Erratum

CORRECTION FOR FLORES ET AL., BIOPHYS. J. 86 (2) PART 2 OF 2

On page 11a, second column, figure was missing from Abstract. Abstract should have appeared as shown below.

52-PLAT

Identification of ^{1}H Endor Lines at 35 GHz Corresponding to Hydrogen Bonds to Q_A^-. in RCs of *Rb. sphaeroides.* Marco Flores[1], E. Abresch[1], W. Lubitz[2], R. Calvo[3], R. Isaacson[1], G. Feher[1], [1]Physics, UCSD, La Jolla, CA, USA, [2]Max Planck Institute for Bioinorganic Chemistry, D-45470 Mülheim an der Ruhr, Germany, [3]Dept. de Física, Facultad de Bioquímica y Ciencias Biológicas and INTEC, Universidad Nacional del Litoral and CONICET, 3000 Santa Fe, Argentina.

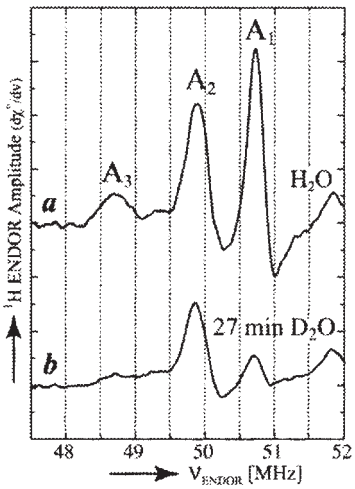


Fig. 1. Low frequency $Q_A^{\cdot-}$ ^{1}H ENDOR spectra (along g_y) at 35 GHz of deuterated RCs in H_2O (a) and after incubation in D_2O for 27 min (b).

Hydrogen bonds to the two carbonyl oxygens of Q_A^-. in fully deuterated reaction centers (RCs) of *Rb. sphaeroides* have been studied by ^{1}H ENDOR at 35 GHz and 80 K. The aim of this study was to identify the ENDOR lines corresponding to each H-bond. We prepared two samples of Q_A^-., one in H_2O and one incubated in D_2O[1] for 27 min to preferentially deuterate one of the hydrogen bonds. Figure 1 shows the ^{1}H ENDOR spectra of Q_A^-. in the two samples, at a magnetic field corresponding to g_y. The peaks labeled A_1, A_2 and A_3 correspond to the hydrogen bonds.[2] Upon incubation in D_2O the intensities of A_1 and A_3 decreased together, indicating that both lines belong to the same H-bond ($A_{//}$ and A). ENDOR spectra obtained at different field positions suggest that A_3 overlaps with the partner of A_2. This may explain why a fourth ENDOR line had never been reported. [1] M. Paddock et al., *Biophys. J.*, 76, A141 (1999). [2] W. Lubitz and G. Feher, Appl. Magn. Reson., 17, 1 (1999). Supported by NIH.

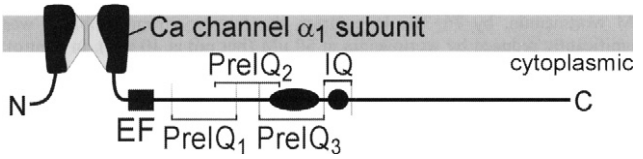
CORRECTION FOR EVANS ET AL., BIOPHYS. J. 86 (2) PART 2 OF 2

On page 275a, first column, figure was missing from Abstract. Abstract should have appeared as shown below.

1427-POS BOARD #B412

FRET Two-Hybrid Analysis Reveals Differences in the Interaction of Calmodulin with CaV1.2 (L-) versus CaV2.1 (P/Q-type) Ca Channels. Jenafer Evans, Lai-Hock Tay, Masayuki X. Mori, Molly Anderson, Michael G. Erickson, David T. Yue, Biomedical Engineering, Johns Hopkins Sch Med, Baltimore, MD, USA.

Calmodulin (CaM) regulates Ca_V1-2 channels through a partially conserved overall design (*Neuron* 2003, 39:951). In common, CaM is a resident Ca^{2+} sensor, because even Ca^{2+}-free apoCaM preassociates with channels; Ca^{2+} binding to either the N- or C-lobe of CaM selectively triggers channel regulation, depending upon channel context; and Ca^{2+}/CaM binding to a channel IQ region often triggers regulation. A valuable clue to the overarching regulatory mechanisms may reside in a striking functional contrast: the CaM C-lobe regulates Ca_V1.2 and Ca_V2.1 channels with inverse function, *inactivating* the former while *facilitating* the latter. We therefore used FRET two-hybrid analysis (*Neuron* 2003, 39:97) to compare CaM interactions with key regulatory segments of Ca_V1.2 and Ca_V2.1, as interactions occur in live cells. ApoCaM preassociates differently between channels: whereas the Ca_V1.2 preIQ$_3$-IQ segment collectively embraces apoCaM, the Ca_V2.1 IQ region alone predominates. Ca^{2+}/CaM interacts throughout the entire preIQ$_2$-IQ region of Ca_V1.2, with CaM lobe-specific preferences therein. By contrast, Ca^{2+}/CaM mainly interacts with flanking preIQ$_2$ and IQ segments of Ca_V2.1, showing little lobe specificity. These distinctive patterns may be fundamental to the inverse regulation of Ca_V1.2 versus Ca_V2.1.



CORRECTION FOR PADDOCK ET AL., BIOPHYS. J. 86 (2) PART 2 OF 2

On page 11a, second column, figure inadvertently appeared Abstract. Abstract should have appeared as shown below.

54-PLAT

Conformations Of QB-. Trapped By B Side Electron Transfer In Reaction Centers From Rhodobacter Sphaeroides.* Mark Paddock, Roger Isaacson, Charlene Chang, George Feher, Melvin Okamura, UCSD, La Jolla, CA, USA.

The electron transfer to Q_B in the native RC has been proposed to be limited by a conformational gating step (1). We have constructed a quintuple mutant RC that allows direct reduction of Q_B by bacteriopheophytin via the normally inactive B-branch utilizing a greater driving force for electron transfer in samples lacking active Q_A. This mutant consists of 5 amino acid replacements - one to displace Q_A (Ala-M260->Trp) (2) three to inhibit A-side transfer to BChlA (Gly-M203->Asp, Tyr-M210->Phe, Leu-M214->His) and one to promote B-side transfer (Phe-L181->Tyr). RCs frozen in the dark and illuminated at 80K showed a light induced EPR signal of $D^+.Q_B^-$. in ~10% of the RC sample. The lack of a narrow Q_B^-. signal indicates a magnetic interaction between QB-. and the Fe^{2+}. These results suggest that ~10% of the RCs have the quinone in the more thermodynamically stable proximal site (3). The quantum yield for formation was ~7%, similar to those previously reported for other B-side mutants (4,5). The lifetime of the dark generated $D^+.Q_B^-$. is $\tau \sim 3$s at 80K. This result is in contrast to RCs frozen in the light, in which ~100% $D^+.Q_B^-$. was formed with a lifetime > 10^5s (6). The large difference in stability is attributed to an undetermined conformational change that occurs only at higher temperatures and stabilizes the reduced form of Q_B. This change may be responsible for the conformational gate.

*Supported by NIH.

(1) Graige et al. (1998) PNAS **95**, 11679-11684.
(2) Wakeham et al. (2003) FEBS L. **540**, 234-240.
(3) Stowell et al. (1997) Science **276**, 812-816.
(4) deBoer et al. (2002) Biochemistry **41**, 3081-3088.
(5) Heller et . (1995) Science **269**, 940-945.
(6) Kleinfeld et al. (1984) Biochemistry **23**, 5780-5786.

CORRECTION FOR WYKES ET AL., BIOPHYS. J. 86 (2) PART 2 OF 2

On page 275a, first column, figure inadvertently appeared in Abstract. Abstract should have appeared as shown below.

1428-POS BOARD #B413

Calmodulin Regulation of Calcium Channels in Bovine Adrenal Chromaffin Cells. Robert C. Wykes, PhD, Elizabeth P. Seward, PhD, Biomedical Sciences, University of Sheffield, Sheffield, United Kingdom.

Studies aimed at elucidating the molecular mechanisms underlying calcium-dependent inactivation of VOCCs have shown that calmodulin may bind directly to and inactivate L and P/Q (Zuhlke et al

1999, Lee et al 1999) type channels. There have been many diverse reports surrounding the inactivation properties of the N-type calcium channel. Studies in sympathetic neurones and in recombinant systems have only detected voltage-sensitive inactivation (Patil et al 1998, Jones et al 1999) In contrast studies in dorsal root ganglia (Cox and Dunlap 1994) favour calcium-dependent inactivation. It has been suggested that calmodulin does not act directly on N type channels, but indirectly through a mechanism involving calcineurin (Burley et al 2000).

We have investigated the inactivation of VOCCs in patch-clamped bovine chromaffin cells. Cells were held at –80 mV in either the whole-cell or perforated-patch configuration and stimulated with a prolonged (200ms) depolarization or a train of fifty brief (10ms) to +20mV. The calcium-dependence of inactivation was investigated by barium replacement of external calcium and by varying the concentration of calcium chelator dialyzed into the cell. The results show that inactivation of VOCC is regulated by calcium in these cells, with the pharmacologically isolated N-type current displaying the more profound sensitivity. Inhibiting calcineurin by 20 mins pre-incubation with 1μM cyclosporine A or by introducing 30μM calmodulin inhibitory peptides through the patch pipette did not significantly reduce the level of calcium-dependent inactivation. In contrast, expression of a mutant calmodulin deficient in Ca^{2+} binding resulted in a profound reduction in inactivation of VOCCs. This was not observed following expression of wild type calmodulin or EGFP. Taken together, these results are consistent with calmodulin acting directly to control N-type channel inactivation in adrenal chromaffin cells.

Correction for Liu et al., Biophys. J. 86 (2) part 2 of 2

Abstract was inadvertently omitted from Abstracts Issue.

A Single Residue in the S0-S1 Cytoplasmic Loop Contributes to the Differential Ethanol Responses of *mslo vs. bslo* Channels. Pengchong Liu, MD, PhD, Jianxi Liu, PhD, Alejandro M. Dopico, MD, PhD. Univ. Tennessee Hlth. Sci. Ctr., Memphis, TN, USA.

Ethanol at clinically relevant concentrations (3-200 mM) differentially modifies the steady-state activity (NP_o) of *slo* channels from different tissues/species when studied in the same expression system under identical conditions. Thus, we hypothesized that nonconserved regions in *slo* are important to determine ethanol responses. Taking advantage of two isoforms (*mslo* and *bslo*) that differentially respond to acute ethanol exposure (consistent increases in NP_o *vs.* heterogenous responses, primarily inhibition), we constructed chimeras by interchanging the core-linker and the tail domains of these *slo* subunits, and studied ethanol responses of the chimeric channels. Data demonstrate that the core-linker is the slo region that critically determines the channel responses to ethanol (Liu et al., 2003). In this region, a few residues are not conserved between *mslo* and *bslo*. Thus, to investigate in more detail the molecular basis of these differential *slo* responses to ethanol, we introduced pinpoint mutagenesis in one of the nonconserved residues (in the S0-S1 cytoplasmic loop), and studied ethanol responses of the mutated channels. Acute ethanol (100 mM) applied to the cytosolic side of *Xenopus* oocyte membrane inside-out patches that expressed T*bslo*V channels markedly increased activity in 100 % of the patches, NP_o reaching 319±24 % of pre-ethanol, isosmotic control values. These responses are similar to those found in wt mslo. Conversely, ethanol evoked varied responses in VmsloT channels: decreases (50% of patches) and increases (50%) in NP_o. This heterogeneous pattern of responses is close to that observed with *wt bslo*. In conclusion, our data indicate that the nonconserved residue T/V in the S0-S1 cytoplasmic loop of bslo/mslo critically contributes to the differential ethanol responses of these *slo* channel proteins.

Support: NIH AA11560 and a grant from Univ. Tennessee COE Cardiov. Biol.

Correction for Chaieb et al., Biophys. J. 86 (2) part 2 of 2

On page 32a, first column,Presenting author was incorrectly listed in Abstracts Issue.

154-Plat

Wrinkling Transition of Partially Polymerizable Membrane for Drug Delivery. Sahraoui Chaieb, PhD[1], **Vinay Kumar Natrajan**[2], [1]TAM/Bioengineering, University of Illinois at Urbana Champaign, Urbana, IL, USA, [2]Tam, University of Illinois at Urbana Champaign, Urbana, IL, USA.

When membranes made of polymerized lipids are cooled below the melting temperature of the phospholipids, they undergo a wrinkling transition whereby the vesicle collapses like a crumpled elastic sheet. We investigate this transition using fluorescence spectroscopy and using AFM measurement of the wrinkled surface. We apply this transition to study drug delivery by monitoring the amount of drug delivered using fluorescence microscopy.

Correction for Lee et al., Biophys. J. 86 (2) part 1 of 2

pp 1105-1117, authors found that the following sentences will help readers understand the results of their paper better.

In Figure 6, a regularization parameter of t1 = 0.01 was used for the folded Gaussian in the b direction, and t2 = 1.0 was used for the Gaussian in the distance direction. The t1 is reported as t in the paper but the t2 value, which also significantly impacts the shape of the contours, was not reported.