The testis-specific Cα2 subunit of PKA is kinetically indistinguishable from the common Cα1 subunit of PKA

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Abstract

Background: The two variants of the α-form of the catalytic (C) subunit of protein kinase A (PKA), designated Cα1 and Cα2, are encoded by the PRKACA gene. Whereas Cα1 is ubiquitous, Cα2 expression is restricted to the sperm cell. Cα1 and Cα2 are encoded with different N-terminal domains. In Cα1 but not Cα2 the N-terminal end introduces three sites for posttranslational modifications which include myristylation at Gly1, Asp-specific deamidation at Asn2 and autophosphorylation at Ser10. Previous reports have implicated specific biological features correlating with these modifications on Cα1. Since Cα2 is not modified in the same way as Cα1 we tested if they have distinct biochemical activities that may be reflected in different biological properties.

Results: We show that Cα2 interacts with the two major forms of the regulatory subunit (R) of PKA, RI and RII, to form cAMP-sensitive PKAI and PKAII holoenzymes both in vitro and in vivo as is also the case with Cα1. Moreover, using Surface Plasmon Resonance (SPR), we show that the interaction patterns of the physiological inhibitors RI, RII and PKI were comparable for Cα2 and Cα1. This is also the case for their potency to inhibit catalytic activities of Cα2 and Cα1.

Conclusion: We conclude that the regulatory complexes formed with either Cα1 or Cα2, respectively, are indistinguishable.

Keywords: PKA, Catalytic subunit, N-terminal, splice variants
Cβ variants have variable N-terminal ends which are encoded by different exons upstream of exon 2 in the Cβ gene [14;15].

PKA-C splice variants are tissue-specifically expressed and some experimental evidence support that they may harbor specific features and non-identical activities when associated with the R subunits to form holoenzymes [18;19]. With regard to this Ca2 it is the sole C subunit expressed in the sperm cell. Moreover, Ca2 was shown to be vital for mouse sperm motility since ablation rendered the sperm cells non-motile and the male individuals infertile [9-11;20].

Ca1 is equipped with an N-terminal of 14 amino acids which undergo three well defined co- and posttranslational modifications. They include \( \text{in vivo} \) myristylation of Gly1 [21]. At position +1 an Asn is encoded which is partly deamidated \( \text{in vivo} \) leading to Ca1-Asp2 and Ca1-iso(\( \beta \))Asp2 [22]. A third modification is identified as a PKA-autophosphorylation site at Ser10 [23-25]. Ca2 on the other hand is encoded with 7 unique amino acids at the N-terminus which to our knowledge do not have the ability to undergo any of the N-terminal modifications seen for Ca1.

Based on the different N-terminal sequences of Ca1 and Ca2 we speculate that they will introduce distinct biological features to these subunits. To investigate this hypothesis we made a thorough characterization of Ca2 activities both \( \text{in vivo} \) and \( \text{in vitro} \) and compared the results to what is known for Ca1 and to results obtained for Ca1 in the present work.

**Methods**

**Sperm cell isolation**

Semen samples were obtained from patients attending infertility investigations at the Andrology Laboratory at Rikshospitalet-Radiumhospitalet HF, Oslo, Norway. All patients signed a letter of approval and all experiments were done according to the recommendation from the Regional Committees for Medical and Health Research Ethics. All men produced their ejaculates on site or at home after 3-5 days of sexual abstinence. Samples were collected by masturbation into a wide-mouthed sterile container (Sarstedt Ltd., Leicester, United Kingdom) and after 30 min of liquefaction at 37°C, sperm parameters were evaluated according to World Health Organization (WHO) methods (World Health Organization, WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction (4th ed.), Cambridge University Press, Cambridge (1999)).

Sperm cells were isolated from the seminal plasma by percoll gradient centrifugation. Sperm samples were pipetted on top of a 90%/45% percoll gradient and centrifuged at 2500 rpm for 20 min, no brake. After centrifugation, the sperm pellet was recovered by first using a sterile glass Pasteur pipette to remove the top layers of the semen sample and sperm gradient, leaving approximately 0.5 mL of the bottom layer. The sperm pellet was subsequently resuspended and washed twice in phosphate buffered saline (PBS) and centrifuged again at 2500 rpm for 8 min.

**Sperm head and tail separation**

Isolated sperm cells were diluted to 1 mill/mL in PBS and sonicated mildly for 10 sec at low frequency. Ten μL samples were taken out to be examined by microscopy to assure head and tail separation. After complete separation the mixture was centrifuged at 400 g for 10 min. The supernatant containing the tails was transferred to a new tube and tails pelleted by centrifugation at 10.000 × g for 15 min. The tail pellet and the pellet from the first centrifugation were separately solubelized in RIPA buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA, 1% Triton X-100, 0.1% SDS, 0.1% Na-deoxycholate and 100 mM NaCl) containing 1 mM DTT, 1 mM PMSF and protease inhibitor cocktail (Roche), and sonicated 2 × 10 seconds at full effect.

**SDS-polyacrylamide gel electrophoresis (SDS-PAGE)**

SDS-PAGE, was performed as described by [26]. Briefly, samples were diluted in SDS sample buffer (62.5 mM Tris-HCl, pH 6.8, 2.3% SDS, 10% glycerol, 5% β-mercaptoethanol, 0.001% bromophenol blue), boiled for 2 min and loaded onto slab gels consisting of a 4.5% stacking gel and a 12.5% separating gel.

**Immunoprecipitation**

Lysates were cleared by centrifugation at 15000 g for 30 min at 4°C, and subsequently incubated with primary antibody [anti-Ca2 (SNO101; 320 μg/mL), mouse anti-RIα (2.5 μg/mL), rabbit anti-RIα serum diluted 1:100] for 2 h to overnight. Antibody-antigen complexes were precipitated using either Dynabeads protein G (Dynal, catalogue number 100.04), anti-mouse agarose beads or anti-rabbit agarose beads (Sigma, catalogue number A6531, A1027). Precipitates were washed three times using appropriate buffer and extracted with buffer in the presence or absence of 1 mm 8-CPT cAMP as indicated in the figure legends.

**Immunoblot analysis**

Total protein was estimated by Bradford protein assay (BioRad). Proteins were separated by SDS-PAGE and transferred to PVDF membranes by electro blotting. Membranes were blocked in 5% skimmed milk powder in Tris-buffered saline containing 0.1% Tween-20 (TBST) for 1 h at room temperature, and then incubated for 1 h at room temperature or overnight at 4°C with the appropriate primary antibodies diluted in TBST...
Expression of Ca2, Ca1 and myristylated Ca1
Recombinant non-myristylated human Ca1 was expressed and purified as described previously [29;30]. Recombinant human Ca2, as well as recombinant human Ca2, were co-transformed with N-myristyl-transferase in Escherichia coli BL21 (DE3) (Novagen) and co-expressed with human Ca1 as well as Ca2 using the same conditions. Both proteins were purified by affinity chromatography using PKI-peptide Affi-Gel. The procedure was first described by Olsen et al. [31] and modified after Thullner et al. [32].

Expression and purification of R subunits
Recombinant human R subunits (hRIα, hRIβ, hRIIα, hRIIβ) were over-expressed in Escherichia coli BL21 (DE3) RIL (Novagen) and purified according to a procedure by Bertinetti et al. [33] using Sp-8-AEA-cAMPS-agarose.

The purity of the R and C proteins was confirmed by SDS-PAGE as well as by immunoblot analysis and the biological activity of the proteins was measured as described before [30]. Primary sequence of Ca2 and the presence of N-myristylation at Ca1 were checked by mass spectrometry (Data not shown).

SPR analysis
All SPR interaction analyses were performed at 25°C in 20 mM MOPS pH 7, 150 mM NaCl plus 0.005% (v/v) surfactant P20, 1 mM ATP, 5 mM MgCl2 and 50 μM EDTA using Biacore 2000 or 3000 instruments (GE Healthcare-Biacore, Sweden). For covalent coupling of the C subunits, carboxymethylated sensor chip surfaces (CM5, research grade, GE Healthcare) were activated with NHS/EDC for 7 min and non-myristylated Ca1, Ca2 and myrCa1 (5 μg/μl in 10 mM sodium acetate plus 200 μM ATP and 500 μM MgCl2 with a pH 6.0) as described [34] were injected on separate flow cells with a flow rate of 5 μl/min until approximately 300 response units (RU) were reached. This amine coupling was described previously [36;37]. Deactivation of the surface was performed using 1 M ethanolamine-HCl (pH 8.5) for 7 min. As a reference one flow cell was activated and deactivated in the absence of any protein.

The interaction experiments with four R subunit were performed at 25°C in running buffer (20 mM MOPS pH 7, 150 mM NaCl, 1 mM ATP, 5 mM MgCl2, 50 μM EDTA, 0.005% surfactant P20) at a flow rate of 30 μl/min. During simultaneous R subunit injection over the four surfaces (150 sec), the dissociation phase was monitored for 150 sec. The binding of R subunit to C subunit was not limited by mass transport according to previous experiments [38]. Response of the activated/deactivated reference cell was subtracted. Surfaces were regenerated with two subsequent 1 min injections of 0.1 mM cAMP, 2.5 mM EDTA in running buffer. Evaluation of non-normalized data was performed with Biaevaluation 3.2 RC1 (GE Healthcare). A Langmuir 1:1 binding model was applied for the kinetic analysis of C subunit/R subunit interactions [37].

The sensor chip was stored at 4°C in running buffer and tested for proper performance prior to each analysis.
Results
Sperm cell-specific Ca2 associates with Rlx and RIIα in a cAMP-dependent fashion to form PKAI and PKAII holoenzymes in vivo

We first determined whether human sperm cells express Ca2. Protein extracts of T cells, whole sperm, sperm tail, and sperm heads were separated by SDS-PAGE transferred to immunoblot filters and filters probed with a pan-C antibody (Figure 1A, left panel, anti-C) and an anti-Ca2 antiserum (SNO 101, anti-Ca2 [10], Figure 1A, right panel). This confirms previous results that Ca2 is not expressed in T cells and is distributed in tail and head of spermatozoa [10]. RI and RII are both expressed in all sperm cell compartments and are known to form PKA type I and type II (PKAI and PKAII) [39-41]. PKAI and PKAII holoenzymes can be separated by DEAE ion exchange chromatography using increasing concentrations of NaCl [42]. Since Ca2 is the sole C subunit in human sperm cells it is expected that it will associate with RI and RII to form PKAI and PKAII, respectively, as has been demonstrated in the mouse [11]. To test this, whole sperm cell extracts (3 mg) were fractionated on DEAE resins by a linear NaCl gradient ranging from 0 to 350 mM). Two peaks of phosphotransferase- (●) and cAMP-binding activity (□) between 50-100 mM and 100-250 mM NaCl, respectively, were observed. This implied formation of both PKAI and PKAII (Figure 1B). C and R subunit identity were documented by immunoblotting using a pan-C antibody (Figure 1, panel CI) and anti-Ca2 (panel CII) as well as anti-Rlx (panel CIH) and anti-RIIα (panel CIV). This showed that immunoreactive Ca2 co-elutes with the two major R subunits in sperm cells, Rlx (peak I) and RIIα (peak II) [43-45]. From the figure it is also seen that some of the C activity was detected before the R subunit activity in the first peak, implying free C subunit. Moreover, we also noted that PKAI and PKAII containing Ca2 eluted at comparable concentrations of NaCl. To further investigate whether human Ca2 forms PKAI and PKAII in cell extracts we immunoprecipitated (IPed) with anti-RIlα and anti-RIIα (Figure 2). Using immunoblotting and anti-Ca2 (upper panels) and anti-C (lower panels) we showed that both RI and RIIα associate with Ca2, implying that Ca2 forms PKAII and II in vivo (lanes 6 and 12). To define whether the R-C interaction is specific and functional, the IPed proteins were challenged with cAMP to dissociate the holoenzyme into R subunit dimers and free C subunits [46]. In these experiments the IPed R subunits would be expected to be immobilized by the precipitating antiserum and remain in the pellet (P) after the cAMP wash whereas the IPed C subunit would be released into the supernatant (S) in the presence of cAMP [46,47]. Figure 2 (lanes 3 and 4, and 9 and 10) depicts that immunoreactive Ca2 is released into the supernatant after the cAMP wash (+cAMP) after both anti-Rlx and anti-RIIα IP (upper and lower panels) whereas the immunoreactive Ca2 subunit remained in the pellet (P) in the absence of cAMP (-cAMP) (Figure 2, lanes 5 and 6 and 11 and 12, upper and lower panels).

Comparable activities of Ca2 and Ca1

Our results and several reports showing that Ca2 associates with both RI and RII subunits [3; 48-50] in a cAMP-sensitive fashion imply that N-terminal differences do not interfere with PKA holoenzyme formation.. To further investigate whether Ca2 has activities that differ from Ca1 we expressed Ca2 using the pREST B
vector. First we noted that Ca2 and Ca1 were captured in the soluble and particulate fractions of the bacteria lysates, respectively, suggesting differences in solubility (results not shown). Furthermore, specific activity of expressed Ca2 was determined to 18 ± 3 units/mg (U/mg, n = 3) which was notably lower than the specific activity of expressed Ca1 (28 ± 4 U/mg, Figure 3A). Taken together this may imply differential features of Ca1 and Ca2. Based on this and to determine the exact activities for Ca2 three features were investigated. These included (i) Ca2’s substrate affinity, (ii) the ability to form holoenzymes with RI and RII in vitro and (iii) the Km values for Kemptide and ATP. In the latter case we found the Km values of Ca2 for Kemptide and ATP to be 27.8 ± 2.3 μM and 11.5 ± 0.5 μM, respectively (Figure 3B and 3C). This is in good agreement with previous results obtained with expressed Ca1 [7].

We next monitored cAMP-sensitivity of type I and II PKA holoenzymes containing Ca2 in vitro. To calculate the accurate cAMP activation constant, PKAI (RIα) and...
PKAII (RIIβ) containing Ca2 holoenzymes were incubated with 250 μM Kemptide and 10 mM ATP in the presence of increasing concentrations of cAMP. Kact values for cAMP were 120 nM and 460 nM for the holoenzyme formed with RIα and RIIβ, respectively, showing that PKAI containing Ca2 are nearly 4 fold more sensitive to cAMP than PKAII containing Ca2 holoenzymes (figure 4A,B). The corresponding values for holoenzymes formed with myrCa1 were 99 nM (RIα) and 350 nM (RIIβ) (figure 4A,B) again demonstrating a 4 fold increased sensitivity for the RI holoenzyme. This is also in agreement with previous in vitro results for PKAI and II holoenzymes containing Ca1 [38;51], and the Kact values for mouse PKAI isolated from sperm cells ablated for PKAII (RIα) [11]. It should be noted that Kact in wild type sperm cells which mainly express PKAII (RIIα-Ca2) [45] is almost identical to the Kact in RIα ablated sperm cells. This may suggest that PKAI and PKAII display comparable Kact’s in vivo and hence contradicts the in vitro results demonstrated previously [38] and by us here. It should also be noted that PKAI although expressed at low levels may skew the observed Kact values, due to its sensitivity for cAMP. To what extent this has biological levels may skew the observed Kact values, due to its action of RIIα in a dose-dependent manner with complete inhibition at stoichiometric concentrations of Ca2 and PKIα (Figure 5C). The inhibitory effects of the various R subunits and PKI have previously been determined for Ca1 [7;29] and indicate that the efficiency in inhibiting Ca1 and Ca2 is similar for all R subunits and PKIα. Using a Biacore technology we next investigated the dissociation equilibrium constants (Kd), association (kass) and dissociation (kdis) rate constants for the various R subunits in association with either Ca1 or Ca2. We immobilized 300 RUs of myristylated Ca1 (myrCa1, see Methods) and Ca2 on separate flow cells of a CM5 Biacore sensor chip. Unmyristylated Ca1 was used as reference (data not shown). In the presence of 1 mM ATP and 5 mM MgCl2 the R subunits were simultaneously run over both C subunits on the sensor chip at a flow rate of 30 μL/min. In the case of RIα and RIIβ they were run over the sensor chip at concentrations between 0.25 and 128 nM, and RIα and RIIβ between 0.5 to 256 nM (raw data not shown). Figure 6 (panel A) shows representative runs of RIα, RIIβ, RIα and RIIβ (64 nM each; panel B) on Ca2 and myrCa1. The shape of the curves indicates that the relative on and off rates for RIα when associated with either Ca2 or myrCa1 were highly similar. The relative kass values were slightly different, 1.6 × 106 and 1.9 × 106 M-1 s-1 for RIα versus myrCa1 and Ca2, respectively. The same was true for RIIβ. However, in this case, although the KD value was almost identical to RIα, the association as well as the dissociation rate constants for RIIβ was 2 times faster. Finally, no differences could be observed for the interaction of RIIα and RIIβ against myrCa1 versus Ca2 (for rate and equilibrium constants see Table 1).

In order to investigate the binding behavior of PKI, GST–PKIα was immobilized on sensor chips as described previously [54], and various concentrations of Ca2, Ca1, myrCa1 and, for comparison, mouse Ca1 were run at a flow rate of 30 μL/min over the sensor chips. This revealed a KD for all C subunits and PKIα at a range around 0.4 nM where the myrCa1 displayed a slightly faster association rate compared to Ca1 (4.9 × 106 and 3.2 × 106 M-1 s-1, respectively) with all the dissociation rates being similar (1.5 × 10-3 s-1) (Table 2). SPR measurements with single concentrations demonstrated almost identical shapes of the curves (Figure 7),
Figure 5 Phosphotransferase activity of Ca2 is inhibited by the R subunits and PKI in dose-dependent manner. Recombinant Ca2 (30 nM) mixed with Kemptide (250 μM) and ATP (10 mM) was incubated in the presence of increasing concentrations (0 - 25 nM) of either Rαa and Rβ (panel A) and Rαa and Rβ (panel B). Ca2 is inhibited by all R subunits in a dose-dependent manner at equimolar concentrations of Rαa, Rβ, Rαa, and Rβ. Panel C: The inhibitory effect of PKI was verified by incubating recombinant Ca2 (28 nM) in the presence of Kemptide (250 μM), ATP (10 mM) and increasing concentrations of recombinant PKI (0-40 nM). Titration curves shown are normalized before linear regression (n = 2).
indicating comparable association and dissociation rates. In order to determine accurate association rate constants, different concentrations of the respective C subunits were applied (results not shown), leading again to the conclusion that the binding activities of Cα2 and Cα1 for PKIn are highly similar.

### Table 1 Association and dissociation constants of RI and RII and Cα1 and Cα2

| Analyt/Ligand (immobilized) | $k_a$ [M$^{-1}$S$^{-1}$] | $K_d$ [s] | $K_d$ [nM] |
|-----------------------------|--------------------------|----------|------------|
| hRIα/PKA-Cα1 myr            | 1.6E + 6                 | 200,0E-6 | 0.13       |
| hRIα/PKA-Cα2                | 1.9E + 6                 | 214,0E-6 | 0.11       |
| hRIβ/PKA-Cα1 myr            | 3.5E + 6                 | 501,0E-6 | 0.15       |
| hRIβ/PKA-Cα2                | 4.4E + 6                 | 482,0E-6 | 0.11       |
| hRIα/PKA-Cα1 myr            | 1.0E + 6                 | 469,0E-6 | 0.48       |
| hRIα/PKA-Cα2                | 1.2E + 6                 | 433,0E-6 | 0.35       |
| hRIβ/PKA-Cα1 myr            | 0.5E + 6                 | 797,0E-6 | 1.5        |
| hRIβ/PKA-Cα2                | 0.9E + 6                 | 793,0E-6 | 0.9        |

### Table 2 Association and dissociation constants of GST-PKIα for Cα1 and Cα2

| Analyt/Ligand (immobilized) | $K_d$ [M$^{-1}$S$^{-1}$] | $K_d$ [s] | $K_d$ [nM] |
|-----------------------------|--------------------------|----------|------------|
| GST-PKIα/PKA-Cα1            | 4.9 × 10$^5$             | 1.5 × 10$^{-3}$ | 0.5       |
| GST-PKIα/PKA-Cα2            | 3.2 × 10$^5$             | 1.5 × 10$^{-3}$ | 0.7       |

### Discussion

At the protein level Cα1 and Cα2 are 97% homologous and only differ at the N-terminal end. Based on this we investigated to what extent differences at the N-terminus may influence splice variant-specific activities that may have biological importance. We found that Cα2 expressed in bacteria was not captured by inclusion bodies as was the case with Cα1. Moreover, the specific activity of Cα2 was lower compared to Cα1. Apart from these differences we observed that Cα2 was highly similar to Cα1 in all parameters measured. This included association of Cα2 with RI and RII to form cAMP-sensitive holoenzymes both in vivo and in vitro. Furthermore, Km values of Cα2 for Kemptide and ATP were comparable to those determined for Cα1. This was also the case for the ability of RI, RII and PKI to inhibit Cα2 enzyme activity in vitro (data not shown). Finally, $K_D$ values as measured by SPR were shown to be comparable between Cα1 and Cα2 towards the RI and RII subunits as well as PKI.

Several reports imply that N-terminal modifications of Cα1 introduce specific features that may have biological consequences. To this end it has been suggested that phosphorylation of Ser10 in Cα1 introduces an electrostatic force which may help the C subunit to remain soluble even when myristylated [55;56]. Moreover, two reports have demonstrated that the N-terminal myristyl moiety of Cα1 is embedded in a hydrophobic pocket.

![Figure 6: The affinity of RI and RII subunits for Cα1 and Cα2 are comparable](image)

![Figure 7: PKIn binds almost identical to hCα1, myristylated hCα1, hCα2 and mCα1. GST-PKια was captured on an α-GST-Antibody sensor chip as described [SS] to surface density of 300 RU. All C-subunits (Cα2, Cα1, myristylated Cα1 and, for comparison, mouse Cα1) were run over the GST-PKια surface using the assay conditions as described in figure legend 6 in the presence of 1 mM ATP and 5 mM MgCl2. The plot shows the normalized binding data at a concentration of 25 nM C-subunit each.](image)
encompassed in the large lobe [57;58]. Mutation of Gly1 to Ala rendering the Ca1 non-myristylated, demonstrated that myristylation was non-essential for conformation and enzyme activation, and was not required for Ca1 interaction with other proteins including various substrates and the R subunits [21,59]. The fact that Ca2 is not myristylated and displays comparable activities with myristylated Ca1 suggests that myristylation is not essential for catalytic activity, holoenzyme formation and inhibition by PKI. This is further supported in that deletion of the entire Ca1 N-terminus did not severely interfere with catalytic activity and inhibitor binding despite that deletion caused thermal instability [25]. This also suggests that the amino acids 2 (Asn) and 10 (Ser) of Ca1 are not essential for activity a suggestion which is supported by our results on Ca2.

Ca1 and Cβ1 which are 100% identical at the N-terminus, but only 91% identical in the sequence encoded by exon 2 through 10, have different apparent sizes (40 and 41 kDa) and possess distinct biochemical properties both in vitro and in vivo [60; 31]. These differences include differential Km values for certain peptide substrates and that Ca1 but not Cβ1 is inhibited by substrate concentrations above 100 μM. In addition, they display distinct IC50 values for PKI and RIIa. Taken together with our results this may suggest that the amino acid sequence encoded by exon 2 through 10 and not exon 1 influence C subunit activities such as holoenzyme formation, enzyme activity and inhibition by R and PKI. This may further imply that Ca1 and Cβ1 have distinct roles in regulating cellular processes. This was recently shown in T cells which express Ca1, Cβ1 and Cβ2. In that study Ca1, but none of the Cβ forms, mediated the inhibitory effect of cAMP on immune cell reactivity in vivo [17;61]. In light of these observations it is also of interest to note that Ca2 but not Ca1 is required for sperm cell forward velocity and male fertility, despite 100% identity at the amino acid sequence encoded by exon 2 through 10 [20;62]. However, since Ca2 is the sole C subunit in sperm cells [9;10;63], the difference observed may only be ascribed to tissue-specific expression and not sequence-specific differences.

In contrast to Ca1 it is expected that the hydrophobic pocket in which the myristyl group is submersed in Ca1, is constitutively empty and exposed to the surroundings at all time in non-myristylated Ca2. It has been speculated whether exposure of the hydrophobic pocket would introduce more lipophilic properties to the Ca2 subunit [64]. Support for such a hypothesis is found in a by a study demonstrating that binding of Ca1 to RII induced a unique conformation that is associated with exposure of the hydrophobic pocket to the surroundings due to increase in N-terminal flexibility of the N-myristate away from the large lobe. This renders Ca1 more hydrophobic and promotes membrane association of the PKA II holoenzyme only [64]. Therefore it may be suggested that exposure of the hydrophobic pocket serves features such as isoform specific features and subcellular localization of the C subunit. To this end it is interesting to note that Ca2 is associated with the sperm tail in the presence of detergent treatment with 1% Triton X-100 and, after a challenge with 2 mM cAMP [10]. This may be indicative of a direct association of Ca2 with subcellular structures. To what extent such attachment involves the hydrophobic pocket remains unknown. In other cells and tissues, C subunits targeted to subcellular structures independent of the R subunit and traditional A-kinase anchoring proteins have been demonstrated. To day a number of C subunit binding proteins have been identified. These include PKI, A-kinase interacting protein 1 (AKIP1), homologous to AKAP95 (HA95), inhibitor of NFkappaB kinase (IKB), Caveolin-1 and p75 neuropilin receptor (p75NTR) [65-69]. To what extent Ca2 is targeted to the sperm cell midpiece through a C interaction protein and if specificity of binding is retained in the hyper variable N-terminal end remains to be shown. However, it should be noted that deamination of the Asn2 moiety in Ca1 helps fine-tuning enzyme distribution within the cell in vivo [70]. Moreover, p75NTR was shown to specifically bind to the Cβ splice variant Cβ4ab [69], which is encoded with unique N-terminal domain that may not undergo the same post translational modifications as Ca1 [15;69]. Together this may imply that the N-terminal end may be important for targeting and specificity of subcellular localization of the various C subunits.

Conclusion

Our study demonstrates that N-terminal sequence encoded by alternative use of exons upstream of exon 2 in the PRKACA gene does not influence C subunit activities such as holoenzyme formation, cAMP sensitivity, enzyme activity as well as inhibition by RI, RII and PKI. Based on several studies it may be suggested that the N-terminus is involved in other C subunit features such as subcellular localization.

Abbreviations

PKA: Protein kinase A; C: Catalytic subunit; Ca: Alpha-form of C, Ca2: Sperm-specific C subunit.

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