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Poster Session Abstracts

CFTR

1★

A DISEASE-RELATED MUTATION IN TM5 ALTERS THE CONDUCTION AND GATING PROPERTIES OF CFTR. M.K. Mansoura[1], T.V. Strong[2], F.S. Collins[2], and D.C. Dawson[3]. Bioengineering Program[1], Departments of Physiology[3] and Human Genetics[2], University of Michigan Medical School, Ann Arbor, MI, USA.

We assayed the ion channel properties of a CFTR bearing a mutation in the fifth putative transmembrane segment, G314E, which has been associated with cystic fibrosis (Golla et al., Hum. Mutation 3:67, 1994). Mutant and wild-type CFTRs were expressed in *Xenopus* oocytes and macroscopic, cAMP-activated currents were measured using a two-electrode voltage clamp. CFTR gating was assayed by measuring the dose-dependent activation of chloride currents in response to increasing concentrations of 3-isobutyl-1-methylxanthine (IBMX) in the presence of 10 mM forskolin. Conduction properties were probed by examining the effect of substituting thiocyanate (SCN^-) for chloride (Cl^-) in the extracellular bath (Tabcharani et al., Nature 366:79, 1993). In the IBMX dose response assay, the G314E mutation substantially reduced the sensitivity to activating conditions. The apparent $K_{1/2}$ was 1.3 mM (compared to 0.12 mM for wild-type CFTR) and was similar to that seen with G551D, a mutation associated with severe disease. A mutant with a more conservative substitution, G314A, was more sensitive than G314E to activating conditions ($K_{1/2}$ = 0.8 mM), but still much less sensitive than wild-type CFTR. Equimolar substitution of SCN^- for Cl^- in the external bath shifted the reversal potential for wild-type CFTR to more negative values, consistent with a $P_{SCN}/P_{Cl} > 1$. The slope conductance, however, was reduced in a concentration-dependent manner when measured at the reversal potential (or at -60 mV). The shift in reversal potential for the G314E mutant was similar to that seen with wild-type, but the slope conductance was either only slightly reduced or increased by SCN^- substitution, as if this substitution alters the interaction of SCN^- with the channel. These results suggest that the glycine residue at position 314 is important for both the conduction and gating properties of wild-type CFTR, and that mutations at this position severely compromise the function of CFTR chloride channels. (Supported by the NIH, CF Foundation, and the University of Michigan.)

2★

IDENTIFICATION OF AMINO ACID RESIDUES IN THE M1 MEMBRANE-SPANNING SEGMENT THAT LINE THE Cl^- CHANNEL OF CFTR. Myles H. Akabas, Christine Kaufmann, Patrick Archdeacon. Center for Molecular Recognition, Columbia University, New York, NY, USA.

In order to understand the structural bases of ion conduction and selectivity in CFTR we combined mutagenesis, heterologous expression and chemical modification to identify amino acid residues that line the ion channel of CFTR. We mutated, one at a time, 14 consecutive residues (91-104) in the M1 membrane-spanning segment of CFTR to cysteine. To date, we have expressed 11 of these mutant CFTR's in Xenopus oocytes and probed the susceptibility of the engineered cysteine to covalent chemical modification by small, charged, sulfhydryl-specific reagents, added extracellularly. The reagents used were derivatives of methanethiosulfonate. We assume that among residues in membrane-spanning segments, only those lining the channel will be susceptible to modification by these highly polar reagents and that such modification would irreversibly alter conduction. In contrast, engineered cysteines facing the interior of the protein or the lipid bilayer would be inaccessible to the reagents. Conduction was irreversibly altered in oocytes expressing the mutants G91C, K95C and Q98C. We infer that the side chains of the corresponding wild-type residues Gly91, Lys95 and Gln98 are exposed in the channel lumen. When the residues in the M1 segment are plotted on a helical wheel, the channel-lining residues lie along one side of an α helix; we infer that the secondary structure of this region is α helical. Supported by the NIH (DK41146), New York City Affiliate of AHA, and a Klingenstein Fellowship in the Neurosciences (MHA).

3★

CFTR ACTIVATION: STIMULATORY AND INHIBITORY PHOSPHORYLATION SITES IN THE R DOMAIN.
D.J. Wilkinson[1], T.V. Strong[2], F.S. Collins[2], D.C. Dawson[1]. Depts. of Physiology[1] and Human Genetics[2], Univ. of Michigan Medical School, Ann Arbor, MI, USA.

Activation of the CFTR Cl^- channel requires cAMP-dependent phosphorylation. Serine (S) residues in the R domain that lie within consensus sequences for phosphorylation by cAMP-dependent protein kinase are candidates for regulatory sites. An initial study (Cheng et al., Cell 66:1027, 1991) suggested that R domain phosphorylation sites might be degenerate, but we showed that when individual phosphorylation sites were eliminated by substituting alanine (A) for serine, the effects on the steady state activation kinetics of CFTR varied widely from one site to another (Strong et al., Ped. Pulmon. S8:249, 1992). In particular, substitution of serine 813 (S813A) substantially reduced the sensitivity to activation, but substitution of serine 768 (S768A) actually increased the sensitivity to activation. To explore the basis for these changes, we compared the effects of these substitutions on the rates of CFTR activation (on rate) and deactivation (off rate). Wild type and mutant CFTRs were expressed in *Xenopus* oocytes, and a two-electrode voltage clamp was used to monitor the activation rate of CFTR Cl^- conductance in response to 10 μM forskolin and 5 mM IBMX (3-isobutyl-1-methylxanthine) as well as the deactivation rate after washout. The mutation S813A reduced the

★ = Also being presented orally in a workshop session.
☆ = Data from these abstracts are being combined into one presentation.
Abstracts 61 & 62 and 67 & 68 in Workshop #11.
Abstracts 231 & 232 in Workshop #24.
Abstracts 372 & 373, 374 & 375 and 378 & 379 in Workshop #5.

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