

Proceedings

Open Access

## Cyclic AMP signalling pathways in the regulation of uterine relaxation

Wei Yuan and Andrés López Bernal\*

Address: University of Bristol, Clinical Science at South Bristol (Obstetrics and Gynaecology), St Michael's Hospital and Dorothy Hodgkin Building, Whitson Street, Bristol, BS1 3NY, UK

Email: Wei Yuan - Wei.Yuan@Bristol.ac.uk; Andrés López Bernal\* - A.LopezBernal@bristol.ac.uk

\* Corresponding author

from Special Non-Invasive Advances in Fetal and Neonatal Evaluation Network of Excellence, First and Second European Workshops on Preterm Labour Tarragona, Spain. 21–22 September 2006 and 22 June 2005

Published: 1 June 2007

*BMC Pregnancy and Childbirth* 2007, **7**(Suppl 1):S10 doi:10.1186/1471-2393-7-S1-S10

This article is available from: <http://www.biomedcentral.com/1471-2393/7/S1/S10>

© 2007 Yuan and López Bernal; licensee BioMed Central Ltd.

This is an open access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

### Abstract

Studying the mechanism(s) of uterine relaxation is important and will be helpful in the prevention of obstetric difficulties such as preterm labour, which remains a major cause of perinatal mortality and morbidity. Multiple signalling pathways regulate the balance between maintaining relative uterine quiescence during gestation, and the transition to the contractile state at the onset of parturition. Elevation of intracellular cyclic AMP promotes myometrial relaxation, and thus quiescence, via effects on multiple intracellular targets including calcium channels, potassium channels and myosin light chain kinase. A complete understanding of cAMP regulatory pathways (synthesis and hydrolysis) would assist in the development of better tocolytics to delay or inhibit preterm labour. Here we review the enzymes involved in cAMP homeostasis (adenylyl cyclases and phosphodiesterases) and possible myometrial substrates for the cAMP dependent protein kinase. We must emphasise the need to identify novel pharmacological targets in human pregnant myometrium to achieve safe and selective uterine relaxation when this is indicated in preterm labour or other obstetric complications.

### Background

Preterm labour is a major reproductive health problem due to a high incidence of severe short- and long-term infant morbidity. In developed countries, social and medical advances have improved the survival rate of the preterm neonate; however, the incidence of preterm birth and related morbidity remain a serious challenge and the physiopathological mechanisms of preterm birth are still a mystery. The use of tocolytic drugs, including those that operate through cyclic AMP such as beta-mimetics, to inhibit uterine contractility in preterm labour is contro-

versial because there is no evidence that currently available drugs improve long term neonatal outcome. Moreover, some tocolytics can cause serious side effects such as tachycardia, hypertension and pulmonary oedema. The potential of drugs of high uterine selectivity, e.g. oxytocin receptor antagonists, for the management of preterm labour is encouraging and further clinical trials are being undertaken. However, there is a need to identify novel pharmacological targets in myometrium to provoke safe and selective uterine relaxation when this is clinically indicated [1].

It is not known whether the cause of preterm labour is the premature loss of uterine quiescence (e.g. removal of inhibitory factors), or the induction of uterine contractility (e.g. release of stimulatory mediators) or a combination of both [2]. The second messenger cyclic adenosine monophosphate (cAMP) is known to promote the relaxation of smooth muscle, and is likely to be implicated in the maintenance of uterine quiescence [2]. Hence, the study of myometrial cAMP regulatory pathways will help to understand the mechanism of labour and highlight possible targets for the development of more specific and effective tocolytics for preterm labour.

### Cyclic AMP signalling pathways

Cyclic AMP is a diffusible intracellular second messenger, which influences many physiological events, by transducing hormone and small molecule effects into activation of protein kinases, modulating calcium transport and regulating gene activation. Its function in the relaxation of uterine and other types of smooth muscle is believed to be via inhibition of calcium mobilization and the contractile apparatus [3], through the activation of cAMP-dependent protein kinase (PRKA), which phosphorylates target proteins such as myosin light chain kinase (MYLK) [4] and phospholipase C (PLC) [5] (Table 1 and Figure 1). However the identification of PRKA substrates in human myometrium is a challenging area of research and more information is required before the mechanism of cyclic nucleotide-induced relaxation is understood.

The synthesis and catabolism of cAMP has been studied in many systems. Adenylyl cyclases (ADCY) catalyse the conversion of ATP to cAMP in response to the actions of hormones and drugs acting upon cell surface G protein coupled receptors. The cAMP formed is hydrolysed by a family of cyclic nucleotide phosphodiesterases (PDE). In myometrium ADCY activity is induced by endogenous agonists (catecholamines, prostaglandins, etc) operating through protein receptors e.g. ADRB2 ( $\beta$ -adrenoceptor),

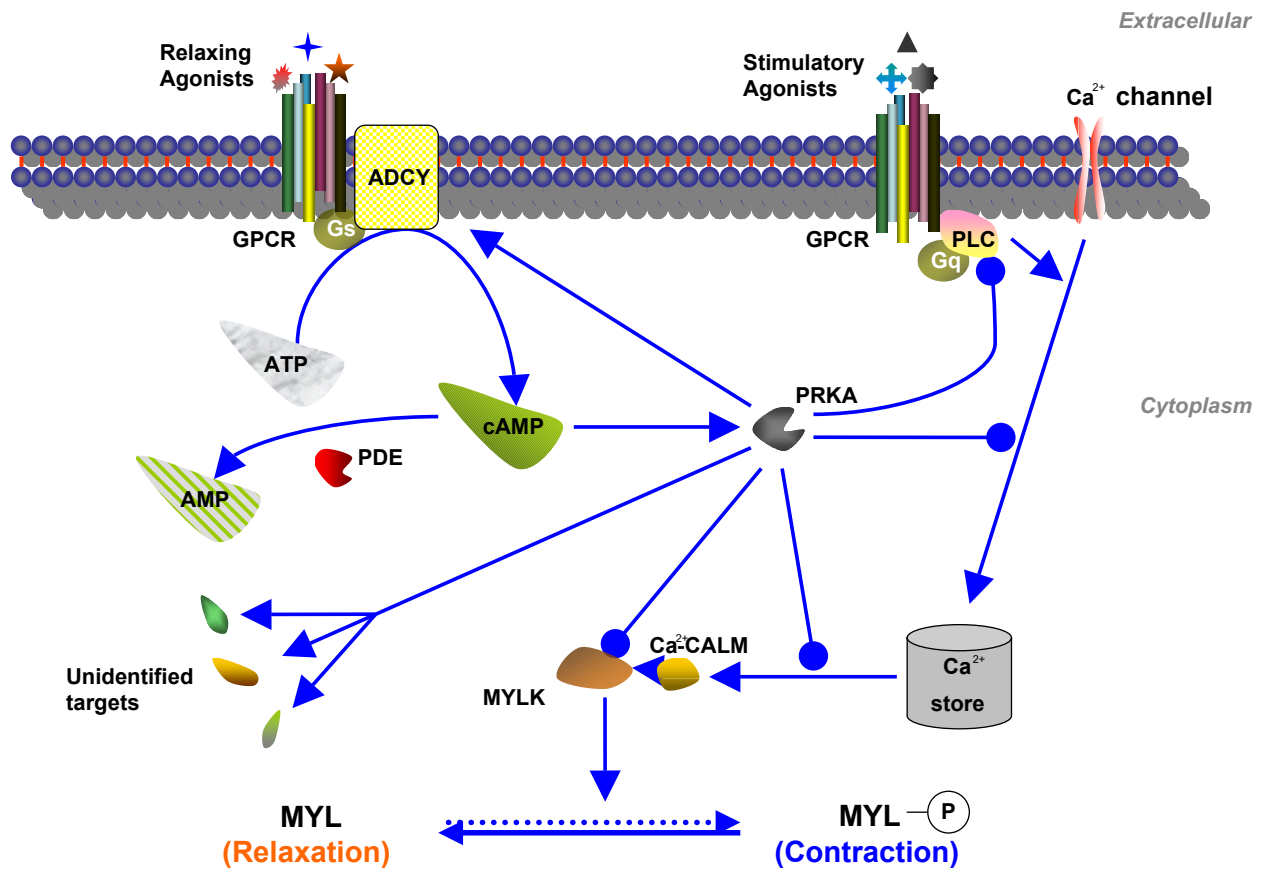
PTGER2 (prostaglandin E2 receptor) coupled to Gs [6]. Activated ADCY removes one pyrophosphate molecule to convert ATP to cAMP and releases it into the cytoplasm. Cyclic AMP activates PRKA by binding to its catalytic subunits. Spatial and temporal changes in cAMP levels can be translated into compartmentalised responses by PRKA anchoring proteins which also bind other protein kinases and PDE isoforms to regulate a variety of signalling activities including feedback phosphorylation of ADCY [7]. The concentration of cAMP is reduced rapidly by PDE hydrolysis. Regulation of PDEs, especially PDE4, the largest myometrial PDE family, is an attractive area of study because of its potential as a new target for tocolysis. Long forms of PDE4 are involved in fast cAMP signalling loops through PRKA activation at conserved phosphorylation domains, while short forms of PDE4 are associated with long term responses promoted by cAMP such as gene transcription. Moreover, prostaglandin E2 up-regulates PDE activity by inducing the synthesis of PDE4 short isoforms [8].

### Adenylyl cyclases and their regulators

Is there a specific myometrial cAMP generation system? This question is important because it would provide a target for pharmacological intervention. ADCY are a family of disparate enzymes directly involved in cAMP synthesis. The catalytic core of mammalian ADCY consists of a pseudosymmetric heterodimer composed of two highly conserved cytosolic regions, namely C<sub>1a</sub> and C<sub>2a</sub>, which can bind Gs and ATP. All isoforms of ADCY are activated by the  $\alpha$  subunit of Gs (G $\alpha$ s) and most are directly activated by the diterpene forskolin, which is a useful tool for functional studies [9,10]. The C<sub>1a</sub> and C<sub>2a</sub> regions alone are sufficient for cAMP generation, and linkage of portions of these cytosolic domains yields a soluble form of ADCY, which is fully activated by both G $\alpha$ s and forskolin [11,12].

**Table 1: Potential protein kinase A substrates involved in the regulation of human uterine relaxation**

Physiological roles (see Shabb (2001) [38])	Protein substrate (HUGO nomenclature)	References
Autophosphorylation cAMP signalling	cAMP dependant protein kinase regulatory subunit type II $\alpha$ (PRKAR2A) $\beta$ -2 adrenoceptor (ADRB2) G protein coupled receptor kinase-2 (ADRBK1) Phosphodiesterase 4 (PDE4)	Zakhary <i>et al.</i> (2000) [39] Daaka <i>et al.</i> (1997) [40]; Iyer <i>et al.</i> (2006) [41] Houslay & Baillie (2006) [42] Murthy <i>et al.</i> (2002) [43]
Phosphoinositide and calcium signalling	InsP3 Type I receptor (ITPR1) Phospholipase-C $\beta$ 3 (PLCB3) Phospholipase-C $\gamma$ 1 (PLCG1) ATPase 2 (ATP2) Regulators of G-protein signalling (RGS) Thromboxane A2 receptor (TBXA1R)	Straub <i>et al.</i> (2004) [44] Yue <i>et al.</i> (1998) [45] Park <i>et al.</i> (1992) [46] Tribe <i>et al.</i> (2000) [47] Suarez <i>et al.</i> (2003) [48] Walsh <i>et al.</i> (2000) [49]
Rho signalling	RhoA small GTP binding protein (RHOA) G $\alpha$ 13	Murthy <i>et al.</i> (2003) [50] Manganello <i>et al.</i> (2003) [51]
Smooth muscle contraction	Myosin light chain kinase (MYLK) Myosin binding subunit of myosin phosphatase (PPP1R12A)	Hirano <i>et al.</i> (2004) [52] Wooldridge <i>et al.</i> (2004) [53]



**Figure 1**

**Cyclic AMP pathways in myometrial tissue.** Activation of membrane receptors (GPCR) coupled to G<sub>s</sub> activates adenylyl cyclase (ADCY) which converts ATP to cAMP. The levels of cAMP are tightly regulated by phosphodiesterases (PDE), especially PDE4 isoforms. It is thought that cAMP induces uterine relaxation via activation of a specific protein kinase (PRKA) which phosphorylates and inhibits myosin light chain kinase (MYLK). PRKA may also oppose the effect of stimulatory receptors which operate through the phospholipase C (PLC)/calcium pathway. However the precise targets for PRKA phosphorylation in human myometrium are under investigation (→ stimulation, ● inhibition)

So far, nine mammalian membrane-bound isoforms of ADCY have been identified, and allocated into one of four subgroups, according to their modes of regulation [9] (table 2). All isoforms are activated by G<sub>αs</sub> binding primarily to a hydrophobic, negatively charged pocket on the C<sub>2a</sub> domain [10]. Since the site of interaction of G<sub>αs</sub> with ADCY is distal to the catalytic site, it has been suggested that the likely mechanism for G<sub>αs</sub> activation involves a modulation of the structure of the active site by the induction of a conformational change. All ADCYs, except ADCY 9, which lacks the required serine and leucine residues, can be activated by forskolin, which binds in a narrow hydrophobic crevice between the C<sub>1a</sub> and C<sub>2a</sub> domains [13]. Activation of ADCYs by forskolin occurs even in the absence of a functional G protein, and is, therefore, a con-

sequence of a direct action on the catalytic subunit of the enzyme [14].

Responses of ADCYs to other modulators can be very different. For example, Group 1 cyclases (ADCY1, ADCY3, ADCY8) are stimulated by calcium/calmodulin (Ca<sup>2+</sup>-CALM) and by PRKA but are inhibited by the α subunits of proteins of the G<sub>αi/o</sub> family and also by G protein βγ subunits (G<sub>βγ</sub>). In contrast, Group 2 isoforms (ADCY2, ADCY4, and ADCY7) are activated by G<sub>βγ</sub> and insensitive to Ca<sup>2+</sup>-CALM [15]. Group 3 isoforms (ADCY5 and ADCY6) are similar to Group 1, in that they are inhibited by G<sub>αi/o</sub> and G<sub>βγ</sub>, but they are also inhibited by the pertussis toxin-insensitive inhibitory G-protein G<sub>αz</sub>, by low concentrations of calcium, and by PRKA phosphorylation

**Table 2: Adenylyl cyclase (ADCY) isoforms**

Sub-family	Mode of regulation	Member	Chromosomal localization	References
1	Activated by calcium, G $\alpha$ s, PRKA inhibited by G $\alpha$ i/o and G $\beta$ $\gamma$	ADCY1 ADCY3 ADCY8	7p12 5p15 2p22-24	Villacres <i>et al.</i> (1993) [54] Diel <i>et al.</i> (2006) [55] Steiner <i>et al.</i> (2006) [56]
2	Activated by G $\alpha$ s and G $\beta$ $\gamma$	ADCY2 ADCY4 ADCY7	14q11.2 3q13.2-q21 12q12-13	Nguyen & Watts (2006) [57] Wang & Burns (2006) [58] Guzman <i>et al.</i> (2005) [59]
3	Activated by G $\alpha$ s, Inhibited by G $\alpha$ i/o, G $\beta$ $\gamma$ calcium, PRKA	ADCY5 ADCY6	16q12-13 8q24	Iwami <i>et al.</i> (1995) [60] Chen <i>et al.</i> (1997) [61]
4	Activated by G $\alpha$ s	ADCY9	16p13	Hacker <i>et al.</i> (1998) [62]

at Ser-674 [16]. ADCY9 belongs in a separate group because it is insensitive to either calcium or G $\alpha$ i/o/G $\beta$  $\gamma$  but is inhibited by the phosphatase calcineurin [17]. The effects of protein kinase C (PRKC) on ADCY are very complex and cut across groups. For instance PRKC phosphorylation enhances the activities of ADCY2 and ADCY7 (Group 2) as well as ADCY5 (Group 3). On the other hand phosphorylation by PRKC reduces the activities of ADCY4 (Group2) and ADCY6 (Group 3) [9,15]. In pregnancy there is upregulation of group 2 and group 3 isoforms in myometrium [18], but the question whether there are dominant ADCY isoforms coupled to specific myometrial receptors or whether there is promiscuity in the coupling of GPCR/G $\alpha$ s complexes to several ADCY isoforms remains unanswered. The development of sensitive fluorescent tags to study molecular proximity in real time will help answer this question in myometrial cells.

### Phosphodiesterases and their regulators

The phosphodiesterases (PDE) multi-gene family of enzymes catalyze the hydrolysis of 3',5'-cyclic nucleotides, and so occupy a key position in modulating intracellular cyclic nucleotide levels [19]. In myometrial tissue, the activity of PDE enzymes is significantly inhibited during pregnancy [20], probably as a consequence of the high progesterone levels [21], suggesting that cAMP-PDE inhibition may be part of the mechanism for myometrial relaxation and pregnancy maintenance.

At present, more than 14 PDE genes have been cloned in mammals and 21 gene products have been identified. PDE isoforms are grouped in 11 distinct but related families (PDE1 to PDE11), according to sequence homology, substrate specificity, inhibitor sensitivity and function. Within these families alternative splicing or mRNA transcription initiation result in additional diversity producing around 100 mRNA products, but it is not clear how many of these are actually translated into different proteins [19,22]. The protein isoforms all share a common structural pattern: a C-terminus whose function is unclear; a conserved catalytic core, which determines substrate specificity and inhibitor sensitivity; and a divergent N-terminus responsible for the enzyme's targeting [19,22].

PDE subfamilies are differentially expressed in human tissues, and only PDE1-5 subtypes have been detected in human term myometrium [23]. PDE1 subtypes are of mixed substrate specificity for cyclic nucleotides (they tend to hydrolyse either cAMP or cGMP) and contain auto-inhibitory substrate-like sequences; such inhibition is relieved by Ca<sup>2+</sup>/CALM [24]. PDE1 isoforms are clearly regulated by PRKA and are involved in cell proliferation [25].

PDE2 and PDE5 have similar structures as both of them contain allosteric cyclic nucleotide binding sites for cGMP. The binding of cGMP to PDE2 stimulates cAMP hydrolysis to mediate cross-talk between cGMP and cAMP pathways [26,27]. PDE5 expression is inhibited by hCG in human myometrium [28], and some evidence suggested that PDE5 may play a role in myometrial cell proliferation and cGMP dependent potassium channel regulation. High doses of sildenafil, a specific PDE5 inhibitor, relax human pregnant myometrium in vitro, but this effect is unlikely to be of tocolytic value [29].

PDE3 has two isoforms PDE3A and PDE3B, which contain a conserved catalytic site for both cAMP and cGMP binding [30]. Functional studies suggest that PDE3 is involved in vascular smooth muscle contractions, heart function, platelet aggregation, oocyte maturation and cell metabolism [31].

PDE4 is the largest PDE subfamily, consisting two classes of isoforms, long or short PDE4, depending upon the presence or absence of upstream conserved regions. Both classes of PDE4 have a much higher affinity for cAMP than for cGMP [32]. In human myometrial cell, the major source (up to 75%) of PDE activity is provided by PDE4 isoforms and membrane targeting can influence the maximal activity of the enzyme and its sensitivity [23,33]. Early functional studies of PDE4 showed that specific inhibition with rolipram leads to inhibition of the spontaneous contraction of myometrial strips [34,35]. However, rolipram has significant side effects, particularly nausea and vomiting. Cilomilast, originally designed as a novel therapy for asthma with fewer side effects, produces

concentration-dependent inhibition of the spontaneous contractions of term myometrium [36]. PDE4 selective inhibitors reduce the incidence of inflammation-induced preterm delivery in mice [37]. A better understanding of the regulation of PDE4 isoforms in pregnant human myometrium may be a useful approach to develop tocolytics of high uterine selectivity [33].

## Conclusion

The role of cAMP as an important mediator in the regulation of myometrial function is beyond dispute. Much is known about the pathways for cAMP synthesis and hydrolysis. However, the mechanisms of action of cAMP and its associated kinase PRKA in myometrial cells are not fully understood. Several candidate PRKA substrates have been proposed based on studies in other types of smooth muscle. More research into the identification of physiological PRKA substrates in pregnant human myometrium and into their regulation by phosphorylation will clarify the role of cAMP in pregnancy maintenance, and provide novel targets for uterine-selective therapeutic intervention.

## List of abbreviations used

ADCY, adenylyl cyclases; ADRB2,  $\beta$ 2-adrenoceptor; CALM, calmodulin; cAMP, cyclic AMP (cyclic adenosine monophosphate); GPCR, G protein-coupled receptor; MYL, myosin light chain kinase; PDE, cyclic nucleotide phosphodiesterase; PLC, phospholipase C; PRKA, cAMP-dependent protein kinase; PRKC, protein kinase C; PTGER2, prostaglandin E2 receptor.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

WY drafted the manuscript. ALB conceived the study, and helped to draft the manuscript. All authors read and approved the final manuscript.

## Acknowledgements

WY is a PhD student supported by the SAFE network of Excellence (LSHB-CT-2004-503243). Also we would like to thank Sarah A. Price and Edward Man-Lik Choi for unpublished material. We are grateful to Dr Ciara O'Sullivan and Dr Jorg Strutwolf for hosting SAFE workshops in Tarragona. Sponsorship by Ferring, PerkinElmer and Serono covered the publication cost.

This article has been published as part of *BMC Pregnancy and Childbirth* Volume 7, Supplement 1, 2007: Proceedings of the First and Second European Workshops on Preterm Labour of the Special Non-Invasive Advances in Fetal and Neonatal Evaluation Network of Excellence. The full contents of the supplement are available online at <http://www.biomedcentral.com/1471-2393/7?issue=S1>.

## References

- Keirse MJ: **New perspectives for the effective treatment of preterm labor.** *Am J Obstet Gynecol* 1995, **173**(2):618-628.
- Price SA, López Bernal A: **Uterine quiescence: the role of cyclic AMP.** *Exp Physiol* 2001, **86**(2):265-272.
- Sanborn BM: **Hormones and calcium: mechanisms controlling uterine smooth muscle contractile activity.** *The Litchfield Lecture.* *Exp Physiol* 2001, **86**(2):223-237.
- Word RA: **Myosin phosphorylation and the control of myometrial contraction/relaxation.** *Semin Perinatol* 1995, **19**(1):3-14.
- Sanborn BM, Ku CY, Shlykov S, Babich L: **Molecular signaling through G-protein-coupled receptors and the control of intracellular calcium in myometrium.** *J Soc Gynecol Investig* 2005, **12**(7):479-487.
- Lopez Bernal A, Europe-Finner GN, Phaneuf S, Watson SP: **Preterm labour: a pharmacological challenge.** *Trends Pharmacol Sci* 1995, **16**(4):129-133.
- Houslay MD, Milligan G: **Tailoring cAMP-signalling responses through isoform multiplicity.** *Trends Biochem Sci* 1997, **22**(6):217-224.
- Mehats C, Tanguy G, Dallot E, Cabrol D, Ferre F, Leroy MJ: **Is up-regulation of phosphodiesterase 4 activity by PGE2 involved in the desensitization of beta-mimetics in late pregnancy human myometrium?** *J Clin Endocrinol Metab* 2001, **86**(11):5358-5365.
- Hanoune J, Pouille Y, Tzavara E, Shen T, Lipskaya L, Miyamoto N, Suzuki Y, Defer N: **Adenylyl cyclases: structure, regulation and function in an enzyme superfamily.** *Mol Cell Endocrinol* 1997, **128**(1-2):179-194.
- Tesmer JJ, Sprang SR: **The structure, catalytic mechanism and regulation of adenylyl cyclase.** *Curr Opin Struct Biol* 1998, **8**(6):713-719.
- Tang WJ, Gilman AG: **Construction of a soluble adenylyl cyclase activated by Gs alpha and forskolin.** *Science* 1995, **268**(5218):1769-1772.
- Sunahara RK, Dessauer CW, Whisnant RE, Kleuss C, Gilman AG: **Interaction of Gsalpha with the cytosolic domains of mammalian adenylyl cyclase.** *J Biol Chem* 1997, **272**(35):22265-22271.
- Hurley JH: **Structure, mechanism, and regulation of mammalian adenylyl cyclase.** *J Biol Chem* 1999, **274**(12):7599-7602.
- Seamon K, Daly JW: **Activation of adenylyl cyclase by the diterpene forskolin does not require the guanine nucleotide regulatory protein.** *J Biol Chem* 1981, **256**(19):9799-9801.
- Taussig R, Gilman AG: **Mammalian membrane-bound adenylyl cyclases.** *J Biol Chem* 1995, **270**(1):1-4.
- Krupinski J, Cali JJ: **Molecular diversity of the adenylyl cyclases.** *Adv Second Messenger Phosphoprotein Res* 1998, **32**:53-79.
- Antoni FA, Palkovits M, Simpson J, Smith SM, Leitch AL, Rosie R, Fink G, Paterson JM: **Ca2+/calcineurin-inhibited adenylyl cyclase, highly abundant in forebrain regions, is important for learning and memory.** *J Neurosci* 1998, **18**(23):9650-9661.
- Price SA, Pochun I, Phaneuf S, Lopez Bernal A: **Adenylyl cyclase isoforms in pregnant and non-pregnant human myometrium.** *J Endocrinol* 2000, **164**(1):21-30.
- Bender AT, Beavo JA: **Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use.** *Pharmacol Rev* 2006, **58**(3):488-520.
- Kofinas AD, Rose JC, Meis PJ: **Changes in cyclic adenosine monophosphate-phosphodiesterase activity in nonpregnant and pregnant human myometrium.** *Am J Obstet Gynecol* 1987, **157**(3):733-738.
- Kofinas AD, Rose JC, Koritnik DR, Meis PJ: **Progesterone and estradiol concentrations in nonpregnant and pregnant human myometrium. Effect of progesterone and estradiol on cyclic adenosine monophosphate-phosphodiesterase activity.** *J Reprod Med* 1990, **35**(11):1045-1050.
- Bushnik T, Conti M: **Role of multiple cAMP-specific phosphodiesterase variants.** *Biochem Soc Trans* 1996, **24**(4):1014-1019.
- Leroy MJ, Lugnier C, Merezak J, Tanguy G, Olivier S, Le Bec A, Ferre F: **Isolation and characterization of the rolipram-sensitive cyclic AMP-specific phosphodiesterase (type IV PDE) in human term myometrium.** *Cell Signal* 1994, **6**(4):405-412.
- Sonnenburg WK, Seger D, Kwak KS, Huang J, Charbonneau H, Beavo JA: **Identification of inhibitory and calmodulin-binding domains of the PDE1A1 and PDE1A2 calmodulin-stimulated**

- cyclic nucleotide phosphodiesterases. *J Biol Chem* 1995, **270**(52):30989-31000.
25. Ang KL, Antoni FA: **Reciprocal regulation of calcium dependent and calcium independent cyclic AMP hydrolysis by protein phosphorylation.** *J Neurochem* 2002, **81**(3):422-433.
  26. Martins TJ, Mumby MC, Beavo JA: **Purification and characterization of a cyclic GMP-stimulated cyclic nucleotide phosphodiesterase from bovine tissues.** *J Biol Chem* 1982, **257**(4):1973-1979.
  27. Wu AY, Tang XB, Martinez SE, Ikeda K, Beavo JA: **Molecular determinants for cyclic nucleotide binding to the regulatory domains of phosphodiesterase 2A.** *J Biol Chem* 2004, **279**(36):37928-37938.
  28. Belmonte A, Ticconi C, Dolci S, Giorgi M, Zicari A, Lenzi A, Jannini EA, Piccione E: **Regulation of phosphodiesterase 5 expression and activity in human pregnant and non-pregnant myometrial cells by human chorionic gonadotropin.** *J Soc Gynecol Invest* 2005, **12**(8):570-577.
  29. Mehats C, Schmitz T, Breuiller-Fouche M, Leroy MJ, Cabrol D: **Should phosphodiesterase 5 selective inhibitors be used for uterine relaxation?** *Am J Obstet Gynecol* 2006, **195**(1):184-185.
  30. Scapin G, Patel SB, Chung C, Varnerin JP, Edmondson SD, Mastracchio A, Parmee ER, Singh SB, Becker JW, Van der Ploeg LH, et al.: **Crystal structure of human phosphodiesterase 3B: atomic basis for substrate and inhibitor specificity.** *Biochemistry* 2004, **43**(20):6091-6100.
  31. Ding B, Abe J, Wei H, Huang Q, Walsh RA, Molina CA, Zhao A, Sadoshima J, Blaxall BC, Berk BC, et al.: **Functional role of phosphodiesterase 3 in cardiomyocyte apoptosis: implication in heart failure.** *Circulation* 2005, **111**(19):2469-2476.
  32. Houslay MD, Adams DR: **PDE4 cAMP phosphodiesterases: modular enzymes that orchestrate signalling cross-talk, desensitization and compartmentalization.** *Biochem J* 2003, **370**(Pt 1):1-18.
  33. Mehats C, Oger S, Leroy MJ: **Cyclic nucleotide phosphodiesterase-4 inhibitors: a promising therapeutic approach to premature birth?** *Eur J Obstet Gynecol Reprod Biol* 2004, **117**(Suppl 1):S15-17.
  34. Leroy MJ, Cedrin I, Breuiller M, Giovagranti Y, Ferre F: **Correlation between selective inhibition of the cyclic nucleotide phosphodiesterases and the contractile activity in human pregnant myometrium near term.** *Biochem Pharmacol* 1989, **38**(1):9-15.
  35. Bardou M, Cortijo J, Loustalot C, Taylor S, Perales-Marin A, Mercier FJ, Dumas M, Deneux-Tharaux C, Frydman R, Morcillo EJ, et al.: **Pharmacological and biochemical study on the effects of selective phosphodiesterase inhibitors on human term myometrium.** *Naunyn Schmiedebergs Arch Pharmacol* 1999, **360**(4):457-463.
  36. Oger S, Mehats C, Barnette MS, Ferre F, Cabrol D, Leroy MJ: **Anti-inflammatory and utero-relaxant effects in human myometrium of new generation phosphodiesterase 4 inhibitors.** *Biol Reprod* 2004, **70**(2):458-464.
  37. Schmitz T, Souil E, Herve R, Nicco C, Batteux F, Germain G, Cabrol D, Evain-Brion D, Leroy MJ, Mehats C: **PDE4 inhibition prevents preterm delivery induced by an intrauterine inflammation.** *J Immunol* 2007, **178**(2):1115-1121.
  38. Shabb JB: **Physiological substrates of cAMP-dependent protein kinase.** *Chem Rev* 2001, **101**(8):2381-2411.
  39. Zakhary DR, Fink MA, Ruehr ML, Bond M: **Selectivity and regulation of A-kinase anchoring proteins in the heart. The role of autophosphorylation of the type II regulatory subunit of cAMP-dependent protein kinase.** *J Biol Chem* 2000, **275**(52):41389-41395.
  40. Daaka Y, Luttrell LM, Lefkowitz RJ: **Switching of the coupling of the beta2-adrenergic receptor to different G proteins by protein kinase A.** *Nature* 1997, **390**(6655):88-91.
  41. Iyer V, Tran TM, Foster E, Dai W, Clark RB, Knoll BJ: **Differential phosphorylation and dephosphorylation of beta2-adrenoceptor sites Ser262 and Ser355,356.** *Br J Pharmacol* 2006, **147**(3):249-259.
  42. Houslay MD, Baillie GS: **Phosphodiesterase-4 gates the ability of protein kinase A to phosphorylate G-protein receptor kinase-2 and influence its translocation.** *Biochem Soc Trans* 2006, **34**(Pt 4):474-475.
  43. Murthy KS, Zhou H, Makhlof GM: **PKA-dependent activation of PDE3A and PDE4 and inhibition of adenylyl cyclase V/VI in smooth muscle.** *Am J Physiol Cell Physiol* 2002, **282**(3):C508-517.
  44. Straub SV, Wagner LE 2nd, Bruce JI, Yule DI: **Modulation of cytosolic calcium signaling by protein kinase A-mediated phosphorylation of inositol 1,4,5-trisphosphate receptors.** *Biol Res* 2004, **37**(4):593-602.
  45. Yue C, Dodge KL, Weber G, Sanborn BM: **Phosphorylation of serine 1105 by protein kinase A inhibits phospholipase Cbeta3 stimulation by Galphaq.** *J Biol Chem* 1998, **273**(29):18023-18027.
  46. Park DJ, Min HK, Rhee SG: **Inhibition of CD3-linked phospholipase C by phorbol ester and by cAMP is associated with decreased phosphotyrosine and increased phosphoserine contents of PLC-gamma 1.** *J Biol Chem* 1992, **267**(3):1496-1501.
  47. Tribe RM, Moriarty P, Poston L: **Calcium homeostatic pathways change with gestation in human myometrium.** *Biol Reprod* 2000, **63**(3):748-755.
  48. Suarez VR, Park ES, Hankins GD, Soloff MS: **Expression of regulator of G protein signaling-2 in rat myometrium during pregnancy and parturition.** *Am J Obstet Gynecol* 2003, **188**(4):973-977.
  49. Walsh MT, Foley JF, Kinsella BT: **The alpha, but not the beta, isoform of the human thromboxane A2 receptor is a target for prostacyclin-mediated desensitization.** *J Biol Chem* 2000, **275**(27):20412-20423.
  50. Murthy KS, Zhou H, Grider JR, Makhlof GM: **Inhibition of sustained smooth muscle contraction by PKA and PKG preferentially mediated by phosphorylation of RhoA.** *Am J Physiol Gastrointest Liver Physiol* 2003, **284**(6):G1006-1016.
  51. Manganello JM, Huang JS, Kozasa T, Voyno-Yasenetskaya TA, Le Breton GC: **Protein kinase A-mediated phosphorylation of the Galpha13 switch I region alters the Galpha13-G protein-coupled receptor complex and inhibits Rho activation.** *J Biol Chem* 2003, **278**(1):124-130.
  52. Hirano K, Hirano M, Kanaide H: **Regulation of myosin phosphorylation and myofilament Ca2+ sensitivity in vascular smooth muscle.** *J Smooth Muscle Res* 2004, **40**(6):219-236.
  53. Wooldridge AA, MacDonald JA, Erdodi F, Ma C, Borman MA, Hartshorne DJ, Haystead TA: **Smooth muscle phosphatase is regulated in vivo by exclusion of phosphorylation of threonine 696 of MYPT1 by phosphorylation of Serine 695 in response to cyclic nucleotides.** *J Biol Chem* 2004, **279**(33):34496-34504.
  54. Villacres EC, Xia Z, Bookbinder LH, Edelhoff S, Distechi CM, Storm DR: **Cloning, chromosomal mapping, and expression of human fetal brain type I adenylyl cyclase.** *Genomics* 1993, **16**(2):473-478.
  55. Diel S, Klass K, Wittig B, Kleuss C: **Gbetagamma activation site in adenylyl cyclase type II. Adenylyl cyclase type III is inhibited by Gbetagamma.** *J Biol Chem* 2006, **281**(1):288-294.
  56. Steiner D, Saya D, Schallmach E, Simonds WF, Vogel Z: **Adenylyl cyclase type-VIII activity is regulated by G(betagamma) subunits.** *Cell Signal* 2006, **18**(1):62-68.
  57. Nguyen CH, Watts VJ: **Dexamethasone-induced Ras protein 1 negatively regulates protein kinase C delta: implications for adenylyl cyclase 2 signaling.** *Mol Pharmacol* 2006, **69**(5):1763-1771.
  58. Wang HY, Burns LH: **Gbetagamma that interacts with adenylyl cyclase in opioid tolerance originates from a Gs protein.** *J Neurobiol* 2006, **66**(12):1302-1310.
  59. Guzman L, Romo X, Grandy R, Soto X, Montecino M, Hinrichs M, Olate J: **A Gbetagamma stimulated adenylyl cyclase is involved in Xenopus laevis oocyte maturation.** *J Cell Physiol* 2005, **202**(1):223-229.
  60. Iwami G, Akanuma M, Kawabe J, Cannon PJ, Homcy CJ, Ishikawa Y: **Multiplicity in type V adenylyl cyclase: type V-a and type V-b.** *Mol Cell Endocrinol* 1995, **110**(1-2):43-47.
  61. Chen Y, Harry A, Li J, Smit MJ, Bai X, Magnusson R, Pieroni JP, Weng G, Iyengar R: **Adenylyl cyclase 6 is selectively regulated by protein kinase A phosphorylation in a region involved in Galphas stimulation.** *Proc Natl Acad Sci USA* 1997, **94**(25):14100-14104.
  62. Hacker BM, Tomlinson JE, Wayman GA, Sultana R, Chan G, Villacres E, Distechi C, Storm DR: **Cloning, chromosomal mapping, and regulatory properties of the human type 9 adenylyl cyclase (ADCY9).** *Genomics* 1998, **50**(1):97-104.