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PHOSPHODIESTERASE FAMILY: CHARACTERIZATION & PROPERTIES

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ABSTRACT

Phosphodiesterases are enzymes which are uniformly distributed in mammalian tissues, play a unique role in cell signalling by hydrolysis of cAMP and cGMP. The PDE family is divided into 11 sub families (PDE 1 to PDE 11). PDE 1 to PDE 6 are well known Phosphodiesterases enzymes because of there presence in various tissues and cells. The main concern is about PDE 7 to PDE 11 as their role is not specified yet. More research is going on to these later families. This review brings together knowledge on overview, distribution, regulation and inhibitors of all PDEs.

KEYWORDS: Phosphodiesterases, Phosphodiesterase inhibitors, PDEs.

INTRODUCTION

As the presence of cyclic nucleotide adenosine monophosphate (cAMP) second messenger in mammalian cells which participates in enhancing function in a variety of human cells, efforts were made to develop pharmacologic substances that could modify cardiac function, and other cellular events by increasing intracellular cAMP concentration. An effective way to increase intracellular level of cAMP is to inhibit the metabolism of the nucleotide with phosphodiesterase inhibitors. After several early studies using high doses of phosphodiesterase inhibitors in patients with severe congestive heart failure raised the possibility that these agents could do more harm than good, their development was largely discontinued. However, the development of new phosphodiesterase inhibitors, an improved understanding of human disease, the recognition of the importance of dosing and concomitant

medications, and a better understanding of some of the properties of these agents have led to a development of interest in these medications.[1]

Phosphodiesterase 1 family

Overview

Cheung and Kakiuchi and Yamazaki simultaneously discovered from bovine and rat brains, respectively, a calciprotein constituted of 148 aa, as a thermostable factor named calcium-dependent activator or regulator (CDA or CDR) or phosphodiesterase activating factor (PAF), which binds 4 Ca^{2+} mol/mol. The name of the protein is calmodulin (CaM), which activate cyclic nucleotide phosphodiesterase in a calcium-dependent manner. This discovery lead to the characterization of the first eluted fraction of PDE activity (Peak 1 or PDE I) isolated from vascular smooth muscle by chromatography. Since this PDE I fraction was specifically activated by Ca^{2+}/CaM, it was also named CaM-PDE9 (Wells et al., 1975). The cooperative binding of 4 Ca^{2+} to calmodulin is required to fully activate CaM-PDE.[2-4]

Distribution

Most PDEI are cytosolic; however, they are found in sub cellular regions. PDEIA is highly expressed in the brain. In human spermatozoa, PDEIA is tightly associated to calmodulin and is permanently activated.[5]

Regulation

In cells expressing PDE1, hormones that increase cytosolic Ca^{2+} would activate PDE1, decreasing thereby cAMP in response to hormones that stimulate cAMP or cGMP synthesis. It was recently shown that sustained Ca^{2+} entry in the cell is required to activate PDE1A in astrocytoma cell line and that PDE1A cannot discriminate between the different sources of Ca^{2+} entry. In contrast, in vivo phosphorylation of PDE1 would likely result in the potentiation of cAMP or cGMP accumulation involving the elevation of cytosolic Ca^{2+} and activation of guanylyl or adenylyl cyclases and would play a major role in the amplification and prolongation of cyclic nucleotide effects. In this regard, PDE1 might contribute to certain forms of synaptic plasticity in neurons. PDE1 is mainly present in cytosolic fraction; nevertheless, it was shown that PDE1 is also found in the fibers of several neurons from dorsal root ganglion.[6,7]

Inhibitor

Nimodipine, a dihydropyridine that antagonizes specifically L-type Ca channel, was firstly described as a CaM-PDE inhibitor,[8] This effect is not related to its calcium antagonist property since it inhibits, in micromolar range, basal and calmodulin stimulated purified PDE1.[9] Since nimodipine at lower concentrations blocks the L-type calcium channel, it can only be used to estimate PDE1 participation in tissue and cell homogenates.[10] Today, there is no any useful and effective specific PDE1 inhibitor that can be used to assess the functional role of PDE1 in tissue. Vinpocetine was described as a specific inhibitor of basal and calmodulin-activated PDE1 and mainly used as a pharmacological tool to implicate PDE1.[11]

Phosphodiesterase 2 family

Overview

PDE2, firstly named cGMP-stimulated PDE (cGS-PDE), was discovered by Beavo[12] by adding cGMP during cAMP-PDE assay to rat liver supernatant or to crude particulate fractions from various rat tissues.[13] Studies performed on purified PDE2 clearly showed that PDE2 hydrolyzes both cAMP and cGMP and is allosterically regulated by cAMP and cGMP with positive cooperative kinetics, with cGMP being preferred both as substrate and effector.[14] In the presence of cGMP, the rate of cAMP hydrolysis is increased by 6-fold. PDE2 was shown to play a major feedback role by restoring the basal level in cyclic nucleotides in response to hormonal stimulation in the adrenal gland.[15]

Distribution

PDE2 superfamily may be associated to cytosolic or to functional membrane structures. PDE2A1 is cytosolic whereas PDE2A2 and PDE2A3 are bound to membrane. PDE2 protein is mainly present in adrenal medulla, heart, rat ventricle, brown adipose tissue, liver, and brain.[5]

Regulation

PDE2 activity is up-regulated in vivo at post-transcriptional level by PKC under 4 beta-phorbol 12-myristate 13 acetate.[16] Furthermore, PDE2 was also upregulated in rat ventricle in response to pressure overload. Under pathophysiological changes, PDE2 is not only regulated at the post-transcriptional level but also at transcriptional level.[17] In endothelial cells, PDE2A is up-regulated during phenotype changes as well as under stimulation by vascular endothelium growth factor (VEGF), indicating PDE2 participation in endothelial

cell proliferation. In another way, PDE2 mRNA and proteins are increased in brown adipose tissue of obese rat.

PDE2 would play a major feedback role by restoring the basal level in cyclic nucleotide in action to hormonal stimulation and would participate in a interaction between cAMP and cGMP when present in the cell. The development of PDE2 inhibitors, as well as PDE2 transgenic mice, will be helpful to precise PDE2 implication in pathophysiology.[18]

Inhibitor

Until 1994, no specific inhibitor of PDE2 was known; therefore, some compounds were chosen to investigate PDE2 function in specific condition. For instance, we used cilostamide, a PDE3 selective inhibitor, as cGMP-stimulated PDE2 inhibitor in bovine aortic endothelial cells that are devoided of PDE3. EHNA (erythro-9-(2-hydroxy-3-nonyl) adenine), previously known as MEP 1, was shown to specifically act on PDE2 by inhibiting cGMP-activated PDE2 with an IC50 value of 3 µM.[19,20]

Phosphodiesterase 3 family

Overview

This enzyme, also called as new cardiotonic drug target in the 1980s, was firstly named cAMP-PDE, PDE III, or PDE IV, according to its elution order, and then cGI-PDE. The discovery of rolipram, both as tissue selective inhibitor[21] and as potent selective inhibitor for cAMP-PDE in regard to CaM-PDE and cGMP-PDE[22], has allowed to pharmacologically distinguish soluble and membrane associated PDE III[23] and to separate by chromatography the soluble cardiac cAMP-PDE in two fractions: ROI-PDE (rolipram-inhibited PDE) and cGI-PDE (cGMP-inhibited PDE).[24] The PDE3 enzyme was originally found mainly in the heart, liver, platelet, and adipocyte. Beavo's and Manganiello's teams first purified PDE3 from the heart and platelet to homogeneity.[25] PDE3 is characterized by its high affinity for cAMP and its capacity to hydrolyze both cAMP and cGMP, with Km values in submicromolar ranges (Km cAMP = 0.2 mM, Km cGMP= 0.1 mM). Since PDE3 hydrolyzes cAMP with a rate 10-fold greater than for cGMP hydrolysis, and since it has a greater affinity for cGMP, cGMP behaves as a competitive inhibitor of cAMP. This property contributes to various NO-induced cAMP/cGMP cross-talks in the platelet, in vascular smooth muscle intracellular signaling and in cardiac myocytes.[26]

Distribution

PDE3 like PDE2 is also cytosolic or membrane bound. It was shown to be associated to plasma membrane, sarcoplasmic reticulum and Golgi apparatus. PDE3A is mainly present in the heart, blood, and vascular smooth muscle whereas PDE3B is mainly present in adipocytes and hepatocytes.[5]

Regulation

Short-term activation of PDE3 was firstly demonstrated in rat fat cells in response to insulin and isoprenaline stimulations, which induce PDE3 phosphorylation. A single phosphorylated serine site (Ser 302) was identified.[27] This site could be phosphorylated either by PKA or by PKB, but the major site of PKB phosphorylation is Ser 273. The possible activation of PDE3B by PI3-K phosphorylation was recently implicated in the hypothalamic action of leptin on feeding as well as in b-cell insulin secretion. Furthermore, hormonal stimulation (insulin, glucagon) was shown to activate in vivo PDE3 associated to Golgiendosomal fraction.[28]

Inhibitor

Cilostamide (OPC-3689) was described as the first potent selective inhibitor of cAMP-PDE in the platelet. The comparison of its effects on cAMP-PDE isolated from platelets and from vascular smooth muscle, as well as its relaxant and antiaggregatory effect, pointed out the specificity of cilostamide for platelet cAMP-PDE (which was later identified as PDE3), indicating that platelet cAMP-PDE differed from vascular smooth muscle cAMP-PDE. Milrinone has been the most studied and used extensively as PDE3 inhibitor, and it is currently used in the acute treatment of heart failure to diminish long-term risks. Enoximone, piroximone, CI-930, sulmazole, pimobendan, and its metabolite UD-CG 212 CL all inhibit PDE3 at concentrations ranging from 10^{-7} to 10^{-5} M.[29-31]

Phosphodiesterase 4 family

Overview

PDE4, a cAMP-specific PDE, previously named cAMP-PDE, ROI-PDE, and PDE IV, is mainly present in the brain, inflammatory cells, cardiovascular tissues, and smooth muscles but is lacking in the platelets. PDE4 was shown to be specifically inhibited by rolipram (Ki=0.8 mM) and Ro-201724 and to be insensitive to cGMP allowing pharmacological discrimination of PDE4 family from PDE3 family. In another way, the antidepressant agent

rolipram binds with high affinity to rat brain, questioning the link between high affinity rolipram-binding site and PDE4.[32-34]

Distribution

PDE4 enzyme are localized in very complex system and it's isoforms may be found in cytosol or in cellular membranes. They are mainly found in the brain, heart, inflammatory cells and smooth muscle.[5]

Regulation

Activity of PDE4 is regulated by phosphorylation, protein association or endogenous mediator, as well as proteolysis. PKA-mediated phosphorylation takes place at acceptor site in UCR1 allows a rapid change in PDE4 activity. In vivo, prolonged elevation of cAMP results in increase of PDE4 activity resulting from hormonal stimulation, and it was proposed and shown to be a short-term feedback mechanism allowing cAMP level to return to basal cellular state. Dimerization was shown to be a requisite for the activation of PDE4 long forms by PKA phosphorylation, indicating that dimerization stabilizes PDE4 long forms in their high-affinity rolipram binding conformation. Furthermore, association of PDE4A4 with tyrosine kinases belonging from SRC family in intact cells, by SH3 interaction with the LR2 region of PDE4A, change the conformation of the PDE4 catalytic unit, increasing it sensitivity to rolipram.[35-37]

Inhibitor

Rolipram (ZK 62711, Schering AG), an antidepressant compound, was shown to be a potent cAMP-PDE inhibitor in brain homogenates. Denbufylline, a xanthine derivative, is also selective for PDE4, but inhibits PDE5 at 10-fold higher concentration. Benzyladenine derivatives were synthesized as potent and selective inhibitors being effective in vivo per oral administration. Presently, some new PDE4 inhibitors, with lesser emetic effects, are currently under clinical investigation, such as cilomilast and roflumilast.[38-41]

Phosphodiesterase 5 family

Overview

PDE5, previously named cGMP-PDE, cGMP-binding-cGMP-specific phosphodiesterase (cG-BPDE), or PDE V, was firstly characterized as a cGMP binding protein different from protein kinase co-purifying with a cGMP phosphodiesterase in rat platelets and in rat lung. In human, bovine, and rat vascular smooth muscle, PDE5 was purified and characterized as a

cytosolic PDE isozyme that specifically hydrolyzes cGMP without being activated by Ca/calmodulin and specifically inhibited by compound zaprinast, the archetype for PDE5 inhibitor, and insensitive to rolipram. Thomas and co-workers purified cG-BPDE from bovine lung to homogeneity. It was found to be a homodimer and having molecular weight of of 93 kDa subunit, this binds to 0.93 mol of cGMP/mol of subunit, and this is inhibited by zaprinast and very strongly to rolipram. The use of zaprinast to pharmacologic alleviation of PDE5 activity has allowed investigating the various functional properties of PDE5.[42,43]

Distribution

PDE5A was found in aortic smooth muscle cells, heart, placenta, skeletal muscle, pancreas, and, to a low concentration, in the brain, liver, and lung.[5]

Regulation

cGMP binding, phosphorylation, and protein – protein interaction mediate short-term regulation of PDE5. The binding of cGMP to allosteric site of PDE5 was shown to be required for its phosphorylation, which increases PDE5 activity with an apparent conformational change and a 10-fold increase in cGMP binding affinity. However, it was clearly demonstrated that cGMP could also directly activate PDE5 without phosphorylation in response to sustained NO in the platelet. These results from the binding of cGMP to the PDE5 GAF A domain, inducing a 9-to 11-fold reversible activation. In opposite, it was shown that proteins, that are immunologically related to the gamma subunit of PDE6, which may exist in smooth muscle, regulate PDE5 by preventing PKA-mediated activation of PDE5. Consequently, the PDE5 activation is depend on intracellular level of cGMP, which regulate at least 3 different steps of activation: a hydrolytic state in absence of cGMP; a reversible activated state when cGMP binds to GAF A, which is required to allow phosphorylation; and a full PDE5 activation.[44-47]

Inhibitor

Zaprinast is the first characterized selective PDE5 inhibitor.[22] MY-5445 was also shown to inhibit PDE5. Dipyridamole, for a long period, was referred and used as a specific inhibitor of PDE5.[48]

Phosphodiesterase 6 family

Overview

After showing that retinal PDE was the main site for light regulation of cyclic GMP metabolism, the partial purification of this PDE reveals that bovine photoreceptor cGMP-PDE was allosterically regulated by cGMP, having an major affinity for cGMP than for cAMP. High purification of rod outer segments cGMP-PDE from retinal frog show that the enzyme has a heterodimeric structure (120 and 110 kDa), with a Km of 70 mM, and that one molecule of bleached rhodopsin activates 1 molecule of cGMP-PDE. A model for cGMP cascade in the retina was proposed, in which photoactivated rhodopsin triggers a cascade of intense reactions mediated by transducin (G protein), which rise to the amplified activation of cGMP-PDE. Since this enzyme displayed a high specificity for cGMP, binding sites for cGMP, and sensitivity to zaprinast similar to smooth muscle PDE5, both enzymes were named cGMP-PDE. However, retinal cGMP-PDE, being specifically distributed in the retina, having a higher Vmax and Km values than other cGMP-PDEs and being regulated by G protein, was first called as photoreceptor cGMP-PDE, or rod outer segments PDE, that is, ROS-PDE and, next, according to Beavo nomenclature named PDE6.[49-52]

Regulation

cGMP binding, phosphorylation, and protein interaction are requisite processes to allow cGMP visualling cascade. The cGMP binding to GAF domains is regulated by the binding of γ and δ subunits to αβ PDE6 heterodimer. The γ subunit, which switches the PDE6 hydrolytic activity, is light regulated by GTPbound a subunit of transducin. Its phosphorylation by PKC may decrease PDE6 activation by transducin and, consequently, the photoresponse. Furthermore, its phosphorylation by cyclin-dependent protein kinase 5 inhibits transducin-activated PDE6, even in the presence of transducin, contributing in the recovery phase of photo-transduction.[53]

Inhibitor

Zaprinast and dipyridamole, as well as E4021, inhibit PDE6 as potently as PDE5. Due to the adverse vision effects of PDE6 inhibitors and the specific localization of PDE6, in the retina, there is no pharmaceutical investment on PDE6 inhibitors.[54]

Phosphodiesterase7 family

Overview

From a human glioblastoma cDNA library a cDNA was identified and isolated and shown to encode a novel cAMP-specific PDE characterized by high affinity for cAMP (*Km* = 0.2 mM) and low *Vmax*, which does not share other properties of PDE3 and PDE4 (insensitivity to cGMP, milrinone, rolipram, and Ro 20-1724). According to the Beavo nomenclature, this new family, insensitive to rolipram, was designed as the PDE7 family. Today, this family having 2 genes encoding PDE7A and PDE7B. The PDE7 family does not contain GAF domains as well as regulatory domains.[55]

Phosphodiesterase 8 family

Overview

Phosphodiesterase 8A

A high-affinity, cAMP-specific PDE (*Km* = 0.15 mM), insensitive to rolipram and IBMX, named PDE8A, was cloned from human and from mouse testis. PDE8 mRNA has highest the concentration in the testis, later on found in eye, liver, skeletal muscle, heart, kidney, ovary, and brain, in decreasing order. This PDE contains an N-terminus that is homologous to the PAS domain found in many signal transduction proteins. PDE8As few variants that is 1–5 splice variants of PDE8A were cloned from testis, T-cells showing various distributions of PAS domains, and a receiver (REC) domain for human PDE8A1 (93 kDa) and PDE8A (88.3kDa). In all tissues, the expression levels of PDE8A1 are much higher than that of PDE8As 2–5. PDE8A1 is induced in response to a combination of T-cell receptor and co-stimulatory receptor pathway activation.[56, 57]

Phosphodiesterase 8B

A second gene in the PDE8 family was discovered through a search of EST data base, encoding PDE8B, and showing 65% of identity to that of PDE8A. The mRNA encoding PDE8B is expressed specifically and abundantly in the thyroid gland. The recombinant PDE8B, like PDE8A, is insensitive to IBMX, rolipram, and milrinone, but is three-fold less sensitive to dipyridamole and more sensitive to PDE5 inhibitors and to erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA). There are at least 4 PDE8B variants: PDE8B1 (Km = 101 nM, 105 kDa), as well as PDE8B4 contains an N-terminal REC domain, a PAS domain, and a C-terminal catalytic domain, whereas PDE8B2 and PDE8B3 are not identified in the PAS domain. Three putative cAMP-and cGMP-dependent protein kinase phosphorylation sites are

identified between the PAS domain and the catalytic domain. RT-PCR analysis revealed that while PDE8B1 is the most abundant variant in the thyroid gland, PDE8B3 is the most abundant form in the brain. Mouse PDE8B1 protein is 96% and 99% identical to human and rat PDE8B1 respectively.[58,59]

Phosphodiesterase 9 family

Overview

In 1998, Beavo's and Cheng's teams independently and simultaneously identified PDE9 using either bioinformactic approach for mouse PDE9A1 or sequence homology for human PDE9A. Recombinant PDE9A1 (62 kDa) is highly specific for cGMP (Km = 0.07 mM, 40 – 170 times lower than that of PDE5 and PDE6, respectively) and is insensitive to IBMX or sildenafil, but is inhibited by the PDE1/PDE5 inhibitor, SCH51866, with an IC50 of 1.55 mM. PDE9A1 mRNA is highly distributed in the kidney and to lower levels in the liver, lung, and brain.[56] Similarly, human PDE9A mRNA is identified in the small intestine, spleen, and brain. Recombinant PDE9A has a Km of 170 nM for cGMP and 230 mM for cAMP. The Vmax for cGMP is about twice as fast as that of PDE4 for cAMP. PDE9A is insensitive to rolipram and IBMX but is inhibited by zaprinast (IC50=35 µM). The murine and human PDE9A cDNAs share 93.5% amino acid identity in the catalytic domain, and the putative regulatory domain of PDE9A does not contain a GAF domain.[60,61]

Phosphodiesterase 10 family

Overview

The PDE10 family was isolated and characterized as a dual-substrate gene family in 1999 from mouse as well as from human fetal lung and fetal brain. The deduced amino acid sequence contains 779 amino acids and includes 2 GAF domains at the N-terminal residue. A study on genomic organization reveals that despite containing two GAF domains, PDE10A has a different gene organization from PDE5A and PDE6B (both containing GAF domains) and suggests that the ancestral gene for PDE10A existed in a lower organism such as C. elegans. PDE10A hydrolyzes cAMP with a Km of 0.05 – 0.26 mM and cGMP with a Km of 3–7.2 mM. Although PDE10A has a lower Km for cAMP, the Vmax ratio cGMP/cAMP is 2–4.7. Because of these kinetics, hydrolysis of cGMP by PDE10 enzyme is potently inhibited by cAMP. PDE10A is mostly inhibited by dipyridamole (IC50=1 AM) and inhibited by IBMX (IC50 = 3–17 mM) and zaprinast (IC50=11–22 mM). PDE10A transcripts are particularly present in the brain, thyroid, and testis. PDE10A2, a novel alternative of human

PDE10A, having a putative phosphorylation site by Protein Kinase-A, was characterized as a major form in human tissues.[62-64]

Phosphodiesterase11 family

Overview

The PDE11 family represents a dual-substrate PDE family having a catalytic site most similar to PDE5 (50% identity and 71% similarity) than to PDE10A (41% identity and 64% similarity). PDE11 mRNA occurs in higher concentration in skeletal muscle, liver, prostate, kidney, pituitary glands and testis. PDE11A1 (491 aa) was cloned from human skeletal muscle and predicted to have a molecular mass of 55,786 Da and contained only one GAF domain. Western blotting of human tissue distinguishes three proteins of 78, 66, and 56kDa. Recombinant PDE11A1 hydrolyzes cGMP and cAMP with Km values of 0.52 and 1.04 mM, respectively, with similar max values. PDE11A is sensitive to IBMX (IC50=50 mM), zaprirnast (IC50=12 mM), and dipyridamole (IC50 = 0.37mM). The 66-kDa protein was characterized as PDE11A2 (576 aa; 65.8 kDa) and the 56-kDa protein as a PDE11A3 (684 aa; 78.1 kDa). Two splice variants were characterized: PDE11A3 (684 aa; 78 kDa), which contains one complete and one incomplete GAF domain in the N-terminal region; and PDE11A4 (934 aa; 100 kDa), which includes two complete GAF domains and a putative phosphorylation site for PKA and PKG.[65-67]

Phosphodiesterase 7/8/9/10/11 inhibitors

There are very few selective inhibitors known for these new families discovered by cloning, since their design is only beginning. IC242 inhibits PDE7A selectively. Recently, BRL 50481 was discovered as a PDE7 inhibitor. Thiadiazoles, a new structural class of potent and selective PDE7 inhibitors, acting in the nanomolar range, was found by Pfizer. For the last PDE families, only their differential sensitivity to known inhibitors was reported. PDE8A, insensitive to IBMX, is inhibited by dipyridamole.[68-70] ICPDE9A is only sensitive to zaprinast.[60] PDE10A is also inhibited by dipyridamole. PDE11A variants are sensitive to dipyridamole. There is no doubt that, in the near future, selective PDE inhibitors for PDE8 to 11 will be discovered, allowing to get insight in the knowledge of the functional role and potential therapeutic effects of these new PDEs.[66]

CONCLUSION

Theophylline and caffeine these are the non-selective PDE inhibitors and have been used therapeutically for over 70 years for a range of diseases. the search of selective PDE inhibitor

led to development of sildenafil in treating erectile dysfunction. Few PDE inhibitors are being investigated in a broad range of infections including the use of PDE2 inhibitors in sepsis; PDE5 inhibitors to treat sexual dysfunction in females, cardiovascular disease and pulmonary hypertension; and PDE4 inhibitors to treat asthma, COPD, allergic rhinitis, psoriasis, multiple sclerosis, depression, Alzheimer's disease and schizophrenia. Currently, the study is going on to inhibit the selective PDEs.

REFERENCES

1. Feldman AM, Mcnamara DM. Reevaluating the Role of Phosphodiesterase Inhibitors in the Treatment of Cardiovascular Disease, Clin. Cardiol, 2002; 25: 256–262.
2. Cheung WY. Biochem Biophys Res Commun, 1970; 38: 533–538.
3. Kakiuchi S., & Yamazaki R. Biochem Biophys Res Commun, 1970; 41: 1104–1110.
4. Huang CY, Chau V, Chock PB, Wang JH & Sharma RK. Proc Natl Acad Sci USA, 1981; 78: 871–874.
5. Ghosh R. et.al., Phosphodiesterase inhibitors: their role and Implications. IJPRIF, 2009; 1(4): 1148-1160.
6. Goraya TA, Masada N, Ciruela A & Cooper DM. J Biol Chem, 2004; 279: 40494–40504.
7. Giorgi M, Giordano D, Rosati J, Tata AM & Augusti-Tocco G. J Neurochem, 2002; 80: 970–979.
8. Epstein PM, Fiss K, Hachisu R & Andrenyak DM. Biochem Biophys Res Commun, 1982; 105: 1142–1149.
9. Lugnier C, Follenius A, Gerard D & Stoclet JC. Eur J Pharmacol, 1984; 98: 157–158.
10. Georget M, Mateo P, Vandecasteele G, Lipskaia L, Defer N, Hanoune J, et al. FASEB, 2003; J 17: 1380–1391.
11. Hagiwara M, Endo T & Hidaka H. Biochem Pharmacol, 1984; 33: 453–457.
12. Beavo JA, Hardman JG, Sutherland EW. J Biol Chem, 1971; 246: 3841–3846.
13. Manganiello VC, Tanaka T, Murashima S. Cyclic-GMP stimulated cyclic nucleotide phosphodiesterases. In J. Beavo, & M. D. Houslay (Eds.), Cyclic Nucleotide Phosphodiesterases: Structure, Regulation and Drug Action. John Wiley and Sons Ltd, 1990; 61-85.
14. Erneux C, Couchie D, Dumont JE, Baraniak J, Stec WJ, Abbad EG, et al. Eur J Biochem, 1981; 115: 503–510.
15. MacFarland RT, Zelus BD, Beavo JA. J Biol Chem, 1991; 266: 136-142.

16. Geoffroy V, Fouque F, Nivet V, Clot JP, Lugnier C, Desbuquois B, et al. Eur J Biochem, 1999; 259: 892-900.
17. Yanaka N, Kurosawa Y, Minami K, Kawai E & Omori K. Biosci Biochem, 2003; 67: 973–979.
18. Coudray C, Charon C, Komas N, Mory G, Diot-Dupuy F, Manganiello V et al. FEBS Lett, 1999; 456: 207–210.
19. Kessler T, Lugnier C. Eur J Pharmacol, 1995; 290: 163–167.
20. Podzuweit T, Nennstiel P, Muller A. Cell Signal, 1995; 7: 733–738.
21. Lugnier C, Stierle A, Beretz A, Schoeffter P, Le Bec A, Wermuth C G et al. Biochem Biophys Res Commun, 1983; 113: 954–959.
22. Lugnier C, Schoeffter P, Le Bec A, Strouthou E, Stoclet JC. Biochem Pharmacol, 1986; 35: 1743–1751.
23. Weishaar RE, Kobylarz-Singer DC, Steffen RP, Kaplan HR. Circ Res, 1987; 61: 539–547.
24. 24.Komas N, Lugnier C, Le Bec A, Serradeil-Le Gal C, Barthelemy G, Stoclet JC. J Cardiovasc Pharmacol, 1989; 14: 213–220.
25. Harrison SA, Reifsnyder DH, Gallis B, Cadd GG, Beavo JA. Mol Pharmacol, 1986; 29: 506–514.
26. Komas N, Lugnier C, Stoclet JC. Br J Pharmacol, 1991; 104: 495–503.
27. Rahn T, Ronnstrand L, Leroy MJ, Wernstedt C, Tornqvist H, Manganiello VC et al. J Biol Chem, 1996; 271: 11575–11580.
28. Geoffroy V, Fouque F, Lugnier C, Desbuquois B, Benelli C. Arch Biochem Biophys, 2001; 387: 154–162.
29. Hidaka H, Hayashi H, Kohri H, Kimura Y, Hosokawa T, Igawa T, et al. J Pharmacol Exp Ther, 1979; 211: 26– 30.
30. Cruickshank JM. Cardiovasc Drugs Ther, 1993; 7: 655–660.
31. Stoclet JC, Keravis T, Komas N, Lugnier C. Expert Opin Investig Drugs, 1995; 4: 1081–1100.
32. Tenor H, Schudt C. Analysis of PDE isoenzyme profiles in cells and tissues by pharmacological methods. In C. Schudt, G. Dent, & K. F. Rabe (Eds.), Phosphodiesterase Inhibitors, Handbook of Immunopharmacology Academic Press, 1996; 21-40.
33. Weishaar RE, Kobylarz-Singer DC, Steffen RP, Kaplan HR. Circ Res, 1987; 61: 539–547.

34. Schneider HH, Schmiechen R, Brezinski M, Seidler J. Eur J Pharmacol, 1986; 127: 105–115.
35. Sette C, Iona S, Conti M. J Biol Chem, 1994; 269: 9245–9252.
36. Richter W, Conti M. J Biol Chem, 2004; 279: 30338–30348.
37. McPhee I, Yarwood SJ, Scotland G, Huston E, Beard MB, Ross AH, et al. J Biol Chem, 1999; 274: 11796–11810.
38. Marivet MC, Bourguignon JJ, Lugnier C, Mann A, Stoclet JC, Wermuth CG. J Med Chem, 1989; 32: 1450–1457.
39. Lugnier C, Muller B, Le Bec A, Beaudry C, Rousseau E. J Pharmacol Exp Ther, 1993; 265: 1142–1151.
40. Bourguignon JJ, Desaubry L, Raboisson P, Wermuth CG, Lugnier C. J Med Chem, 1997; 40: 1768–1770.
41. Hatzelmann A, Schudt C. J Pharmacol Exp Ther, 2001; 297: 267–279.
42. Francis SH, Corbin JD. Crit Rev Clin Lab Sci, 1999; 36: 275–328.
43. Lugnier C, Schoeffter P, Le Bec A, Strouthou E, Stoclet JC. Biochem Pharmacol, 1986; 35: 1743–1751.
44. Francis SH, Bessay EP, Kotera J, Grimes KA, Liu L, Thompson WJ, et al. J Biol Chem, 2002; 277: 47581–47587.
45. Mullershausen F, Friebe A, Feil R, Thompson WJ, Hofmann F, Koesling D. J Cell Biol, 2003; 160: 719–727.
46. Lochhead A, Nekrasova E, Arshavsky VY, Pyne NJ. J Biol Chem, 1997; 272: 18397–18403.
47. Souness JE, Brazdil R, Diocee BK, Jordan R. Br J Pharmacol, 1989; 98: 725–734.
48. Goridis C, Virmaux N. Nature, 1974; 248: 57–58.
49. Coquil JF, Virmaux N, Mandel P, Goridis C. Biochim Biophys Acta, 1975; 403: 425–437.
50. Miki N, Baraban JM, Keirns JJ, Boyce JJ, Bitensky MW. J Biol Chem, 1975; 250: 6320–6327.
51. Chabre M, Bigay J, Bruckert F, Bornancin F, Deterre P, Pfister C et al. Visual signal transduction: the cycle of transducin shuttling between rhodopsin and cGMP phosphodiesterase. Cold Spring Harb Symp Quant Biol, 1988; 1: 313–324.
52. Hayashi F, Matsuura I, Kachi S, Maeda T, Yamamoto M, Fujii Y et al. J Biol Chem, 2000; 275: 32958–32965.
53. Gillespie PG, Beavo JA. Mol Pharmacol, 1989; 36: 773–781.

54. Michaeli T, Bloom TJ, Martins T, Loughney K, Ferguson K, Riggs M et al. J Biol Chem, 1993; 268: 12925–12932.
55. Soderling SH, Bayuga SJ, Beavo JA. Proc Natl Acad Sci USA, 1998B; 95: 8991–8996.
56. Wang P, Wu P, Egan RW, Billah MM. Gene, 2001; 280: 183–194.
57. Hayashi M, Matsushima K, Ohashi H, Tsunoda H, Murase S, Kawarada Y et al. Biochem Biophys Res Commun, 1998; 250: 751–756.
58. Hayashi M, Shimada Y, Nishimura Y, Hama T, Tanaka T. Biochem Biophys Res Commun, 2002; 297: 1253–1258.
59. Fisher DA, Smith JF, Pillar JS, St Denis SH, Cheng JB. J Biol Chem, 1998B; 273: 15559–15564.
60. Guipponi M, Scott HS, Kudoh J, Kawasaki K, Shibuya K, Shintani A et al. Hum Genet, 1998; 103: 386–392.
61. Soderling SH, Bayuga SJ, Beavo JA. Proc Natl Acad Sci USA, 1999; 96: 7071–7076.
62. Fujishige K, Kotera J, Yuasa K, Omori K. Eur J Biochem, 2000; 267: 5943–5951.
63. Kotera J, Fujishige K, Yuasa K, Omori K. Biochem Biophys Res Commun, 1999; 261: 551–557.
64. Fawcett L, Baxendale R, Stacey P, McGrouther C, Harrow I, Soderling S, et al. Proc Natl Acad Sci USA, 2000; 97: 3702–3707.
65. Hetman JM, Robas N, Baxendale R, Fidock M, Phillips SC, Soderling SH et al. Proc Natl Acad Sci USA, 2000; 97: 12891–12895.
66. Yuasa K, Kotera J, Fujishige K, Michibata H, Sasaki T, Omori K. J Biol Chem, 2000; 275: 31469–31479.
67. Lee R, Wolda S, Moon E, Esselstyn J, Hertel C, Lerner A. Cell Signal, 2002B; 14: 277–284.
68. Smith SJ, Cieslinski LB, Newton R, Donnelly LE, Fenwick PS, Nicholson AG. et al. Mol Pharmacol, 2004; 66: 1679–1689.
69. Vergne F, Bernardelli P, Lorthiois E, Pham N, Proust E, Oliveira C. et al. Bioorg Med Chem Lett, 2004; 14: 4615–4621.
70. Fisher DA, Smith JF, Pillar JS, St Denis SH, Cheng JB. Biochem Biophys Res Commun, 1998a; 246: 570–577.