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### A DEESTERIFIED FORM OF INO-4995, (INO-4913) STIMULATES WHOLE CELL CL- CURRENTS IN CF HUMAN NASAL EPITHELIAL CELLS

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We previously demonstrated that INO-4995 inhibits basal I<sub>sc</sub> in CF human nasal epithelia (CFHNE). In the current study, we employed the patch clamp recording technique to investigate whether breakdown products of INO-4995 elicit whole cell Cl<sup>-</sup> currents in CFHNE cells. INO 4995 is a membrane-permeant compound protected by ester linked hydrophobic groups masking the charged phosphates. Hydrolysis of the protecting ester groups occurs once the compound is inside the cell, releasing the active moiety. Hydrolysis of the propionoxy protecting groups concealing the phosphates of the prodrug, D-1-O-octyl- 2-O- butyryl - myo -inositol 3,4,5,6-tetrakisphosphate octakis (propionoxymethyl)ester (INO-4995) results in 1-O-octyl-2-O- butyryl- myo-inositol 3,4,5,6-tetrakisphosphate (INO-4913). INO-4913 is a small, highly charged, hydrophilic molecule that is limited in its bioavailability to the inside of the cell (its site of action) due to its poor membrane permeability. In contrast to the prodrug, INO-4995, and in keeping with its low membrane permeability, exposure to INO-4913 at concentrations up to 100 μM had no effect on I<sub>sc</sub> when measured in Ussing chambers. However, by employing patch clamp recording techniques it is possible to deliver highly charged compounds through the patch recording pipette to intracellular sites of action. This approach obviates the need for hydrophobic protecting groups present on the prodrug INO-4995. To test the effect of the de-esterified and unprotected form of INO-4995, we delivered INO-4913 intracellularly to single human CFHNE cells grown on collagen coated coverslips via conventional whole cell recording patch electrodes and assayed for Cl<sup>-</sup> specific whole cell currents. 25 μM INO-4913 applied in this manner stimulated a large increase in whole cell Cl<sup>-</sup> current in 7 out of 13 recordings (1326 +/- 630 pA (Mean +/- SEM), 60 mV; -913 +/- 463 pA, -60mV at 4 min post patch rupture). 2 μM INO-4913 stimulated a moderate increase in 2 of 4 recordings (159 and 689 pA, 60 mV; -59 and -324 pA, -60 mV). The IV relationship of the currents were consistent with an outwardly rectifying Cl<sup>-</sup> channel (E<sub>rev</sub> (observed) = 2.6 mV; E<sub>rev</sub> (Cl<sup>-</sup>) = 0 mV). Similar currents were observed in 4 out of 9 cells bath-exposed to 10 μM INO-4995 using perforated patch recordings whereas no response was observed to its enantiomer. Both INO-4995 and INO-4913 triggered currents were inhibited with 100 μM niflumic acid. Minimal changes were observed in vehicle (1/9) or butyrate(2/9) control experiments. These data demonstrate that a metabolite of INO-4995 when introduced intracellularly induces a similar increase in Cl<sup>-</sup> current as induced by extracellularly applied INO-4995.