[^{3}H]-dofetilide can be applied to cells expressing the hERG channel or a membrane preparation containing the hERG channel. Candidate compounds are then examined for their ability to displace the radiolabel from the channel using the appropriate detection methods (e.g., scintillation counters).[37] While this method is relatively low cost and available in high-throughput mode, it is has an important draw back. The assay will only identify compounds that target the dofetilide binding site. Since there are multiple compound binding sites on the hERG channel, a negative result (no binding) in this assay is not necessarily an indication that the compound in question is clear of hERG issues. Also, this assay is not capable of providing information on the electrophysiological impact of a compound on targeted cells.

As an alternative, hERG activity can be assessed using a rubidium (Rb^+) efflux assay (Figure 8.19). Rubidium can move through the hERG channel,

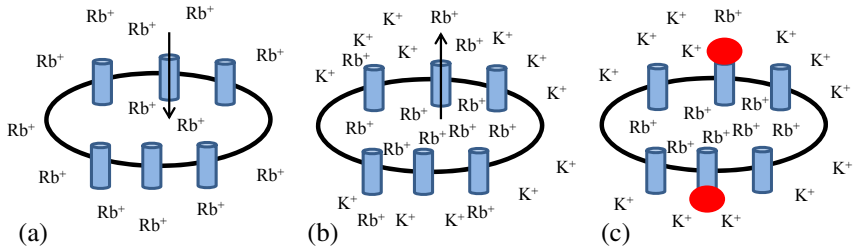

FIGURE 8.19 Rubidium (Rb^+) ions are capable of moving through potassium channels such as the hERG channel (blue) as a result of the similar size and charge. The rubidium (Rb^+) efflux assay takes advantage of this phenomenon. (a) Cells expressing the hERG channel are loaded with Rb^+ by placing them in a media rich in the ion. (b) Replacing the Rb^+ with K^+ will cause the cells to release Rb^+, which can be measured using atomic absorption spectroscopy or scintillation counting ($^{86}Rb^+$). (c) Compounds that block the hERG channel (red) will slow the exit of Rb^+, indicating a potential hERG liability.

as it is has the same charge and size as K^+. Since it is not normally present in cells or media, background Rb^+ concentration is negligible. When cells expressing the hERG channel, such as CHO cells that have been transfected with the hERG gene, are exposed to Rb^+ containing media, they will absorb the Rb^+ until equilibrium has been achieved between the inside and outside of the cells. Replacing the media with Rb^+-free media containing high concentrations of the K^+ will cause the hERG channels to open, allowing the Rb^+ to escape the cells. Measuring the concentration of Rb^+ that enters the media over a fixed period of time provides insight into hERG channel activity. Compounds can be screened for hERG channel activity by including the test compound in the K^+-containing media. Compounds that block hERG channel activity will prevent Rb^+ from leaving the cells, leading to decreased concentration in the media. Rb^+ concentration inside the cells can also be assessed at the end of the assay by lysing the cells. In either case, Rb^+