REVIEW

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Forskolin and derivatives as tools for studying the role of cAMP

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Forskolin (7ß-acetoxy-1α,6ß,9α-trihydroxy-8,13-epoxy-labd-14-en-11-one) is the first main labdane diterpenoid isolated from the roots of the Indian *Plectranthus barbatus* ANDREWS and one of the most extensively studied constituents of this plant. The unique character of forskolin as a general direct, rapid and reversible activator of adenylyl cyclase not only underlies its wide range of pharmacological effects but also renders it as a valuable tool in the study of the role of cAMP. The purpose of this review is to provide data presenting the utility of forskolin - as a cAMP activator - for studying the function of cAMP from different biological viewpoints as follows: 1) Investigation on the role of cAMP in various cellular processes in different organs such as gastrointestinal tract, respiratory tract, reproductive organs, endocrine system, urinary system, olfactory system, nervous system, platelet aggregating system, skin, bones, eyes, and smooth muscles. 2) Studies on the role of cAMP activation and inhibition to understand the pathogenesis (e.g. thyroid autoimmune disorders, leukocyte signal transduction defect in depression, acute malaria infection, secretory dysfunction in inflammatory diseases) as well as its possibly beneficial role for curing diseases such as the regulation of coronary microvascular NO production after heart failure, the attenuation of the development or progression of fibrosis in the heart and lungs, the augmentation of myo-protective effects of ischemic preconditioning especially in the failing hearts after myocardial infarction, the stimulation of the regeneration of injured retinal ganglion cells, the curing of glaucoma and inflammatory diseases, the reducing of cyst formation early in the polycystic kidney disease, and the management of autoimmune disorders by enhancing Fas-mediated apoptosis. 3) Studies on the role of cAMP in the mechanism of actions of a number of drugs and substances such as the effect of the protoberberine alkaloid palmatine on the active ion transport across rat colonic epithelium, the inhibitory effect of retinoic acid on HIV-1-induced podocyte proliferation, the whitening activity of luteolin, the effect of cilostazol on nitric oxide production, an effect that is involved in capillary-like tube formation in human aortic endothelial cells, the apoptotic effect of bullatacin, the effects of paraoxon and chlorpyrifos oxon on nervous system. Moreover, cAMP was found to play a role in acute and chronic exposure to ethanol, in morphine dependence and withdrawal and in behavioral sensitization to cocaine as well as in the protection against cisplatin-induced oxidative injuries.

1. Introduction

Forskolin (7ß-Acetoxy-1α,6ß,9α-trihydroxy-8,13-epoxy-labd-14-en-11-one) is the first main labdane diterpenoid isolated from the roots of the Indian *Plectranthus barbatus* ANDREWS and one of the most extensively studied constituents of this plant (Alasbahi and Melzig 2010a). Forskolin was found to possess a unique character as a direct, rapid and reversible activator of cAMP in a variety of mammalian membranes, broken cell preparations and intact tissues (Seamon et al. 1981, 1983; Fradkin et al. 1982; Stengel et al. 1982; Daly et al. 1982; Birnbaumer et al. 1983; Mettauer et al. 1983; Daly 1984; Seamon and Wetzel 1984; Seamon 1984, 1985, 1987; Purdy et al. 1991). Adenylyl cyclase (AC) exists in at least nine different membrane-associated isoforms (or types) and each isoform shows distinct patterns of tissue distribution and biochemical/pharmacological properties. Forskolin is a general activator of all but one of the adenylyl cyclase isoforms, namely, adenylyl cyclase 9 (Hacker et al. 1998; Defer et al. 2000; Dahle et al. 2005). Interaction of forskolin with different AC isoforms in different tissues is considered responsible for a great number of pharmacological effects of forskolin (Alasbahi and Melzig 2010b). Attempts to enhance the isoform selectivity, with the ultimate goal of developing pharmacotherapeutic agents and/or therapeutic strategies that target adenylyl cyclase isoforms to regulate various neuro-hormonal signals in a highly tissue-organ–specific manner (Ishikawa 2003; Iwatsubo et al. 2003), have led to the development of several forskolin derivatives such as 6-[*N*-(2-isothiocyanatoethyl) aminocarbonyl]-forskolin and 6-(4-acrylbutyryl)-forskolin, which were found to enhance the selectivity for type II and 7-deacetyl-7-hydroxamylforskolin or 5,6-dehydroxy-7-deacetyl-7-nicotinoylforskolin that were found to have enhanced selectivity for type III (Onda et al. 2001), as well as the compounds, 6-[3-(dimethylamino)propionyl]-forskolin (NKH477) and 6-[3-(dimethylamion)propionyl]-14,15-dihydro-forskolin, which were reported to stimulate cardiac adenylyl cyclase isoform type V more potently than the other tissue adenylyl cyclases (lung, brain, kidney) versus

forskolin (Toya et al. 1998; Onda et al. 2001). Recently, NKH477 has been widely used in the treatment of acute heart failure (Alasbahi and Melzig 2010b).

The ability of forskolin to activate AC has been utilized by investigators for studying AC and cAMP formation in experiments with different cell preparations. In addition, forskolin affinity columns have been developed for the purification of the enzyme, sometimes termed C, the catalyst that generates cAMP (Pfeuffer et al. 1985a, 1985b; Smigel 1986). Moreover, iodinated photoaffinity forskolin derivatives with higher specific activities have been developed such as *N*-(3-(4-azido-3-^{125}I-phenyl)propionamide)-6-aminoethylcarbamyl-forskolin and [^{125}I]-2-[3-(4-hydroxy-3-iodophenyl)propanamido]-*N*-ethyl- 6- (aminocarbonyl)-forskolin for labeling adenylyl cyclase (Morris et al. 1991; Appel et al. 1992; Robbins et al. 1992; Sutkowski et al. 1994). Radiolabeled forskolin was also utilized as means to quantitate the number of molecules of adenylyl cylcase in intact-cell preparations, to assess the impact of various treatments on adenylyl cyclase expression and to detect the role of heterotrimeric guanine nucleotide (G)-protein, Gs, and cellular released agonists in contributing to the response to forskolin (Insel and Ostrom 2003). Although forskolin has been used in a great number of studies for more than two decades, it still motivates scientific investigations of a variety of cellular processes. In this paper, we briefly review the exploitation of forskolin as a tool for studying the role of cAMP from different biological aspects.

2. Forskolin as a tool for studying the role of cAMP in cellular processes

In a great number of *in vivo* and *in vitro* studies, forskolin, as a cAMP activator, has been utilized as a valuable tool to reveal the functions of cAMP in a wide range of cellular events in various organs. In rabbit gastric glands (Hersey et al. 1983) as well as in dispersed rabbit parietal cells (Takahashi et al. 1983), forskolin was found to stimulate gastric acid secretion and pepsinogen release by activating gastric adenylyl cyclase. Forskolin was more effective as an activator of AC than NaF and histamine and stimulated cAMP content to levels much higher than histamine or isoproterenol did, providing evidence for the major role of cAMP in gastric acid secretion. Litosch et al. (1982) reported that the addition of forskolin (0.1–10 μM) to salivary-gland homogenates of blowfly (*Calliphora erythrocephala*) resulted in an increase in cAMP accumulation and salivary secretion. Forskolin also activated adenylyl cyclase and enhanced cyclic AMP accumulation in slices of dog thyroid. It was found to reproduce two known cyclic AMP-mediated thyrotropin effects which are the activation of the thyroid secretion and of protein iodination (Van Sande et al. 1983). Studies using forskolin have revealed the role of rat cholangiocyte cilia as sensory organelles containing polycystin-1, polycystin-2, and adenylyl cyclase isoform 6, through which luminal bile fluid flow affected both intracellular Ca^{2+} and cAMP signaling in the cell (Masyuk et al. 2006). The importance of the intracellular cAMP signaling as a regulator of cholangiocyte proliferation was demonstrated by chronic administration of forskolin to normal rats. Compared to control animals, forskolin administration to normal rats led to an increase in the number of bile ducts, intracellular cAMP levels and secretin-induced choleresis as observed in animals with BDL (bile duct ligation). Forskolin-induced increases in cholangiocyte proliferation and secretion were found to be devoid of cholangiocyte necrosis, inflammation and apoptosis. *In vitro*, in pure isolated cholangiocytes, forskolin was also found to increase cholangiocyte proliferation, which can be blocked by inhibiting the PKA-Src-MEK-ERK1/2 pathway (Protein kinase A – Src kinase-mitogen-activated protein kinase kinase – extracellular signal-regulated kinase 1/2-pathway) providing the first evidence that this signaling pathway is critical for cholangiocytes proliferation and its modulation may be important in the regulation of cholangiocyte growth and secretion observed in cholestatic liver diseases (Francis et al. 2004). Maintenance of the cholangiocyte cAMP levels by forskolin administration was reported to prevent the effects of vagotomy - in bile duct ligated rats - on cholangiocyte proliferation, apoptosis, and secretion, which highlights the importance of cholinergic innervation in the regulation of biliary mass and, as mentioned above the dependence of cholangiocyte proliferation on intracellular cAMP-dependent signaling mechanisms (LeSage et al. 1999). Forskolin has demonstrated that β-adrenergic receptor activation leads to parallel events, the cAMP accumulation and calcium movements, which together lead to maximal secretion of the rat parotid gland (Dreux et al. 1986). Forskolin as a potent activator of cAMP-dependent fluid secretion in the intestinal epithelium was used in the study of the differential regulation of the basolateral Na^+ -K^+-2Cl-co-transporter (NKCC1) expression and activity using a novel *ex vivo* 3D tissue culture model of the native human colonic epithelium. NKCC1 is regarded as a central integrator of cellular signals that determines the secretory status of the intestinal epithelium. Studying the differentially modulation of this secretory machinery of the intestinal epithelium by the second messengers (the intercellular Ca^{2+} and cAMP) has revealed, for the first time, that NKCCl was dynamically and differentially regulated by trafficking events in response to cholinergic Ca^{2+} signals and cAMP, alone or in combination. The observed NKCC1 trafficking events were found to be in consistence with a major role in negatively regulating Ca^{2+} mediated fluid secretion, promoting sustained fluid secretion by elevated cAMP, and curtailing the synergistic response invoked by co-stimulation (Reynolds et al. 2007). Forskolin has demonstrated that the cytosolic cAMP pool dominated over the plasma membrane cAMP pool in the control of pulmonary microvascular endothelial cell barrier function (Sayner and Stevens 2006). In addition to the role of α-melanocyte stimulating hormone (α-MSH) and adrenocorticotropic hormone in the regulation of melanogenesis in cultured human melancytes and mouse melanoma cells, forskolin has clearly confirmed the pivotal role of cAMP up-regulation that can act on different signaling cascades which control melanogenesis and melanocyte dendricity (Buscà and Ballotti 2000). It has been reported that the addition of forskolin to the incubation medium of cultured pineal glands of Syrian hamsters collected in the second half of the dark period caused a marked increase in both cAMP and melatonin levels and consequently supported the primary role of cAMP in the nocturnal increase of melatonin production in the Syrian hamster pineal gland (Santana et al. 1990). The ability of forskolin to potentiate the steroidogenic effect of ovine luteinizing hormone (oLH) in chicken granulosa cells (ED_{50} (ng/ml) for oLH alone = 9, in combination with 10 μM forskolin is = 0.4 and in combination with 0.1 μM forskolin and 0.1 mM 3-isobutyl-1-methylxanthine (IBMX) (a phosphodiesterase inhibitor) is = 0.1), has indicated the role of the adenylyl cyclase-cAMP effector system, in addition to other mechanisms, in the oLH-induced steroidogenesis (Asem and Hertelendy 1983). Forskolin was used to reveal the function of cAMP in the porcine oocyte-cumulus physiology. It was found that forskolin (0–100 μM) stimulated a dose-dependent increase in the cAMP content of cumulus masses, cumulus-enclosed oocytes and denuded oocytes indicating that pig oocytes can synthesize cAMP. Forskolin also stimulated progesterone secretion and cumulus mass expansion with maximal increases in both measures occurring at 6.25 μM forskolin, with subsequent dose-dependent declines up to 100 μM forskolin, while cumulus cAMP remained elevated. This result denoted that progesterone

secretion may be regulated by some mechanism(s) in addition to that attributable to elevated amounts of cAMP. Moreover, forskolin was found to induce a dose-dependent increase in heterologous metabolic coupling by which cumulus cAMP may be transferred to the oocyte and cause dose-dependent increases in percentage of germinal vesicle (% GV) of cumulus-enclosed and denuded oocytes with 0.23 and 4.84 μM forskolin maintained 50% GV, respectively. Maintenance of meiotic arrest and stimulation of oocyte-cumulus cAMP were found to be reversible. Many of the results of this study were found to be consistent with those obtained from studies with forskolin and a number of other species. However, this study did not support the hypothesis that meiotic resumption results from a reduction in intra-oocyte cAMP, but was consistent with the proposal that some 'factor' other than cAMP in the oocyte may also be involved in maintenance of meiotic arrest (Racowsky 1985). Forskolin was also used to elucidate the role of cAMP in the regulation of catfish oocyte maturation (Haider and Chaube 1996) and in the gonadotropes of ovariectomized rats revealing that ovariectomy did not result in a change in the role of cAMP, which appears to be a pivotal, but an indirect mediator of protein synthesis-dependent component of gonadotropin-releasing hormone-stimulated luteinizing hormone secretion (Das and Bourne 1992). Forskolin has demonstrated that cAMP mediated the relaxation of a variety of smooth muscles such as cultured human and rabbit corpus cavernosum smooth muscle cells and strips (Palmer et al. 1994; Baba et al. 2004), rat aorta, bovine coronary artery, canine coronary artery, guinea pig taenia caeci, and rabbit small intestine (Muller and Baer 1983). It was found that administration of NaHS at the concentration range of 10–100 μM (yields ~ 3–30 μM H_2S) either before, during, or after addition of forskolin all attenuated/reversed the vasorelaxant effect of forskolin. More importantly, H_2S (5–100 μM) also attenuated forskolin-induced cAMP accumulation. With this pharmacological evidence, it has been demonstrated for the first time that the contractile effect of the endogenous mediator H_2S observed in isolated rat aorta was, at least partially, associated with reducing cAMP level (Lim et al. 2008). The regulation of nicotinic acetylcholine receptor (AcChoR) phosphorylation by cAMP was explored by the addition of forskolin and the phosphodiesterase inhibitor Ro 20-1724 or by addition of cAMP analogues. Addition of the phosphodiesterase inhibitor Ro 20-1724 for up to 1 h did not influence the basal phosphorylation level of the AcChoR. However, treating myotube cultures for 45 min with 20 μM forskolin in the presence of 35 μM Ro 20-1724 increased phosphorylation of the α, δ, and δ' subunits. The concentrations of forskolin that were effective in stimulating phosphorylation of the AcChoR were in the dose response range known to activate adenylyl cyclase in other cell systems. It has been therefore suggested that the effect of forskolin on phosphorylation of the AcChoR is most likely mediated through activation of cAMP-dependent protein kinase. The rapid time course of phosphorylation of the δ and δ' subunits of the AcChoR following treatment with forskolin was found consistent with direct phosphorylation of the AcChoR by cAMP-dependent protein kinase. In contrast, phosphorylation of the α subunit was found to follow a much slower time course, after a considerable lag time, and that may reflect an indirect effect of cAMP-dependent protein kinase or that another protein kinase whose activity or synthesis was regulated by cAMP-dependent protein kinase may phosphorylate the α subunit of the AcChoR. Forskolin was shown to accelerate AcChoR desensitization in rat myotubes with a half-maximal effect that occurred at 8 μM and was complete within 5 min after forskolin treatment. This was found to correspond most closely to the time course and dose dependency of δ and δ' subunit phosphorylation observed after stimulating muscle cell cultures with forskolin. In contrast, since the increase in phosphorylation of the α subunit was found to undergo a longer time course, the stimulation of α-subunit phosphorylation by forskolin appeared not to be correlated directly to AcChoR desensitization rates (Miles et al. 1987). It has also been demonstrated that both spontaneous and PTH (parathyroid hormone)-stimulated bone resorption in 24 h calvarial bone cultures was inhibited when cyclic AMP levels were raised in the tissue by forskolin with calculated IC_{50} values at 1.6 and 0.6 μmol/L respectively. The interpretation that forskolin inhibited bone resorption via cyclic AMP was supported by the findings that in 24 h cultures, forskolin-induced inhibition of PTH-stimulated ^{45}Ca release could be potentiated by several structurally different phosphodiesterase (PDE) inhibitors. The inhibitory effect of forskolin on ^{45}Ca release from PTH-stimulated bones was significant after 3 h, indicating that the decreased bone resorption was due to a direct inhibitory effect on the activity of the osteoclasts. Moreover, forskolin inhibited PTH-stimulated release of ^{3}H from [^{3}H]proline-labelled bones, at the same concentrations as those that inhibited ^{45}Ca release indicating that not only mineral mobilization, but also organic matrix degradation, was affected by forskolin. Furthermore, forskolin-induced inhibition of bone resorption was found to be associated with decreased lysosomal enzyme release. These results strongly indicated that an increase of cyclic AMP inhibited bone resorption and lysosomal degranulation. The inhibitory action of forskolin on bone resorption in short-term cultures (1–24 h) was found to be transient (Lerner et al. 1986). On the other hand, forskolin at a concentration of 0.1 μmol/L was found to stimulate Ca^{2+} mobilization in 6 h cultures but the effect was not sustained, and the magnitude was less than the effect by PTH at a dose giving the same cyclic AMP response (Löwik et al. 1985). Martz and Thomas (1983) also reported that, in 2–8 h incubations, forskolin, at a concentration of 1 μmol/L, stimulated Ca^{2+} efflux from a chick-embryo system. Forskolin was also found to produce a dose-dependent stimulation of ^{45}Ca (EC_{50} = 16 nmol/L), and ^{3}H release in long-term cultures (120 h) (Lerner et al. 1986). It has been demonstrated that the dissociation between the dose-response curves for inhibition (IC_{50} 1.6 μmol/L) and stimulation (EC_{50} 16 nmol/L) on mineral mobilization by forskolin indicated that, at low concentrations of forskolin, a delayed stimulation without a preceding inhibition can be obtained. The stimulatory effect by forskolin in long-term cultures was found to be cell-mediated, as supported by the findings that forskolin not only stimulated mineral mobilization (^{45}Ca, Ca^{2+}, Pi), but also the release of lysosomal enzymes and the degradation of bone matrix (release of ^{3}H from [^{3}H]proline-labelled bones, amounts of hydroxyproline at the end of culture). Furthermore, no stimulation of ^{45}Ca release by forskolin was seen in devitalized bones. Stimulation of ^{45}Ca release was blocked by calcitonin, indicating that the stimulatory effect of forskolin was at least partially osteoclast-mediated. Indomethacin did not affect the stimulatory effect of forskolin on the release of ^{45}Ca, thus suggesting that the stimulation was not prostaglandin-mediated. As indicated by the studies with forskolin, dibutyryl cyclic AMP, PDE inhibitors and cholera toxin, the initial bone-resorptive effect by PTH was found not to be mediated by cyclic AMP; instead, the nucleotide can be a mediator of the late bone-resorptive effect by PTH (Lerner et al. 1986). The increase in the intracellular cAMP by adenylyl cyclase activation caused by forskolin was found to induce stimulation of renin release from the isolated perfused rat kidney (Schwertschlag and Hackenthal 1982). It has also been demonstrated that forskolin-induced transepithelial Cl-secretion caused by cAMP activation of apical Cl-channel and Na^{+}/K^{+}/2Cl-co-transporter in renal epithelial A6 cells was mediated not only through a PKA-dependent pathway and a PKA-independent pathway but also through an activation of

protein tyrosine kinase (PTK)-dependent pathway (Niisato and Marunaka 2001). To study the role of cAMP and the Rho-family small GTPases in the molecular mechanism regulating the formation of podocyte cell-cell contact of the renal glomerular epithelial cells, forskolin was used to investigate the effect of cAMP and three Rho-family small GTPases (RhoA, Cdc42, and Rac1) on the regulation of cell-cell contact formation in a murine podocyte cell line. It has been shown that cAMP supported the integrity of cell-cell contacts, resulting in the closure of an intercellular adhesion zipper, accompanied by a redistribution of cell adhesion molecules and actin-associated proteins in a continuous linear pattern at cell-cell contacts. The Rho-family small GTPases Rac1 and Cdc42 were found to be activated during closure of the adhesion zipper, whereas RhoA was suppressed (Gao et al. 2007). It has been demonstrated that repetitive odor stimulation elicited a potentiation of the subsequent responses in isolated mudpuppy olfactory sensory neurons. This potentiation was found to be mimicked by stimulating the cAMP pathway through the application of a combination of (0.1 mM IBMX + 30 μM forskolin) and not to be related to phosphorylation of ion channels since protein kinase inhibitors could not block it. Activation of cAMP was found to increase the intracellular $[Ca^{2+}]_i$, which mediated the pulse-elicited potentiation. In the first odor application, entry of Ca^{2+} through cyclic nucleotide-gated channels was found to be buffered, but repetitive stimulation allowed local increases in $[Ca^{2+}]_i$, recruiting more Ca^{2+}-dependent Cl^- channels with each subsequent odor pulse (Zhang and Delay 2006). The elevation of cAMP by a low concentration of forskolin (5 μM) was shown to produce a phosphorylation-dependent inhibition of outward potassium currents in rat taste receptor cells. cAMP was also found to be effective in altering the waveform of the gustatory action potential, implying that it may modify transmission of gustatory information to the brain (Herness et al. 1997). It has been found that stimulation of mouse taste buds with 1 μM forskolin + 100 μM IBMX, which elevates cellular cAMP, triggered Ca^{2+} transients through L-type voltage gated Ca^{2+} channels in 38% of presynaptic cells that lack glutamic acid decarboxylase 67 (GAD 67). The response to elevated cAMP was found to be a PKA-dependent influx of Ca^{2+}. These results indicated that GAD-lacking presynaptic cells may represent a functionally distinct set of cells within the taste bud, in which cross-talk between cAMP and Ca^{2+} signaling may integrate tastant-evoked signals (Roberts et al. 2009). Several studies on albino rabbit, monkey and human eyes have demonstrated the ability of forskolin - via cAMP activation - to lower intraocular pressure and to reduce aqueous humor inflow (Bartels et al. 1982; Caprioli and Sears 1983, 1984; Caprioli et al. 1984; Badian et al. 1984; Burstein et al. 1984; Smith et al. 1984; Sears 1985; Seto et al. 1986; Bartels et al. 1987; Lee et al. 1987; Meyer et al. 1987). Investigations of the intracellular signaling that activated relaxin-3-gene-transcription in mouse neuroblastoma cell line using forskolin indicated that relaxin 3 transcription was activated via the cAMP-PKA pathway in the downstream of corticotropin-releasing factor R1 receptor on the cells (Tanaka et al. 2009). In a concentration response study using slices from rat cerebral cortex and cerebellum, which contain mainly β_1-and almost exclusively β_2-adrenoceptors, respectively, isoproterenol and forskolin stimulations of cAMP production were studied either alone or in combination with increasing concentrations of forskolin and isoproterenol, respectively. In the cerebral cortex isoproterenol and forskolin were found both able to potentiate the cAMP accumulation induced by the other compound, whereas, in the cerebellum, isoproterenol was found unable to increase the stimulation induced by forskolin. These results indicated that β_1- and β_2-adrenoceptors may display distinct mechanisms of action in the signaling system by which they stimulate the accumulation of cAMP (Morin et al. 2000). It has been demonstrated that reduction of cAMP level in cultured rat motoneurons by the adenylyl cyclase inhibitor SQ22536 inhibited, and elevation of cAMP by forskolin increased outgrowth and extension of neurites. These cAMP-mediated effects were found to occur via activation of PKA but were independent of Erk activation, which is an essential downstream component of neurotrophin signaling. These findings provided both evidence of the key role of cAMP in promoting peripheral nerve regeneration after nerve injuries as well as indicated that this effect is unusual in not being mediated via Erk phosphorylation (Aglah et al. 2008). Furthermore, forskolin revealed the role of cAMP in processes such as the stimulation of lipolysis in human adipocytes (Burns et al. 1982), and the increase of the endothelial-type nitric oxide synthase (eNOS) activity of human platelets by the cAMP/PKA pathway, which is involved in nitric oxide (NO) synthesis induced by forskolin and potentially by every anti-aggregating substance enhancing intra-platelet cAMP via receptor-dependent and-independent mechanisms (Russo et al. 2004). It has been illustrated that forskolin in the presence of 10 μM IBMX, was able to inhibit rises in the intracellular $[Ca^{2+}]$ evoked by thrombin and platelet-activating factor (PAF) and to completely inhibit the aggregation evoked by thrombin and PAF. But forskolin in this case failed to abolish shape change induced by PAF and thrombin. Moreover, forskolin was found unable to affect the rise in $[Ca^{2+}]i$ evoked by Ca^{2+} ionophore ionomycin, but can, in the presence of 10 μM IBMX, suppress aggregation induced by ionomycin while shape-change persisted. These results showed that cAMP not only suppressed the generation of Ca^{2+} signal by natural agonist, but also reduced the effectiveness of Ca^{2+} in producing the aggregatory response and was ineffective in interfering with the ability of an adequate Ca^{2+} stimulus to produce shape change (Sage and Rink 1985). Using forskolin to increase cAMP and to study its role in the secretion of tissue-type plasminogen activator (tPA) and von Willebrand factor (vWF) in cultured human umbilical vein endothelial cells revealed that cAMP-induced secretion represents a novel mechanism for causing regulated secretion of tPA and vWF from endothelial cells (Hegeman et al. 1998). A dose-dependent increase in platelet cAMP in response to forskolin correlated with progressive inhibition of fibrinogen binding to thrombin-stimulated human platelets was considered as an evidence, beside others, to illustrate that the inhibition of fibrinogen binding by prostaglandin I_2 – a potent activator of platelet adenylyl cyclase and antagonist of platelet aggregation – is linked to its effect on cAMP levels and that elevation of platelet cAMP levels, from any cause, prevents exposure of the fibrinogen receptor (Graber and Hawiger 1982). Studying the role of cAMP/PKA in the regulation of bone morphogenetic protein 4 (BMP-4)-expression in coronary arterial endothelial cells by using forskolin has demonstrated that laminar shear stress activated the cAMP/PKA pathway, and this pathway down-regulated BMP-4 expression in the vascular endothelium. Because BMP-4 can elicit endothelial activation and dysfunction, hypertension, and vascular calcification, the inhibition of BMP-4 expression mediated by cAMP/PKA was found to contribute to the anti-atherogenic and vaso-protective effects of laminar shear stress (Csiszar et al. 2007).

3. Forskolin as a tool for studying the beneficial role of cAMP with possible therapeutic potential for curing diseases

Forskolin was used in a number of studies to reveal the role of cAMP activation as a beneficial action that could be utilized therapeutically, for example, Zhang et al. (2002) reported

that stimulation of cAMP signal transduction – by a number of cAMP-increasing agents including forskolin – increased NO release in microvessels isolated from pacing-induced failing canine hearts, perhaps via activation of cAMP dependent PKA and the subsequent phosphorylation of eNOS by protein kinase B through a phosphatidylinositol 3-kinase (PI3-kinase)-mediated mechanism. The authors suggested that stimulation of the cAMP signal transduction may be an important potential compensatory pathway to increase myocardial microvascular NO production after heart failure, a state in which eNOS is down-regulated. cAMP has been identified as a negative regulator of fibroblast activation and differentiation in a number of organs, for examples Swaney et al. (2005) have found that forskolin (10 μM) and thus an activated cAMP/PKA pathway was able to inhibit α-smooth muscle actin (α-SMA) protein expression and collagen production of adult rat cardiac fibroblasts (CFs) stimulated by serum or pro-fibrotic agents. Moreover, CFs that overexpressed type 6 adenylyl cyclase was shown to enhance forskolin-promoted cAMP formation, and forskolin-inhibition of transforming growth factor-β (TGF-β)-stimulated α-SMA expression and therefore attenuating the fibroblast-to-myofibroblast transformation in parallel with a decrease in collagen synthesis. cAMP elevation by forskolin was also found to block the pro-fibrotic effects of TGF-β in rat cardiac fibroblasts largely by inhibiting ERK1/2 and c-Jun N-terminal kinase activation and also by reducing of cAMP response element binding protein binding protein 1 (CREB-binding protein 1 (CBP1)) recruitment to Smad transcriptional complexes (Liu et al. 2006). Moreover, enhancement of cAMP formation in a human pulmonary fibroblast cell line (WI-38 cells) by multiple approaches, including direct stimulation of adenylyl cyclase by forskolin, and overexpression of adenylyl cylcase type 6, was shown to reduce pulmonary fibroblast cell proliferation and total collagen synthesis (Liu et al. 2004). The authors (Liu et al. 2004, 2006; Swaney et al. 2005) suggested that augmenting cAMP generation may be useful for attenuating the development or progression of fibrosis in the heart and lungs and perhaps other organs. Administration of forskolin to post myocardial infarction of rat hearts prior to ischemic preconditioning (IPC) was found able to restore the myoprotective effects of IPC in terms of the infarct size reduction. Forskolin alone, however, was found unable to provide a myoprotective effect. These results may indicate that AC activation is a requisite to trigger the protective effect of IPC. Since the IPC response has also been shown to exist in human hearts the author implied that pre-ischemic infusion of forskolin preceding IPC may prove useful in augmenting myo-protective effects of IPC especially in the failing hearts after myocardial infarction (Mieno et al. 2002). Using forskolin to reveal the mechanism underlying the anti-mitogenic action of cAMP in airway smooth muscle cells has led to the finding that, in cultured bovine tracheal myocytes, cAMP attenuated cyclin D_1 promoter activation via phosphorylation and activation of cAMP response element-binding protein (CREB) and because cyclin D_1 is required for DNA synthesis in these cells, the authors suggested that this is one mechanism for cAMP-induced growth inhibition of airway smooth-muscle, which may help to understand the potential role of human airways smooth-muscle hyperplasia in the pathogenesis of human airways disease (Musa et al. 1999). It has been shown that cAMP-elevating agents including forskolin can inhibit epidermal growth factor (EGF)-, lysophosphatidic acid (LPA)- and LPA + EGF-stimulated proliferation of cultured human airway smooth muscle (HASM) cells indicating the involvement of cAMP. More importantly, exchange protein directly activated by cAMP (EPAC) rather than PKA was found to be the relevant effector of cAMP-mediated inhibition of proliferation of HASM cells. These findings were the first to implicate EPAC proteins as candidate cAMP effectors for clinically important drug effects in the lung and raise the hope that new drugs targeted at EPAC proteins and pathways and/or selective forskolin analogs could be effective for preventing or reversing the hyperproliferation of airway smooth muscle, providing therapeutic benefit beyond that attainable with the current therapies (Kassel et al. 2008). Forskolin, and therefore cAMP activation, was found to promote the regeneration of injured retinal ganglion cells via the PKA-CREB and the PI3K-Akt pathways (Liang and Li 2003). A recently developed ophthalmic delivery system of forskolin (*in situ* gel forming system) has proved to be effective for the treatment of glaucoma in albino New Zealand rabbits indicating the role of cAMP in reducing the intraocular pressure (Gupta and Samanta 2010).

Forskolin revealed the contribution of cAMP in a number of anti-inflammatory processes such as the chondro-protective effect of increased levels of cAMP in chondrocytes, which was found to inhibit matrix metalloproteinase-mediated cartilage degradation in an *ex vivo* model of bovine articular cartilage explants (Karsdal et al. 2007), as well as the blockage of the neuro-inflammatory process by inhibiting the induction of the pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β in lipopolysaccharide-stimulated astrocytes and microglia and the normalization of the very long chain fatty acids (considered as metabolic abnormality) in cultured skin fibroblasts of x-adrenoleukodystrophy (X-ALD) (Pahan et al. 1998). These findings demonstrated the therapeutic potential of compounds increasing cAMP in the treatment of such inflammatory diseases. A study of adenylyl cyclase-mediated responses in peripheral blood mononuclear cells from 95 drug-free patients with a major depressive episode and 69 healthy controls has demonstrated blunted cAMP response to activation of the beta-adrenergic receptor and prostaglandin receptor in depressed patients. On the other hand, forskolin-generated cyclic AMP response, which involves direct activation of adenylyl cyclase, was not blunted. These results indicated an abnormality at the level of the coupling protein in these adenylyl-coupled receptors in depressed patients (Mann et al. 1997). Forskolin also disclosed the role of the increase of cAMP in the potentiation of Fas-induced apoptosis in normal human T cells and activation-induced cell death in Jurkat cells providing a rationale for investigating the possibility of using cAMP elevating agents therapeutically to potentiate apoptosis in T cells with aberrant Fas signaling and therefore be useful in the management of autoimmune disorders (Naderi and Blomhoff 2008). In an experiment using cultured β-cell line (HIT-T15 cells) and 2-deoxy-D-ribose (dRib) – a reducing sugar with high reactivity to produce cytotoxicity and apoptosis – cAMP-stimulating agents including forskolin was found to protect pancreatic β-cells by reducing dRib-induced damage without influencing the dRib-induced rise in intracellular H_2O_2 levels (Koh et al. 2005). Szabo-Fresnais et al. (2008) reported that forskolin and interleukin-1 (IL-1) synergistically enhanced interleukin-6 (IL-6) mRNA expression in FRTL-5 thyroid cells by mechanisms involving the cAMP/PKA pathway, both stabilization of the IL-6 mRNA and activation of the IL-6 promoter, and activator protein 1 (AP-1) transcription factors and suggested that such mechanisms may be involved in the development of thyroid autoimmune disorders. Activation of cAMP system in Madin-Darby canine kidney cell line and in human cells from normal kidneys and from patients with autosomal dominant polycystic kidney disease (ADPKD) grown in polarized monolayers and exposed to forskolin was found to be involved in the progressive expansion of hereditary and acquired renal cysts (Grantham et al. 1989). Moreover, cAMP signaling was demonstrated to play a key role in renal cyst formation by cultured mammalian metanephric organ (Magenheimer et al. 2006) and

collecting duct cells (Montesano et al. 2009), and this action was found to be mediated by cystic fibrosis transmembrane regulator (CFTR)- and NKCC1-dependent fluid secretion. The authors suggested that if localized, intermittent increases in cAMP levels in (ADPKD) kidneys actually trigger the early stages of cyst formation, then a combination of therapies to inhibit both cell proliferation and fluid secretion, including CFTR and NKCC1 inhibitors, may be effective in reducing cyst formation early in the PKD disease process (Magenheimer et al. 2006). In contrast to the role of an elevated cAMP mentioned above, a reversible defect in cAMP metabolism was observed in peripheral blood lymphocytes during acute malaria infection. The defect was characterized by decreased intracellular cAMP levels in lymphocytes and by hypo-responsiveness to forskolin stimulation of cAMP production and by a reduction in the proliferative response of lymphocytes to concanavalin A and consequently may underlie immunosuppression in malaria infection (Webster et al. 1990). Moreover, inhibition of cAMP-dependent trafficking of CFTR from cytoplasmic stores to the apical plasma membrane in the SCBN cell line induced by nitric oxide provided insight into the mechanism of secretory dysfunction in inflammatory diseases of the gut where mucosal nitric oxide is elevated (Skinn and MacNaughton 2005).

4. Forskolin as a tool for studying the role of cAMP in the pharmacodynamics effects of drugs

Forskolin was used to illustrate the role of cAMP in the mechanism of the pharmacological/toxicological actions of a number of drugs and substances, for example, concerning the effect of the protoberberine alkaloid palmatine on active ion transport across rat colonic epithelium, it was found that palmatine inhibited Ca^{2+} activated Cl^--secretion through inhibiting basolateral charybdotoxin-sensitive SK4 K^+ channels (Small conductance Ca^{2+} activated K^+ channels), and blocked cAMP-activated Cl^--secretion by inhibiting apical CFTR Cl^--channels and basolateral chromanol 293B-sensitive KvLQT1 K^+ channels (voltage-gated potassium channel protein) (Wu et al. 2008). All-*trans* retinoic acid (atRA) was shown to reverse the effects of HIV-1 infection in podocytes by stimulating the retinoic acid receptor isoform RARα- mediated cAMP/PKA activation. Treatment with atRA was found to reduce cell proliferation rate by causing G1 arrest and restore the expression of the differentiation markers (synaptopodin, nephrin, podocin, and WT-1) in HIV-1-infected podocytes (He et al. 2007). The whitening activity of luteolin was found to be related to the inhibition of adenylyl cyclase involved in the signal pathway of alpha-melanocyte stimulating hormone (alpha-MSH)-induced melanin production in B-16 melanoma cells (Choi et al. 2008). Investigating the effect of cilostazol on NO production in human aortic endothelial cells (HAEC) showed that cilostazol induced NO production by endothelial nitric oxide synthase activation via a cAMP/PKA- and PI3K/Akt-dependent mechanism and that this effect was involved in capillary-like tube formation in HAEC (Hashimoto et al. 2006). Testing the effect of ethanol on cAMP signal transduction in primary cultures of rat hepatocytes has indicated that acute exposure to ethanol had a biphasic effect on glucagon receptor-dependent cAMP production in intact cells; 25–50 mM ethanol decreased cAMP, whereas treatment with 100–200 mM ethanol increased cAMP. On the other hand, chronic exposure to ethanol (50–200 mM for 38 h) was found to increase glucagon-receptor-dependent cAMP production in hepatocytes by decreasing the quantity of α1 protein at the plasma membrane and thereby decreasing the inhibitory effects of Gi on adenylyl cyclase activity (Nagy and De Silva 1992). It has been demonstrated that an increase in cAMP content in para-gigantocellularis (PGi) neurons of morphine dependent rats resulted from enhancement of adenylyl cyclase activity was considered as one of the cellular basis of morphine dependence and withdrawal in PGi neurons (Hassanpour et al. 2005). Co-administration of intracerebroventricular injections of a water soluble form of forskolin (7DMB-forskolin) 10 min prior to intra-peritoneal injection of cocaine to rats, for 7 consecutive days, was found to enhance cocaine-induced hyper-locomotor activity of rats from the third day of experiment to day 7 compared to rats receiving cocaine alone. When challenged with cocaine on day 14, animals that had previously received forskolin paired with cocaine on days 1–7 displayed similar locomotor activity to animals that received cocaine only. These alterations in adenylyl cyclase activity and/or cAMP levels were implied to underlie the hyperlocomotor response to cocaine and to play a role in behavioral sensitization (Schroeder et al. 2004). Bullatacin, a potential antitumor annonaceous acetogenin, was found to induce apoptosis as well as to cause a decrease in intracellular cAMP and cGMP levels in 2.2.15 cells (human hepatoma HepG2 cells transfected with hepatitis B virus DNA plasmid) in a concentration and time-dependent manner. The bullatacin-induced apoptosis was inhibited by the addition of cAMP and cGMP elevating agents (forskolin and S-nitrosoglutathione) indicating that a decrease of both cAMP and cGMP levels may play a crucial role in bullatacin-induced apoptosis in 2.2.15 cells (Chiu et al. 2003). The potent acetylcholinesterase inhibitors, paraoxon and chlorpyrifos oxon formed from the organophosphorus insecticides parathion and chlorpyrifos following biotransformation were reported to directly inhibit forskolin-stimulated cAMP formation in cortical slices from rats of different ages (neonatal, juvenile, and adult) via muscarinic receptor-dependent and independent mechanisms and that the developing nervous system may be more sensitive to these non-cholinesterase actions (Olivier et al. 2001). Studying the acute and chronic effects of morphine on forskolin-stimulated pro-enkephalin mRNA levels in rat striatum showed that the acute effect of morphine inhibited the stimulation of pro-enkephalin mRNA levels whereas this inhibitory effect was lost by chronic effect of morphine. These results indicated the relevance of opioid-inhibited adenylyl cyclase in the control of pro-enkephalin mRNA levels, and showed that this model is useful for studying how this signal transduction system is attenuated during the development of tolerance (Kluttz et al. 1995). Stimulation of cAMP by several compounds including forskolin was found to protect rat tubular cells against cisplatin-induced oxidative injury by obliterating reactive oxygen species and subsequent inhibition of TNF-α synthesis through blockade of p38 mitogen-activated protein kinase (p38MAPK) activation (Mishima et al. 2006).

5. Conclusion

The discovery of forskolin, as a unique direct, rapid and reversible activator of adenylyl cyclase by Seamon et al. (1981), has provided investigators with a valuable means for *in vivo* and *in vitro* studies of the role of cAMP not only in physiological cellular processes of almost all organs of the animal and human body but also in the pathogenesis of several diseases such as autoimmune and inflammatory disorders. Consequently, cAMP elevating agents have been considered by the investigators as a possible valuable therapeutic potential for curing diseases of several organs such as failing heart (e.g. regulation of coronary microvascular NO production after heart failure), pulmonary fibrosis (reduction of the development or progression of fibrosis), injured retinal ganglion cells, glaucoma, inflammation (e.g. neuro-inflammation), polycystic kidney disease (reduction

of cyst formation) and autoimmune disorders (e.g. by enhancing Fas-mediated apoptosis). In addition, forskolin was used in studies of the molecular basis of the action of drugs and substances such as palmatine, luteolin, cilostazol, bullatacin, paraoxon, chlorpyrifos oxon, and cisplatin. Moreover, forskolin has helped to clarify the role of cAMP in acute and chronic effects of ethanol, and in morphine dependence and withdrawal as well as in the hyper-locomotor response to cocaine. In this review we have shown how the utility of forskolin – as an activator of cAMP – has provided comprehensive information about the role of cAMP in physiological and pathological processes and shed light into the mechanisms of actions of a number of drugs. Our review add to the extensive literature on forskolin, which despite its use for over two decades will continue to be an important tool in studies of the molecular and cellular basis of biological actions.

References

Aglah C, Gordon T, Posse de Chaves EI (2008) cAMP promotes neurite outgrowth and extension through protein kinase A but independently of Erk activation in cultured rat motoneurons. Neuropharmacology 55: 8–17.

Alasbahi RH, Melzig MF (2010a) *Plectranthus barbatus*: a review of phytochemistry, ethnobotanical uses and pharmacology - part 1. Planta Med 76: 653–661.

Alasbahi RH, Melzig MF (2010b) *Plectranthus barbatus*: a review of phytochemistry, ethnobotanical uses and pharmacology - part 2. Planta Med 76: 753–765.

Appel NM, Robbins JD, De Souza EB, Seamon KB (1992) [^{125}I]-labeled forskolin analogs which discriminate adenylyl cyclase and a glucose transporter: pharmacological characterization and localization of binding sites in rat brain by *in vitro* receptor autoradiography. J Pharmacol Exp Ther 263: 1415–1423.

Asem EK, Hertelendy F (1983) Effects of forskolin on progesterone and cyclic adenosine monophosphate production in avian granulosa cells. Biol Reprod 29: 1098–1104.

Baba K, Iwamoto T, Takahashi T (2004) Smooth muscle regulation of PGE1 and forskolin in rabbit cavernosal tissue by cyclic GMP- and cyclic AMP-dependent mechanisms. Sei Marianna Ika Daigaku Zasshi 32: 527–533.

Badian M, Dabrowski J, Grigoleit HG, Lieb W, Lindner E, Rupp W (1984) Effect of forskolin eyedrops on intraocular pressure in healthy males. Klin Monbl Augenheilkd 185: 522–526.

Bartels SP, Lee SR, Neufeld AH (1982) Forskolin stimulates cyclic AMP synthesis, lowers intraocular pressure and increases outflow facility in rabbits. Curr Eye Res 2: 673–681.

Bartels SP, Lee SR, Neufeld AH (1987) The effects of forskolin on cyclic AMP, intraocular pressure and aqueous humor formation in rabbits. Curr Eye Res 6: 307–320.

Birnbaumer L, Stengel D, Desmier M, Hanoune J (1983) Forskolin regulation of liver membrane adenylyl cyclase. Eur J Biochem 136: 107–112.

Burns TW, Langley PE, Terry BE, Bylund DB, Forte LR (1982) Alpha-2 adrenergic activation inhibits forskolin-stimulated adenylate cyclase activity and lipolysis in human adipocytes. Life Sci 31: 815–821.

Burstein NL, Sears ML, Mead A (1984) Aqueous flow in human eyes is reduced by forskolin, a potent adenylate cyclase activator. Exp Eye Res 39: 745–749.

Buscà R, Ballotti R (2000) Cyclic AMP a key messenger in the regulation of skin pigmentation. Pigment Cell Res 13: 60–69.

Caprioli J, Sears M (1983) Forskolin lowers intraocular pressure in rabbits, monkeys, and man. Lancet 1: 958–960.

Caprioli J, Sears M (1984) The adenylate cyclase receptor complex and aqueous humor formation. Yale J Biol Med 57: 283–300.

Caprioli J, Sears M, Bausher L, Gregory D, Mead A (1984) Forskolin lowers intraocular pressure by reducing aqueous inflow. Invest Ophthalmol Vis Sci 25: 268–277.

Chiu HF, Chih TT, Hsian YM, Tseng CH, Wu MJ, Wu YC (2003) Bullatacin, a potent antitumor Annonaceous acetogenin, induces apoptosis through a reduction of intracellular cAMP and cGMP levels in human hepatoma 2.2.15 cells. Biocheml Pharmacol 65: 319–327.

Choi MY, Song HS, Hur HS, Sim SS (2008) Whitening activity of luteolin related to the inhibition of cAMP pathway in alpha-MSH-stimulated B16 melanoma cells. Arch Pharm Res 31: 1166–1171.

Csiszar A, Labinskyy N, Smith KE, Rivera A, Bakker EN, Jo H, Gardner J, Orosz Z, Ungvari Z (2007) Downregulation of bone morphogenetic protein 4 expression in coronary arterial endothelial cells: role of shear stress and the cAMP/protein kinase A pathway. Arterioscler Thromb Vasc Biol 27: 776–782.

Dahle MK, Myhre AE, Aasen AO, Wang JE (2005) Effects of forskolin on Kupffer cell production of interleukin-10 and tumor necrosis factor alpha differ from those of endogenous adenylyl cyclase activators: possible role for adenylyl cyclase 9. Infect Immun 73: 7290–7296.

Daly JW, Padgett W, Seamon KB (1982) Activation of cyclic AMP-generating systems in brain membranes and slices by the diterpene forskolin: augmentation of receptor-mediated responses. J Neurochem 38: 532–544.

Daly JW (1984) Forskolin, adenylate cyclase, and cell physiology: an overview. Adv Cyclic Nucleotide Protein Phosphorylation Res 17: 81–89.

Das S, Bourne GA (1992) The use of flufenamate and forskolin to evaluate the role of cAMP in gonadotropin-releasing hormone-stimulated luteinizing hormone secretion from pituitaries of ovariectomized rats. Pharmacol Toxicol 71: 395–400.

Defer N, Best-Belpomme M, Hanoune J (2000) Tissue specificity and physiological relevance of various isoforms of adenylyl cyclase. Am J Physiol Renal Physiol 279: F400–F416.

Dreux C, Imhoff V, Huleux C, Busson S, Rossignol B (1986) Forskolin, a tool for rat parotid secretion studies: 45Ca efflux is not related to cAMP. Am J Physiol 251: C754–C762.

Fradkin JE, Cook GH, Kilhoffer MC, Wolff J (1982) Forskolin stimulation of thyroid adenylate cyclase and cyclic 3',5'-adenosine monophosphate accumulation. Endocrinology 111: 849–856.

Francis H, Glaser S, Ueno Y, Lesage G, Marucci L, Benedetti A, Taffetani S, Marzioni M, Alvaro D, Venter J, Reichenbach R, Fava G, Phinizy JL, Alpini G (2004) cAMP stimulates the secretory and proliferative capacity of the rat intrahepatic biliary epithelium through changes in the PKA/Src/MEK/ERK1/2 pathway. J Hepatol 41: 528–537.

Gao SY, Li CY, Shimokawa T, Terashita T, Matsuda S, Yaoita E, Kobayashi N (2007) Rho-family small GTPases are involved in forskolin-induced cell-cell contact formation of renal glomerular podocytes *in vitro*. Cell Tissue Res 328: 391–400.

Graber SE, Hawiger J (1982) Evidence that changes in platelet cyclic AMP levels regulate the fibrinogen receptor on human platelets. J Biol Chem 257: 14606–14609.

Grantham JJ, Mangoo-Karim R, Uchic ME, Grant M, Shumate WA, Park CH, Calvet JP (1989) Net fluid secretion by mammalian renal epithelial cells: stimulation by cAMP in polarized cultures derived from established renal cells and from normal and polycystic kidneys. Trans Assoc Am Physicians 102: 158–162.

Gupta S, Samanta MK (2010) Design and evaluation of thermoreversible *in situ* gelling system of forskolin for the treatment of glaucoma. Pharm Dev Technol 15: 386–393.

Hacker BM, Tomlinson JE, Wayman GA, Sultana R, Chan G, Villacres E, Disteche C, Storm DR (1998) Cloning, chromosomal mapping, and regulatory properties of the human type 9 adenylyl cyclase (ADCY9). Genomics 50: 97–104.

Haider S, Chaube SK (1996) The *in vitro* effects of forskolin, IBMX and cyanoketone on meiotic maturation in follicle-enclosed catfish (*Clarias batrachus*) oocytes. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 115: 117–123.

Hashimoto A, Miyakoda G, Hirose Y, Mori T (2006) Activation of endothelial nitric oxide synthase by cilostazol via a cAMP/protein kinase A- and phosphatidylinositol 3-kinase/Akt-dependent mechanism. Atherosclerosis 189: 350–357.

Hassanpour Ezati M, Semnanian S, Fathollahi Y, Nadermanesh H, Altarihi T (2005) Evaluation of adaptive changes in the cyclic adenosine monophosphate (cAMP) of the paragigantocellularis nucleus neuron nucleus in morphine dependent ruts using NMR spectroscopy. Yakhteh 7: 132–139, 201.

He JC, Lu TC, Fleet M, Sunamoto M, Husain M, Fang W, Neves S, Chen Y, Shankland S, Iyengar R, Klotman PE (2007) Retinoic acid inhibits HIV-1-induced podocyte proliferation through the cAMP pathway J Am Soc Nephrol 18: 93–102.

Hegeman RJ, van den Eijnden-Schrauwen Y, Emeis JJ (1998) Adenosine 3':5'-cyclic monophosphate induces regulated secretion of tissue-type plasminogen activator and von Willebrand factor from cultured human endothelial cells. Thromb Haemost 79: 853–858.

Herness MS, Sun XD, Chen Y (1997) cAMP and forskolin inhibit potassium currents in rat taste receptor cells by different mechanisms. Am J Physiol 272: C2005–2018.

Hersey SJ, Miller M, Norris SH (1983) Forskolin: a new biochemical tool for studying gastric secretion. Prog Clin Biol Res 126: 329–341.

Insel PA, Ostrom RS (2003) Forskolin as a tool for examining adenylyl cyclase expression, regulation, and G protein signaling. Cell Mol Neurobiol 23: 305–314.

Ishikawa Y (2003) Isoform-targeted regulation of cardiac adenylyl cyclase. J Cardiovasc Pharmacol 41 Suppl 1: S1–S4.

Iwatsubo K, Tsunematsu T, Ishikawa Y (2003) Isoform-specific regulation of adenylyl cyclase: a potential target in future pharmacotherapy. Expert Opin Ther Targets 7: 441–451.

Karsdal MA, Sumer EU, Wulf H, Madsen SH, Christiansen C, Fosang AJ, Sondergaard BC. (2007) Induction of increased cAMP levels in articular chondrocytes blocks matrix metalloproteinase-mediated cartilage degradation, but not aggrecanase-mediated cartilage degradation. Arthritis Rheum 56: 1549–1558.

Kassel KM, Wyatt TA, Panettieri RA Jr, Toews ML (2008) Inhibition of human airway smooth muscle cell proliferation by beta 2-adrenergic receptors and cAMP is PKA independent: evidence for EPAC involvement. Am J Physiol Lung Cell Mol Physiol 294: L131–L138.

Kluttz BW, Vrana KE, Dworkin SI, Childers SR (1995) Effects of morphine on forskolin-stimulated pro-enkephalin mRNA levels in rat striatum: a model for acute and chronic opioid actions in brain. Mol Brain Res 32: 313–320.

Koh G, Suh KS, Chon S, Oh S, Woo JT, Kim SW, Kim JW, Kim YS (2005) Elevated cAMP level attenuates 2-deoxy-D-ribose-induced oxidative damage in pancreatic β-cells. Arch Biochem Biophys 438: 70–79.

Lee PY, Podos SM, Serle JB, Camras CB, Severin CH (1987) Intraocular pressure effects of multiple doses of drugs applied to glaucomatous monkey eyes**.** Arch Ophthalmol 105: 249–252.

Lerner UH, Fredholm BB, Ransjö M (1986) Use of forskolin to study the relationship between cyclic AMP formation and bone resorption *in vitro*. Biochem J 240: 529–539.

LeSage G, Alvaro D, Benedetti A, Glaser S, Marucci L, Baiocchi L, Eisel W, Caligiuri A, Phinizy JL, Rodgers R, Francis H, Alpini G (1999) Cholinergic system modulates growth, apoptosis, and secretion of cholangiocytes from bile duct-ligated rats. Gastroenterology 117: 191–199.

Liang Y, Li H (2003) Effects of H-89 and wortmannin on the promoting effects of forskolin and IBMX on the regeneration of injured retinal ganglion cells. Jiepou Xuebao 34: 45–48.

Lim JJ, Liu YH, Khin ES, Bian JS (2008) Vasoconstrictive effect of hydrogen sulfide involves downregulation of cAMP in vascular smooth muscle cells. Am J Physiol Cell Physiol 295: C1261–C1270.

Litosch I, Saito Y, Fain JN (1982) Forskolin as an activator of cyclic AMP accumulation and secretion in blowfly salivary glands. Biochem J 204: 147–151.

Liu X, Ostrom RS, Insel PA (2004) cAMP-elevating agents and adenylyl cyclase overexpression promote an antifibrotic phenotype in pulmonary fibroblasts. Am J Physiol Cell Physiol 286: C1089–1099.

Liu X, Sun SQ, Hassid A, Ostrom RS (2006) cAMP inhibits transforming growth factor-beta-stimulated collagen synthesis via inhibition of extracellular signal-regulated kinase 1/2 and Smad signaling in cardiac fibroblasts. Mol Pharmacol 70: 1992–2003.

Löwik CW, van Leeuwen JP, van der Meer JM, van Zeeland JK, Scheven BA, Herrmann-Erlee MP (1985) A two-receptor model for the action of parathyroid hormone on osteoblasts: a role for intracellular free calcium and cAMP. Cell Calcium 6: 311–326.

Magenheimer BS, St John PL, Isom KS, Abrahamson DR, De Lisle RC, Wallace DP, Maser RL, Grantham JJ, Calvet JP (2006) Early embryonic renal tubules of wild-type and polycystic kidney disease kidneys respond to cAMP stimulation with cystic fibrosis transmembrane conductance regulator/Na(+),K(+),2Cl(−) Co-transporter-dependent cystic dilation. J Am Soc Nephrol 17: 3424–3437.

Mann JJ, Halper JP, Wilner PJ, Sweeney JA, Mieczkowski TA, Chen JS, Stokes PE, Brown RP (1997) Subsensitivity of adenylyl cyclase-coupled receptors on mononuclear leukocytes from drug-free inpatients with a major depressive episode. Biol Psychiatry 42: 859–870.

Martz A, Thomas ML (1983) Effects of forskolin on bone. Stimulation of cyclic AMP accumulation and calcium efflux from chick embryo tibiae in organ culture. Biochem Pharmacol 32: 3429–3433.

Masyuk AI, Masyuk TV, Splinter PL, Huang BQ, Stroope AJ, Larusso NF (2006) Cholangiocyte cilia detects changes in luminal fluid flow and transmits them into intracellular Ca2+ and cAMP signaling. Gastroenterology 131: 911–920.

Mettauer M, Giesen EM, Imbs JL, Schmidt M, Schwartz J (1983) Forskolin increases cAMP production in a kidney cell line (MDCK). Libr Compend 11: 887.

Meyer BH, Stulting AA, Müller FO, Luus HG, Badian M (1987) The effects of forskolin eye drops on intra-ocular pressure. S Afr Med J 71: 570–571.

Mieno S, Horimoto H, Watanabe F, Nakai Y, Furuya E, Sasaki S (2002) Potent adenylate cyclase agonist forskolin restores myoprotective effects of ischemic preconditioning in rat hearts after myocardial infarction. Ann Thorac Surg 74: 1213–1218.

Miles K, Anthony DT, Rubin LL, Greengard P, Huganir RL (1987) Regulation of nicotinic acetylcholine receptor phosphorylation in rat myotubes by forskolin and cAMP. Proc Natl Acad Sci USA 84: 6591–6595.

Mishima K, Baba A, Matsuo M, Itoh Y, Oishi R (2006) Protective effect of cyclic AMP against cisplatin-induced nephrotoxicity. Free Radic Biol Med 40: 1564–1577.

Montesano R, Ghzili H, Carrozzino F, Rossier BC, Feraille E (2009) Cyclic AMP-dependent Chloride Secretion Mediates Tubule Enlargement and Cyst Formation by Cultured Mammalian Collecting Duct Cells. Am J Physiol Renal Physiol 296: F446–F457.

Morin D, Sapena R, Tillement JP, Urien S (2000) Evidence for different interactions between β1- and β2-adrenoceptor subtypes with adenylyl cyclase in the rat brain: a concentration-response study using forskolin. Pharmacol Res 41: 435–443.

Morris DI, Robbins JD, Ruoho AE, Sutkowski EM, Seamon KB (1991) Forskolin photoaffinity labels with specificity for adenylyl cyclase and the glucose transporter. J Biol Chem 266: 13377–13384.

Muller MJ, Baer HP (1983) Relaxant effects of forskolin in smooth muscle. Role of cyclic AMP. Naunyn-Schmiedebergs Arch Pharmacol 322: 78–82.

Musa NL, Ramakrishnan M, Li J, Kartha S, Liu P, Pestell RG, Hershenson MB (1999) Forskolin inhibits cyclin D1 expression in cultured airway smooth-muscle cells. Am J Respir Cell Mol Biol 20: 352–358.

Naderi S, Blomhoff HK (2008) Activation of cAMP signaling enhances Fas-mediated apoptosis and activation-induced cell death through potentiation of caspase 8 activation. Hum Immunol 69: 833–836.

Nagy LE, DeSilva SEF (1992) Ethanol increases receptor-dependent cyclic AMP production in cultured hepatocytes by decreasing Gi-mediated inhibition. Biochem J 286: 681–686.

Niisato N, Marunaka Y (2001) Forskolin activation of apical Cl-channel and $Na^+/K^+/2Cl$-cotransporter via a PTK-dependent pathway in renal epithelium. Biochem Biophys Res Commun 285: 880–884.

Olivier K Jr, Liu J, Pope C (2001) Inhibition of forskolin-stimulated cAMP formation *in vitro* by paraoxon and chlorpyrifos oxon in cortical slices from neonatal, juvenile, and adult rats. J Biochem Mol Toxicol 15: 263–269.

Onda T, Hashimoto Y, Nagai M, Kuramochi H, Saito S, Yamazaki H, Toya Y, Sakai I, Homcy CJ, Nishikawa K, Ishikawa Y (2001) Type-specific regulation of adenylyl cyclase. Selective pharmacological stimulation and inhibition of adenylyl cyclase isoforms. J Biol Chem 276: 47785–47793.

Pahan K, Khan M, Singh I (1998) Therapy for X-adrenoleukodystrophy: normalization of very long chain fatty acids and inhibition of induction of cytokines by cAMP. J Lipid Res 39: 1091–1100.

Palmer LS, Valcic M, Melman A, Giraldi A, Wagner G, Christ GJ (1994) Characterization of cyclic AMP accumulation in cultured human corpus cavernosum smooth muscle cells. J Urol 152: 1308–1314.

Pfeuffer E, Dreher RM, Metzger H, Pfeuffer T (1985a) Catalytic unit of adenlyate cyclase: purification and identification by affinity crosslinking. Proc Natl Acad Sci USA 82: 3086–3090.

Pfeuffer E, Mollner S, Pfeuffer T (1985b) Adenylate cyclase from bovine brain cortex: purification and characterization of the catalytic unit. EMBO J 4: 3675–3679.

Purdy SJ, Whitehouse BJ, Abayasekara DR (1991) Stimulation of steroidogenesis by forskolin in rat adrenal zona glomerulosa cell preparations. J Endocrinol 129: 391–397.

Racowsky C (1985) Effect of forskolin on maintenance of meiotic arrest and stimulation of cumulus expansion, progesterone and cyclic AMP production by pig oocyte-cumulus complexes. J Reprod Fertil 74: 9–21.

Reynolds A, Parris A, Evans LA, Lindqvist S, Sharp P, Lewis M, Tighe R, Williams MR (2007) Dynamic and differential regulation of NKCC1 by calcium and cAMP in the native human colonic epithelium. J Physiol 582: 507–524.

Robbins JD, Appel NM, Laurenza A, Simpson IA, De Souza EB, Seamon KB (1992) Differential identification and localization of adenylyl cyclase and glucose transporter in brain using iodinated derivatives of forskolin. Brain Res 581: 148–152.

Roberts CD, Dvoryanchikov G, Roper SD, Chaudhari N (2009) Interaction between the second messengers cAMP and Ca^{2+} in mouse presynaptic taste cells. J Physiol 587: 1657–1668.

Russo I, Doronzo G, Mattiello L, De Salve A, Trovati M, Anfossi G (2004) The activity of constitutive nitric oxide synthase is increased by the pathway cAMP/cAMP-activated protein kinase in human platelets. New insights into the antiaggregating effects of cAMP-elevating agents. Thromb Res 114: 265–273.

Sage SO, Rink TJ (1985) Inhibition by forskolin of cytosolic calcium rise, shape change and aggregation in quin2-loaded human platelets. FEBS Lett 188: 135–140.

Santana C, Guerrero JM, Menendez-Pelaez A, Reiter RJ (1990) The role of cyclic AMP on the induction of melatonin production in the Syrian hamster pineal gland. Adv Pineal Res 4: 65–68.

Sayner S, Stevens T (2006) Soluble adenylate cyclase reveals the significance of compartmentalized cAMP on endothelial cell barrier function. Biochem Soc Trans 34: 492–494.

Schroeder JA, Hummel M, Unterwald EM (2004) Repeated intracerebroventricular forskolin administration enhances behavioral sensitization to cocaine. Behav Brain Res 153: 255–260.

Schwertschlag U, Hackenthal E (1982) Forskolin stimulates renin release from the isolated perfused rat kidney. Eur J Pharmacol 84: 111–113.

Seamon KB, Padgett W, Daly JW (1981) Forskolin: unique diterpene activator of adenylate cyclase in membranes and in intact cells. Proc Natl Acad Sci USA 78: 3363–3367.

Seamon KB, Daly JW, Metzger H, de Souza NJ, Reden J (1983) Structure-activity relationships for activation of adenylate cyclase by the diterpene forskolin and its derivatives. J Med Chem 26: 436–439.

Seamon KB (1984) Forskolin and adenylate cyclase: new opportunities in drug design. Annu Rep Med Chem 19: 293–302.

Seamon KB, Wetzel B (1984) Interaction of forskolin with dually regulated adenylate cyclase. Adv Cyclic Nucleotide Protein Phosphorylation Res 17: 91–99.

Seamon KB (1985) Activation of hormone-sensitive adenylate cyclase by forskolin. Drug Dev Res 6: 181–192.

Seamon KB (1987) Forskolin and adenylate cyclase. ISI Atlas of Science: Pharmacology 1: 250–253.

Sears ML (1985) Regulation of aqueous flow by the adenylate cyclase receptor complex in the ciliary epithelium. Am J Ophthalmol 100: 194–198.

Seto C, Eguchi S, Araie M, Matsumoto S, Takase M (1986) Acute effects of topical forskolin on aqueous humor dynamics in man. Jpn J Ophthalmol 30: 238–244.

Skinn AC, MacNaughton WK (2005) Nitric oxide inhibits cAMP-dependent CFTR trafficking in intestinal epithelial cells. Am J Physiol Gastrointest Liver Physiol 289: G739-G744.

Smigel MD (1986) Purification of the catalyst of adenylate cyclase. J Biol Chem 261: 1976–1982.

Smith BR, Gaster RN, Leopold IH, Zeleznick LD (1984) Forskolin, a potent adenylate cyclase activator, lowers rabbit intraocular pressure. Arch Ophthalmol 102: 146–148.

Stengel D, Guenet L, Desmier M, Insel P, Hanoune J (1982) Forskolin requires more than the catalytic unit to activate adenylate cyclase. Mol Cell Endocrinol 28: 681–690.

Sutkowski EM, Tang WJ, Broome CW, Robbins JD, Seamon KB (1994) Regulation of forskolin interactions with type I, II, V, and VI adenylyl cyclases by Gs alpha. Biochemistry 33: 12852–12859.

Swaney JS, Roth DM, Olson ER, Naugle JE, Meszaros JG, Insel PA (2005) Inhibition of cardiac myofibroblast formation and collagen synthesis by activation and overexpression of adenylyl cyclase. Proc Natl Acad Sci USA 102: 437–442.

Szabo-Fresnais N, Blondeau JP, Pomérance M (2008) Activation of the cAMP pathway synergistically increases IL-1-induced IL-6 gene expression in FRTL-5 thyroid cells: Involvement of AP-1 transcription factors. Mol Cell Endocrinol 284: 28–37.

Takahashi S, Moriwaki K, Himeno S, Kuroshima T, Shinomura Y, Hamabe S, Kurokawa M, Saito R, Kitani T, Tarui S (1983) Forskolin-induced cyclic AMP production and gastric acid secretion in dispersed rabbit parietal cells: novel evidence for a major role of cyclic AMP in acid release. Life Sci 33: 1401–1408.

Tanaka M, Watanabe Y, Yoshimoto K (2009) Regulation of relaxin 3 gene expression via cAMP-PKA in a neuroblastoma cell line. J Neurosci Res 87: 820–829.

Toya Y, Schwencke C, Ishikawa Y (1998) Forskolin derivatives with increased selectivity for cardiac adenylyl cyclase. J Mol Cell Cardiol 30: 97–108.

Van Sande J, Cochaux P, Mockel J, Dumont JE (1983) Stimulation by forskolin of the thyroid adenylate cyclase, cyclic AMP accumulation and iodine metabolism. Mol Cell Endocrinol 29: 109–119.

Webster HK, Wiesmann WP, Ward GS, Permpanich B, Pavia CS (1990) Reversible defect in cAMP metabolism in lymphocytes in malaria infection. Immunopharmacology 19: 169–175.

Wu DZ, Yuan JY, Shi HL, Hu ZB (2008) Palmatine, a protoberberine alkaloid, inhibits both Ca^{2+}- and cAMP-activated Cl^- secretion in isolated rat distal colon. Br J Pharmacol 153: 1203–1213.

Zhang XP, Tada H, Wang Z, Hintze TH (2002) cAMP Signal Transduction, A Potential Compensatory Pathway for Coronary Endothelial NO Production After Heart Failure. Arterioscler Thromb Vasc Biol 22: 1273–1278.

Zhang W, Delay RJ (2006) Pulse stimulation with odors or IBMX/forskolin potentiates responses in isolated olfactory neurons. Chem Senses 31: 197–206.