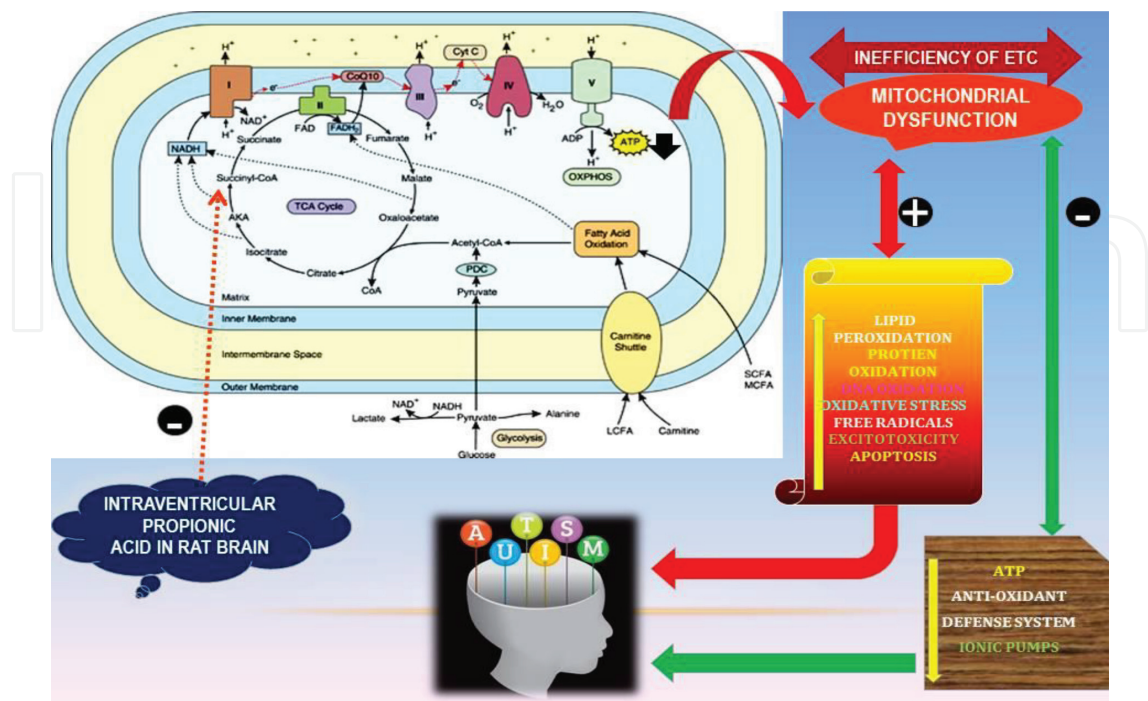


Figure 2. Intraventricular injection of PPA-inducing neurotoxic effect in mitochondrial respiratory chain (ETC).

function, lipid metabolism, immune functions, gap junctional gating, and modulation of gene expression through DNA methylation and histone acetylation [47]. Initial studies using this rodent model revealed that repeated brief infusions of PPA into the lateral cerebral ventricles (i.e., AP 1.3 mm, ML 1.8 mm, and DV 3.0 mm) of adult rats produced behavioral, biochemical, electrophysiological and neuropathological effects consistent with those seen in autism [43]. PPA through oxidative mechanisms inhibits Na⁺/K⁺ ATPase and increases glutamate receptor sensitivity which can enhance neural depolarization leading to neural hyper excitability in brain regions linked to locomotor activity (**Figure 2**).

Mitochondrial dysfunction has been well established to occur and play an important role in the pathogenesis of autism [48]. Preliminary magnetic resonance spectroscopy studies showed decreased synthesis of ATP and a disturbance of energy metabolism in the brain of individuals with autism. PPA is also capable of altering dopamine, serotonin, GABA and glutamate systems in a manner similar to that observed in autism [49].

3. List of proposed parameters can be evaluated on the basis of behavioral and biochemical alterations in neurotoxic experimental animal models of autism

Proposed experimental design of propionic acid-induced behavioral and biochemical estimations (**Figure 3**)

1. Measurement of body weight
2. Measurement of brain weight
3. Behavioral parameters

Spatial navigation task in Morris water maze, spontaneous locomotor activity, string test for grip strength, elevated plus maze test, beam crossing task, force swim test, rota rod apparatus

4. Estimation of biochemical parameters

Preparation of homogenate, estimation of biochemical parameters in serum and tissue homogenate such as protein estimation, lactate dehydrogenase (LDH) assay, estimation of malondialdehyde (MDA) levels, glutathione levels, superoxide dismutase (SOD) activity, catalase activity, acetyl cholinesterase (AChE) levels, determination of protein carbonyl (PC), nitrite levels

5. Estimation of biochemical parameters in serum and urine

Estimation of total urea, estimation of uric acid, estimation of biochemical parameters in tissue homogenate for mitochondrial complex activity

6. Preparation of crude mitochondrial fraction from rat whole brain homogenate

Complex-1 activity (NADPH dehydrogenase), complex-II activity (succinate dehydrogenase/SDH), complex IV activity (cytochrome oxidase), complex-V activity (ATP synthase)

7. Estimation of biochemical parameters in serum

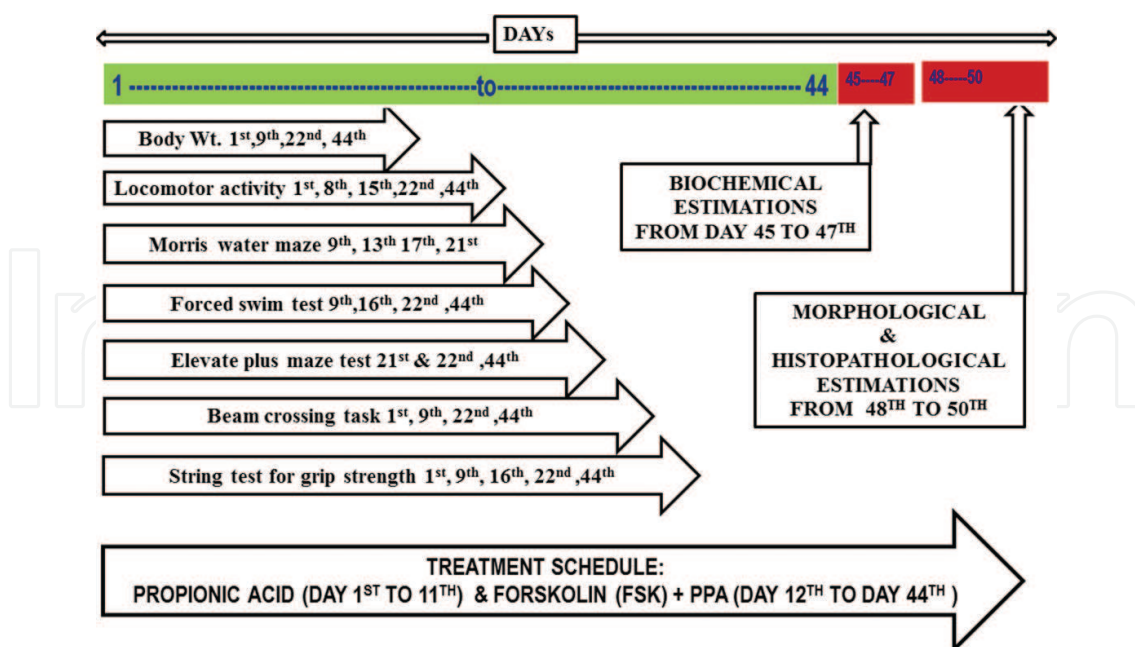


Figure 3. Proposed experimental design: propionic acid-induced behavioral and biochemical estimations.

Estimation of complete blood count (CBC) such as determination of different hematological parameters, such as red blood cells (RBC), white blood cells (WBCs), hemoglobin (HB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), neutrophils%, lymphocytes%, monocytes%, eosinophil's%, basophils%, mean platelet volume (MPV), platelet distribution width (PDW)%, plateletcrit (PCT)% and platelets (PLTs) was measured in rat serum or blood sample

8. Miscellaneous

Estimation of blood glucose levels, triglycerides levels, total cholesterol levels, serum C-reactive protein (CRP) levels

9. Inflammatory parameters in tissue homogenate-enzyme-linked immunosorbent assay (ELISA)

Estimation of $\text{TNF-}\alpha$, interleukin-1 β (IL-1 β), interleukin-6 (IL-6), interleukin-10 (IL-10)

10. Estimation of biochemical parameters in urine

Urine output, urine dipstick test

11. Histopathological and morphological sections studies

12. Immunohistochemistry

4. Future perspectives and treatment approach

Phytochemicals drugs have been used since ancient times as medicines for treatment of a range of diseases. Medicinal plants have played a key role in world health. In spite of the great

advances observed in modern medicine in recent decades, plants still make an important contribution to health care. Medicinal plants are distributed worldwide, but they are most abundant in tropical countries. Over the past decade, interest in drugs derived from higher plants, especially the phototherapeutic ones, has increased expressively. It is estimated that about 25% of all modern medicines are directly or indirectly derived from higher plants. Phytomedicines are standardized herbal preparation consisting of complex mixtures of one or more plants which are used in most countries for the management of various diseases. Other characteristics of phytochemicals are their wide therapeutic use and great acceptance by the population. In contrast to modern medicines, phytochemicals are frequently used to treat chronic diseases. Phytochemicals are normally marketed as standardized preparations in the form of liquid, solid, or various preparations. Compared with well-defined synthetic drugs, phytochemicals exhibit some marked differences, namely:

- The empirical use in folk medicine is a very important characteristic.
- They have a wide range of therapeutic use and are suitable for chronic treatments.
- The occurrence of undesirable side effects seems to be less frequent with herbal medicines, but well-controlled randomized clinical trials have revealed that they also exist.
- They usually cost less than synthetic drugs

5. Forskolin (*Coleus forskohlii*)

Coleus forskohlii known as phashana bedi (Telugu) a medicinal plant found in the Indian subcontinent is widely used in the Indian system of medicine. Forskolin (FSK) (also known as Colonels) is labdane diterpene that is obtained from the tuberous roots of *Coleus forskohlii*, which belongs to the family of Lamiaceae. *Coleus Forskohlii* is one of the world's most researched plant in which FSK is believed to be the plant's most active constituent. *C. forskohlii* has been used as an important folk medicine in India. *C. forskohlii* is a perennial herb and grows wild in arid and semi-arid regions of India, Nepal and Thailand; the roots have long been used in Ayurvedic medicine [50]. In traditional medicine, *C. forskohlii* is commonly used in different countries for various health disorders including cardiovascular diseases, hypertension, asthma, glaucoma and Alzheimer's disease. Its further use in promoting lean body mass, treating mood disorders and its anticancer activities is well known.

6. Medicinal properties of forskolin

Traditionally, the roots have been used as condiments in pickles, for preparation of pickles. Forskolin has positive effect against a wide range of conditions such as asthma, glaucoma, hypertension, hair loss, cancer, and obesity [51]. *C. forskohlii* extract (standardized to contain 95% forskolin) is potentially useful in skin care formulations, particularly as a conditioning agent. In traditional Indian systems of medicine, the roots of *C. forskohlii* are used as a tonic. Other therapeutically relevant properties include anthelmintic action and efficacy in the

management of skin infections and eruptions. The plant is also used traditionally in veterinary practice (**Table 1**). Essential oil in tubers of this plant has potential uses in food flavoring industry and can be used as an antimicrobial agent and has very attractive and delicate odor with spicy note. A labdane diterpenoid is considered the active secondary metabolite because of its ability to activate the enzyme adenylyl cyclase (Ac) thereby increasing the intracellular level of cAMP and leading to various physiological effects [52]. FSK is shown to exert a 6–400 fold increase in levels of cAMP. Cyclic AMP is a “second messenger” hormone signaling system as its synthesis triggers the action of various hormones, enzymes and other biological activities that have profound effects on local cells, as well as systemic effects, in some instances, on the entire body [53]. FSK by passes the adrenoreceptors, increasing cAMP levels directly, thereby stimulating lipolysis. FSK has also been shown to counteract the decreased response of fat cells to epinephrine, associated with aging. FSK also accelerates lipolysis through the activation of hormone-sensitive lipase [54]. It is primarily via the increased synthesis of cyclic AMP that *C. Forskohlii* may exert its medicinal influences on a significant number of common health conditions.

| S.No. | Pharmacological activity | Mechanism of action | Ref. No |
|-------|--|--|---------|
| i. | Anti-depressant | FSK stimulated AC activity in rat brain and leads to enhancement of the coupling between stimulatory GTP-binding protein (G protein) and AC catalytic molecules FSK stimulates AC and regulates brain-derived neurotrophic factor (BDNF) and TrkB expression in the rat brain | 92 |
| ii. | Anti-Alzheimer's | FSK-induced abipolar neuron-like cell morphology and it enables neurogenin-2 (Ngn2) to convert human fibroblasts into cholinergic neurons Neuronal differentiation of adult rat neural progenitor cells (NCP's) was achieved | 93 |
| iii. | Anti-cancer | Restoration of PP2A activity with forskolin that inhibit Akt and ERK activity and block proliferation and induce caspase-dependent apoptosis in AML cell lines. Forskolin inhibited the <i>in-vivo</i> leukemogenesis of imatinib sensitive and resistant BCR/ABL+ 32Dcl3 cells in mice | 94 |
| iv. | Antispasmodic activity | increase of cAMP inhibit cramping or smooth muscle contraction | 95 |
| v. | Anti-Glaucoma | Stimulates Adenylate cyclase which stimulates the ciliary epithelium to produce cyclic adenosine monophosphate (cAMP) that results in decreased aqueous humor inflow there by decrease in IOP Reduction of intra ocular pressure | 96 |
| vi. | Cardioprotective amelioration of Mitochondrial dysfunction in cardiomyopathy | It reduces diastolic blood pressure without increasing myocardial oxygen consumption. Reduction of I_{Na} (cardiac Na ⁺ current) and overproduction of mitochondrial ROS in deoxycorticosterone acetate (DOCA) mouse myocytes by activating PKA and PKC | 97 |
| vii. | Anti-asthmatic | Forskolin activation of cAMP inhibits human basophil and mast cell degranulation, resulting in subsequent bronchodilation | 98 |

| S.No. | Pharmacological activity | Mechanism of action | Ref. No |
|-------|--|---|---------|
| viii. | Anti-psoriasis | Decreased cGMP levels that are associated with cell proliferation and thus decrease cell division. Normalizing the cAMP /cGMP ratio | 99 |
| ix. | Hepatoprotective activity | Repair of hepatic tissue damage, normalization of inflammatory hepatic and necrosis Forskolin increases cAMP accumulation, and therefore stimulates lipolysis. | 100 |
| x. | Anti-inflammatory | Reduction in the level of Interleukin-1 β , 6 and 8 Overexpression of TANK-binding kinase 1 (TBK1) reduced phosphorylation of hormone-sensitive lipase (HSL) in response to FSK Inhibit mast cell degranulation | 101 |
| xi. | Anti-diabetic activity | FSK predominantly decreased basal glucose in healthy rats and attenuated the severity of hyperglycemia in diabetic rats FSK increase intracellular cAMP, which, together with the increase in ATP, enhance the priming of insulin granules | 102 |
| xii. | Anti-platelet aggregation | Antagonizes the action of platelet-activating factor (PAF). Reduction in the extent of platelet aggregation Induced a partial deaggregation of ADP- or collagen-aggregated human platelets | 103 |
| xiii. | Inhibition of human neutrophil degranulation Anti-histaminic activity | cAMP-mediated phosphodiesterase inhibition. Reduction in the histamine release from human basophiles and mast cells by modulating the release of mediators of the immediate hypersensitivity reaction, through activation of AC | 104 |
| xiv. | Smooth muscle relaxant | Increase both the cytosolic Ca ²⁺ concentration and the cytosolic NO concentration ([NO]c) in the endothelial cells leads to cause vasodilatation Increases uterine smooth muscle AC | 105 |
| xv. | Hydrodynamic alterations in collecting tubule Anti-cystic fibrosis | FSK resulted in increase in osmotic water flux and hydraulic conductivity of the rabbit cortical collecting tubule FSK leads to cyst formation in culture media | 106 |

Table 1. Pharmacological action of FSK.

7. FSK and brain

7.1. FSK-binding sites

3H-forskolin has, for example, been found to bind to both a high and a low affinity site in rat brain membranes [55] and the capacity of the high affinity forskolin-binding site has been shown to be increased by the activation of N-proteins by guanine nucleotides [56]. High affinity [3H] FSK-binding sites have been mapped autoradiographically in rat brain area such as caudate-putamen, nucleus accumbens, olfactory tubercle, globus pallidus, substantia nigra and the hilus of the area dentata [57] and exhibit a markedly heterogeneous distribution.

7.2. Role of FSK in brain

FSK may activate Ac by interacting with two sites, one which may be directly located on the cyclase molecule, and the other which is associated with OJ somehow formed by the interactions with the N₁ protein. FSK, a commonly used activator of Ac [55], elevates the stimulation-induced release of several transmitters, such as acetylcholine, noradrenaline and 5-hydroxytryptamine, from brain or synaptosomes and markedly increasing the rate of conversion of ATP to cyclic AMP [58]. FSK directly reduces certain K⁺ –potassium currents in addition to its action on Ac. cAMP could increase the apparent number of Na, K-ATPase sites by either direct or indirect mechanisms. cAMP could increase the number of Na, K-ATPase sites by increasing cell Na⁺ or decreasing K⁺ though there are reports of Na, K-ATPase stimulation that may be independent of cation changes. FSK elevates electrically evoked acetylcholine release in the hippocampus independently of Ac activation [58]. FSK appears to provide a new clue for elucidating the physiological role of cAMP in the synaptic transmission in the sympathetic ganglia. FSK exerts two opposite pharmacological actions at the synapse, i.e. a facilitation of transmitter release at the presynaptic site and a depressant action on nicotinic acetylcholine receptor at the postsynaptic site. FSK reduced the amplitude shock stimulation of preganglionic nerve. FSK induces a reversible AChR desensitization at the junctional and extrajunctional regions in rat [59]. FSK, an activator of Ac, could increase transmitter release presynaptically in CA1 neurons. FSK directly stimulates Ac and thereby increases cyclic AMP activity, which is known to influence neurite outgrowth and membrane trafficking in neurons. Increased cyclic AMP activity may have multiple effects on cells including changing the direction of growing neurites [60] and increasing the density of clathrin-coated pits and coated vesicles at plasma membranes coincident with an increased synthesis of clathrin light chain. The cAMP effector system enhanced by FSK is involved in the release of dopamine from dopaminergic nerve endings in the neostriatum [61]. FSK increased dopamine formation in rat striatal slices, rat striatal synaptosomes, rat hypothalamic synaptosomes and bovine retinal slices [62].

8. Neuroprotective action of FSK

8.1. FSK against neuroinflammation

An increase in intracellular cAMP levels through FSK to play an important role in modulating the cytokine production. Intracellular cAMP has been reported to depress the accumulation of tumor necrosis factor (TNF- α) mRNA by inhibiting the transcriptional processes. Elevation of intracellular cAMP levels induced by PDE inhibitors, FSK, prostaglandin E₂, or cell-permeable cAMP analogue also inhibited the secretion of IL-1 β , whereas it increased IL-1 β mRNA levels from lipopolysaccharide-stimulated human monocytes. Although the regulatory modality of IL-8 production by cAMP is still unclear and depends on the cell type, enhanced cAMP appears to have favorable effects at least on airway cells by suppressing IL-8 production [63]. Therefore, enhanced cAMP levels by have also FSK been recognized to reverse the increased pulmonary microvascular permeability associated with ischemia reperfusion (**Figure 4**) [64].

8.2. Forskolin against neurooxidation

Oxidative stress may play a role in the development and clinical manifestations of autism. Both central and peripheral markers of oxidative stress have been reported in autism. Peripheral markers have included lipid peroxidation levels. Increases in these markers correlated with loss of previously acquired language skills in autism. Furthermore, metabolic markers of oxidative stress have been identified including abnormal levels of metabolites signifying impaired methylation and increased oxidative stress in autism [65]. The oxidative stress in autism may be caused by an imbalance between the generation of ROS and the defense mechanism against ROS by antioxidants. An increase in reactive oxygen species (ROS) results in damage to proteins, DNA, and lipids. Specifically, the interaction between ROS and nitric oxide (NO) results in the nitration of tyrosine residues in proteins and can alter protein conformation and function [66]. Oxidative DNA damage is also considered to play an important role in the pathology of a number of diseases like Parkinson's disease, tardive dyskinesia, metal intoxication syndromes, Down's syndrome, and possibly also in schizophrenia, Huntington's disease, and Alzheimer's disease. Reactive oxygen species including superoxide (O_2^-), hydroxyl ($\cdot OH$), hydrogen peroxide (H_2O_2), singlet oxygen (1O_2) and nitric oxide ($NO\cdot$) can cause cellular injury when they are generated excessively or the enzymatic and nonenzymatic antioxidant defense systems are impaired [67].

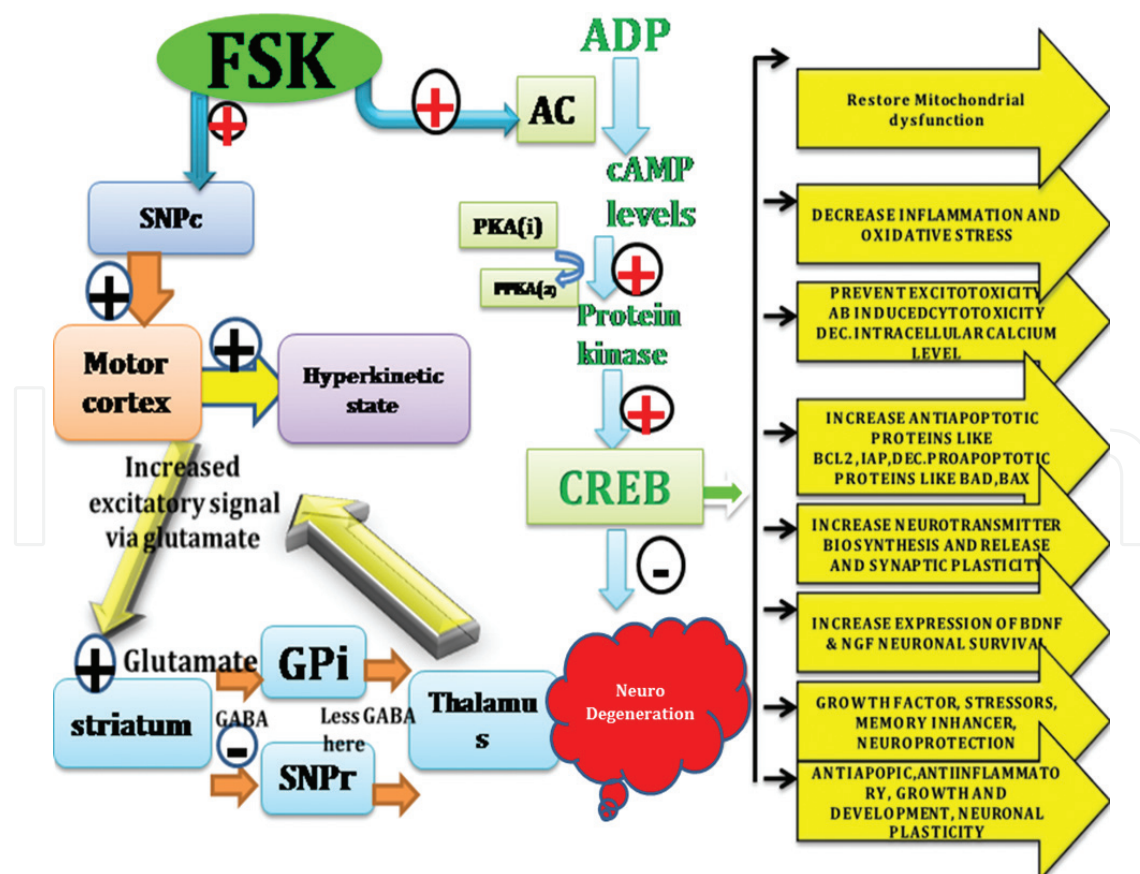


Figure 4. Neuroprotective action of forskolin-mediated AC/cAMP/PKA/CREB activation.

Moreover, FSK-mediated cAMP/PKA/CREB activation were found to inhibit LPS- and cytokine-mediated production of NO as well as the expression of iNOS, whereas compounds (H-89 and (Rp)-cAMP) that decrease PKA activity stimulated the production of NO and the expression of iNOS in rat primary astrocytes [68].

8.3. Forskolin against mitochondrial dysfunctioning

The brain is strongly dependent on the ATP production of the cell energy-producing organelle, the mitochondrion. There is a large body of evidence involving mitochondrial dysfunctions in ASD. Palmieri and Persico, regarding ASD, oxidative phosphorylation (OXPHOS) in the mitochondrion requires at least 80 proteins, of which only 13 are encoded by the mtDNA, while mitochondrial functioning has been estimated to need the participation of approximately 1500 nuclear genes. Mitochondrial dysfunction is present in the brains of individuals with ASD and may play a role in its core cognitive and behavioral symptoms. Alternatively, mitochondria can be damaged by endogenous stressors associated with ASD such as elevated pro-inflammatory cytokines resulting from an activated immune system or other conditions associated with oxidative stress. Oxidative stress may be a key link between mitochondrial dysfunction and ASD as reactive oxygen species (ROS) generated from pro-oxidant environmental toxicants and activated immune cells can result in mitochondrial dysfunction. Excess production of free radicals or impaired antioxidant mechanisms may cause oxidative stress: impaired mitochondrial function then leads to further oxidative stress and a vicious negative cycle can ensue. Instead, abnormal functioning appears secondary to excessive Ca^{2+} levels. Mitochondrial dysfunctioning caused depletion of ATP, that further decrease the level of cAMP. Forskolin, increase in intracellular cAMP, through the phosphorylation of CREB which perform neuroprotective functioning associate with mitochondrial dysfunctioning [69].

8.4. Forskolin against cognitive dysfunction

Autistic brain which may reflect enhanced cortical plasticity which is defined as the process of microstructural construction of synapses occurring during development and the remodeling of these synapses during learning [70]. Enhanced synaptic plasticity triggers a regional reorganization of brain functions that account for both the unique aspects of autism and its variability [71]. Activation of cAMP/PKA has been mainly implicated in stimulating learning and memory. FSK activate cAMP/CREB in hippocampal region [72].

8.5. Possible involvement of FSK in PPA-induced autism

Summarizing the whole information given above, FSK confirmed a versatile role in autism where it activates the AC/cAMP-mediated PK_A /CREB activation. Moreover, on other side FSK act as a co-activator in brain that follows the G_s pathway through the activation of D1 receptor. There is least availability of selective AC activation and so far only limited reports suggest beneficial effect of FSK in neurodegeneration animal model.

9. Conclusion

In conclusion, the current study strongly confirms that the administration of propionic acid induces brain lesions that are similar to the behavioral, histological, morphological, biochemical, neurochemical, and pathological features of autism. After Chronic administration of propionic acid in the rats as proven by motor dysfunctions, biochemical and neurochemical alternations. The literature finding in the current study reveals that adenylyl cyclase activator, that is, FSK-mediated cAMP/CREB activation, might be a unique platform for the prevention of neurodegenerative diseases. Thus in conclusion, neuroprotective and neuro restoration effects of FSK may be due to favorable modulation of CREB-mediated signaling. The involvement of cAMP/PK_A/CREB pathway, anti-oxidant, anti-inflammatory and neuroprotective effect of test drug FSK may be the possible mechanisms at least in part underlying the observed effects (**Figure 4**).

Furthermore, with cAMP/PK_A/CREB signaling in regulation of neuronal functioning, the future studies can be designed to investigate the protective and therapeutic potency of forskolin in animal models of brain hemorrhage, Huntington's disease and Parkinson's disease and to find out if cAMP-mediated CREB pathway is equally implicated in the disease pathogenesis or progression. So, now we can finally conclude the significant mitochondrial restorative effects of the FSK may be due to showing its improved motor and cognitive functions as well as to restore the energy levels and antioxidant and anti-inflammatory defense system.

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Author details

Sidharth Mehan*, Himanshi Khera and Ramit Sharma

*Address all correspondence to: sidh.mehan@gmail.com

Department of Pharmacology, ISF College of Pharmacy, Moga, Punjab, India

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