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***P. falciparum* Na⁺ / H⁺ Exchanger (PfNHE) Function and Quinine Resistance (QNR)**

Paul D. Roepe[#] and Michael T. Ferdig^{\$}

[#]Dept. Chemistry, Dept. Biochemistry & Molecular Biology, Program in Tumor Biology, Lombardi Cancer Center, and Center for Infectious Diseases, Georgetown University, 37th and O Streets, Washington, DC 20057

^{\$}Eck Institute for Global Health, Department of Biology, University of Notre Dame, Notre Dame, IN 46556

Sir: Two recent papers in this journal differ on connections between PfNHE function and QNR for *P. falciparum* [1,2]. Authors of [2] challenge that PfNHE function is measureable for *P. falciparum* via Na⁺ induced recovery of cytosolic pH (pH_{cyt}), and question our interpretation [1] that PfNHE is localized to plasma membrane (pm) and helps to regulate pH_{cyt}. However, the closest known PfNHE homologue is *T. gondii* NHE1 which has clearly been localized to pm [3]. Structural criteria for predicting localization also points very strongly to pm for PfNHE [4].

Quantification of NHE function requires transient cytosolic acidosis. Various methods exist, including weak base loading [5] and nigericin - BSA treatment [6]. In [2], concerns regarding the latter are raised; however, we note that in [1] BSA is administered by perfusion, rather than added as a single dose into a cuvette as in [2]. Use of perfusion ensured that constant free BSA was always present since BSA - nigericin is washed away. Other variables (e.g. fatty acid free BSA) may also affect reliability. These different procedures likely lead to different efficiencies in quenching nigericin. Indeed, in [2] pH_{cyt} "... reach[ed] a final pH_i which was ... equal to the extracellular pH ...". In [1] this is not the case, suggesting our approach clamps pH_{cyt}, as shown (Fig. 4A, ref. [1]). Another key difference [1,2] is the pH_{cyt} at which NHE activity is initiated. The lower the pH prior to adding Na⁺, the faster Na⁺/H⁺ exchange will be. Calibration of pH_{cyt} is therefore critical, as is how one distinguishes "faster" vs. "slower". Calibration is not shown in [2], but was presumably obtained via bulk populations of detergent excised parasites injected into cuvettes (- CO₂/HCO₃). Calibration was shown in detail in [1], and was done under perfusion (+ CO₂/HCO₃) in real time for each live, intact cell whose pH or NHE activity is measured, eliminating the requirement that multiple bulk samples of detergent treated cells [2] behave similarly. Also, proper quantification of "faster" is multiplication of the measured change in pH vs. time by the cytosolic buffering capacity (β_i). In [1] NHE is quantified this way and averaged for many replicate experiments and 13 strains. In [2] β_i is not measured and "representative" recovery from acidosis is plotted as pH units vs. time for only 1 strain. Also, representative recovery from acidosis is shown only for samples with pH_{cyt} ≥ 6.6 in [2]. In [1] it is quantified at many pH_{cyt} including 6.2. NHE activity is much stimulated at lower pH_{cyt}. Proper quantification of NHE [1] requires β_i measurements and H⁺ flux data at many pH_{cyt} values between (typically) 6.0 and 7.0.

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We measure a K_i of $\sim 0.9 \mu\text{M}$ for ethyl isopropyl amiloride (EIPA) for Na^+ dependent recovery from acidosis. In [2] EIPA does not inhibit, however, only a single dose is examined. We have no simple explanation, but differences in assay precision, e.g. single iRBC SCP under physiologic perfusion [1] vs. a bulk detergent extracted parasite and cuvette– based approach [2] have likely led to distinct interpretations.

Notably [2] ignores that [1] correlates both PfNHE activity and pH_{cyt} vs. genetically characterized QNR status [7]. Chr 13 $\times$ chr 9 epistatic loci defined for QNR correlate with elevated pH_{cyt} [1] and the absence of any reference in [2] to extensive strain analysis in [1] indicates these authors do not disagree. Any interpretation of [1] should acknowledge that the activity we measure must reside in the chr 13 region and interact epistatically with chr 9. But a key experiment missing in [1] is measurement of steady state PfNHE activity. We do not yet know the relative importance of PfNHE in setting resting pH_{cyt} . Clearly, the pm VAPase plays a key role [8], thus [2] is correct to further highlight VAPase. It may be that at certain pH_{cyt} the more essential role of PfNHE is to balance Na^+ transport, and that VAPase is more essential to pH equilibrium *per se*. We are grateful that Kirk and colleagues have raised these important points, nevertheless we are not convinced that [2] has ruled out our observation of a role for PfNHE in pH_{cyt} regulation.

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