unidirectionally relative to the liposome interior, led to channel activation,[61,253] 1a channels increased activity when gradients were altered in either direction. Thus, genotype 1a channels displayed a more open-form, pore-like behaviour compared with genotype 1b, explaining why His17 may be dispensable for channel opening. Intriguingly, variation amongst patient-derived 1a sequences at key ionisable positions switched p7 gating behaviour back to a genotype 1b pattern, whereas others enhanced/reduced channel activity. Specifically, Ser21Pro and Tyr31His induced 1b-like behaviour, whereas His17Asn dramatically enhanced channel activity, but in a pore-like pattern.[253] Interestingly, Asn17 is also present in the genotype 2a J6 isolate and transferring this p7 into other genotype backgrounds dramatically enhances virion production.[232]

One hypothesis explaining the observed behaviour of p7 from different genotypes and mutant backgrounds is that channel opening is dependent on the hydrophobic/hydrophilic balance of the channel interior, which in turn is influenced by both overall channel structure and variation of the lumenal amino acid sequence. Hydrophobic/bulky residues brought into close proximity would represent a barrier to the formation of a water column through the channel, and this must be overcome for ions to flow. The energy required to achieve this may be minimal in a pore-like fashion or may require events such as ionisation to effect necessary structural changes. In the case of genotype 1a channels, this barrier appears low, resulting in a large proportion of the channel population tending towards an open state, even at neutral pH where His17 remains uncharged. This barrier may be further lowered by the presence of Asn17. Changes where polar residues are altered such as Ser21Pro and Tyr31His would increase the hydrophobicity of the lumen at neutral pH (His pK_a in solution is 6.04) and so make the energy obtained from His17 protonation at lower pH now necessary to effect opening. This scenario seems to be the baseline for genotype 1b channels, so explaining why His17Ala mutations abrogate activity.[241] Lastly, some isolates such as genotype 2a JFH-1 possess both His17 and His31, generating a hydrophobic lumen at neutral pH with a potentially redundant means of effecting channel opening *via* protonation of one or other His residue; JFH-1 p7 is potently activated by reduced external pH in liposomes (E. Atkins and S. Griffin, unpublished observations). Therefore, mutation of one or other JFH-1 His residue in isolation would not prevent channel opening and explains the lack of virion production phenotypes when individual changes are introduced into the JFH-1 genome.[233,254] Extensive mutagenesis programmes and characterisation of pH-dependent gating will be necessary to formalise this hypothesis, and future investigations must allow for the clear genotype- and subtype-dependent differences in p7 function, which appear to affect virtually all facets of this channel examined to date.

9.2.3.4 *How Do Adamantanes, Amilorides and Alkyl Imino-Sugars Inhibit p7 Channels?*

Work done using prototype p7 inhibitors initially generated considerable controversy, particularly in the light of a perceived lack of clinical efficacy.[84,85]