Chapter 1
Background: Problem and Methods

Drugs are generally devised to alter the function of cells in a favorable manner. The actions of drugs can in some cases be represented by mathematical models often phrased in terms of differential equations. Our aim in these notes is to study such models and show how the effect of drugs can be optimized. More precisely, drugs are represented in terms of a set of parameters and we show how optimal drugs can be characterized by tuning the parameters. Our approach is to consider models of a healthy cell and a non-healthy cell and a model of a non-healthy cell to which a drug has been applied. The problem we are trying to resolve is how to tweak the parameters of the drug such that the drugged non-healthy cell behaves as similarly to the healthy cell as possible.

We will use this approach to address two processes of immense importance in physiology: (1) voltage-gated ion channels and (2) calcium release from storage structures inside the cell. We will also study combinations of these processes occurring in a space in which the release through voltage-gated ion channels interacts with calcium release from the internal storage structures.

Both processes can be affected by disease and by mutations. In these notes we will concentrate on wild type (healthy) cells and mutant cells. We will assume that the behavior of the wild type cell can be described in terms of Markov models and that a Markov model can represent the effects of the mutation.

1.1 Action Potentials

Suppose a group of engineers were given the task of developing a pump weighing about 300 g that is supposed to work uninterruptedly and basically without maintenance for about 80 years, pump about 7,0001 of blood every day, and beat every second. The group would—and should—agree that the task is impossible but,

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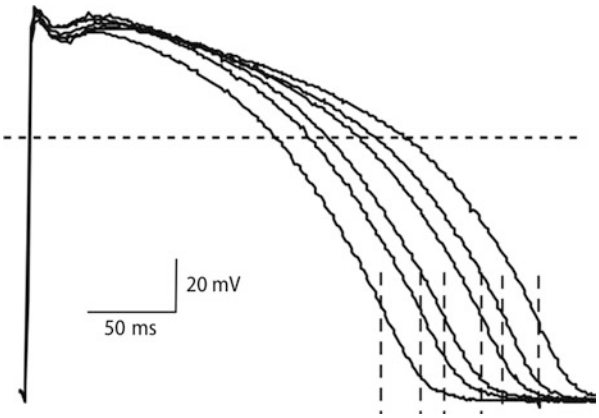

Fig. 1.1 Action potentials obtained by measurements taken from Jost et al. [40]

under pressure from their employer, they would probably agree that the mechanism would have to be extremely simple. Fortunately for us all, the pump has already been developed by evolution, but it is very far from being simple; it is an extremely complex piece of machinery, so complex that how it works is still not completely understood. For an intriguing illustration of this, the reader is encouraged to consult the fascinating joint paper by Lakatta and DiFrancesco [47] in which they debate the following fundamental question: How is the heartbeat initiated? It is remarkable that such a basic question is still open. Two plausible and completely different mechanisms are discussed, with supporting experimental data and mathematical models for both. The interested reader can also consult Li et al. [50] for an introduction to this discussion.

Even if the exact mechanism for initiating the heartbeat is still under debate, it is completely clear that every normal heartbeat is initiated in the sinoatrial node. From that node, an electrochemical wave spreads throughout the cardiac muscle. With every beat, billions of cardiac cells undergo an action potential that is a characteristic temporal change of the transmembrane potential of the cell V, defined by

$$V = V_i - V_e,$$

where V_i and V_e are the intracellular and extracellular electrical potentials, respectively.

In Fig. 1.1 we show an action potential obtained by measurements. The recordings are taken from the paper by Jost et al. [40]. Mathematical models have been used to represent action potentials ever since the groundbreaking paper by Hodgkin and Huxley [33] from 1952. The first models of cardiac cells were developed by Noble [61, 62] in 1960–1962. In Fig. 1.2 an action potential is presented based on the mathematical model of ventricular cardiac cells developed by Grandi et al. [29].

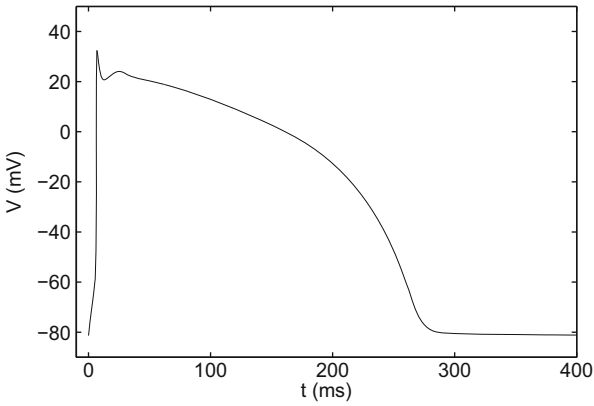

Fig. 1.2 Action potential computed using the model of Grandi et al. [29]

When an electrical wave of the increased transmembrane potential approaches a cell, the cell's transmembrane potential is elevated above a critical value. This elevation leads to the opening of sodium channels, resulting in a huge influx of sodium ions into the cell. This rapid process dramatically increases the transmembrane potential and is referred to as the upstroke of the action potential. When the transmembrane potential increases, voltage-gated calcium channels in the cell membrane open and calcium ions flow into the cell because of the huge difference in concentrations; the extracellular concentration of calcium ions is much greater than the intracellular (cytosolic) concentration when the cell is at rest. The increased concentration of calcium ions within the cell triggers the opening of channels to internal stores and a great deal more calcium floods into the cytosol. The increased level of calcium in the cytosol leads to the cell's contraction, which is basically the main goal of the whole operation. Then everything returns to the resting state: Calcium is pumped out of the cell and into internal stores—every cell prepares for a new wave.

Even if this process is amazingly stable and versatile and a masterpiece by any standard in the universe, it is not infallible. It can be harmed by disease, by the side effects of drugs, and by mutations. In these lecture notes, we shall focus on the effect of mutations and search for theoretical drugs that can, in principle, repair the effect of dangerous mutations. The study of mutations affecting cardiac cells is a huge field and we will simply look at prototypical models that capture the characteristic effects of well-known mutations. Our main objective is to present methods for computing characterizations of optimal theoretical drugs using prototypical models of ion release.

Most of these lecture notes will be focused on what happens in single ion channels. However, in the final chapter we will return to the action potential of the whole cell.

1.2 Markov Models

The cell membrane is densely populated with ion channels that can open and close to control the flow of ions across the cell membrane. In Fig. 1.3, we show the recordings of a single channel and we note the frequent transitions between the open and closed states and how the frequency changes with the transmembrane potential. It is commonly believed that the state of a single channel is adequately modeled using a stochastic approach. Actually, it is common to claim that the process is stochastic. It is hard, if not impossible, to prove that something is stochastic, but for modeling purposes it suffices to state that a stochastic approach leads to reasonable models of the gating dynamics.

A Markov model in its simplest form is usually written as the chemical reaction scheme

$$C \underset{k_{co}}{\overset{k_{oc}}{\leftrightarrows}} O, \tag{1.1}$$

where k_{oc} and k_{co} are reaction rates that may depend on the transmembrane potential. We will return to the interpretation of this notation many times, but let us just roughly describe what it means. Suppose at a given time t that the gate is open so the channel is in state O and suppose that Δt is a very short time interval. Then (1.1) states that the probability that the channel changes state from open to closed is given by $k_{oc}\Delta t$. Similarly, if the channel is closed (C), the probability for a change to the open state is given by $k_{co}\Delta t$.

More formally, we let $S = S(t)$ denote a random variable representing the state of the channel at time t, so $S \in \{O, C\}$. Then the transition rates k_{oc} and k_{co} give the probability of changing state during a small time interval Δt :

$$k_{oc}\Delta t = \text{Prob}\left[S(t + \Delta t) = C \mid S(t) = O(t)\right]$$

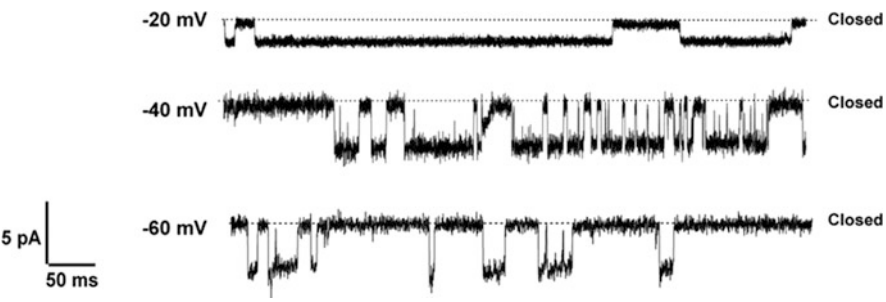

Fig. 1.3 Single-channel recording of a sodium current (from Shaya et al. [81]). The levels of the current indicate whether the channel is closed (as indicated in the figure) or open. The probability that the channel is open is low at −60 mV, higher at −40 mV, and even higher at −20 mV

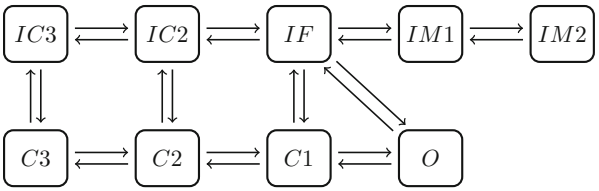

Fig. 1.4 The sodium channel model of Clancy et al. [15]; O is the open state, C1, C2 and C3 are the closed states, while the rest of the states represent different kinds of inactivation

and

$$k_{co}\Delta t = \text{Prob}\left[S(t+\Delta t) = O \mid S(t) = C(t)\right],$$

respectively. With this notation, we easily see that we can play with the properties of the channels by changing the values of the parameters k_{oc} and k_{oc}. We also see that we can make the reaction scheme dependent on the transmembrane potential V (mV) by allowing the reaction terms to depend on V.

The case of just one closed and one open state is particularly simple but it is still the base model and it is frequently used in modeling ion channels. However, much more intricate models have been derived and one is shown in Fig. 1.4. It represents a Markov model with one open state, three closed states, and five inactivated[1] states.

The popularity of these models stems from the fact that it is possible to adjust the parameters involved to obtain a model that reflects data quite well. However, it should also be mentioned that models can be so complex that it is virtually impossible to uniquely determine all the parameters involved. In these notes, we shall confine ourselves to relatively simple Markov models but the methods we describe can be applied, at least in principle, to Markov models of higher complexity.

1.2.1 The Master Equation

From the Markov model written on the form (1.1), we can derive an equation giving the evolution of the probability of the two states, open (O) and closed (C). Let $o = o(t)$ be the probability that the channel is in the open (O) state at time t and let $c = c(t)$ denote the probability that the channel is closed (C). We assume that the probabilities o and c are known at time t and then use the Markov model (1.1) to compute the probabilities at time $t + \Delta t$. Here Δt is assumed to be so small that the

[1] Inactivated states are discussed in Chap. 11.

channel changes state at most once during the time step from t to $t + \Delta t$. Then the scheme (1.1) states that the open probability at time $t + \Delta t$ is given by

$$o(t + \Delta t) = \text{Prob}\,[(S(t) = C) \text{ and } (C \to O \text{ during } \Delta t)] \tag{1.2}$$

$$+ \text{Prob}\,[(S(t) = O) \text{ and not}(O \to C \text{ during } \Delta t)] \tag{1.3}$$

$$= c(t) \cdot (\Delta t k_{co}) + o(t) \cdot (1 - \Delta t k_{oc}) \tag{1.4}$$

so

$$o(t + \Delta t) = o(t) + \Delta t(k_{co} c(t) - k_{oc} o(t)).$$

From this equation, we obtain

$$\frac{o(t + \Delta t) - o(t)}{\Delta t} = k_{co} c(t) - k_{oc} o(t),$$

and, therefore, by passing to the limit $\Delta t \to 0$, we get the differential equation

$$o'(t) = k_{co} c(t) - k_{oc} o(t). \tag{1.5}$$

Similarly, we find that the probability of being in the closed state evolves according to

$$c'(t) = k_{oc} o(t) - k_{co} c(t). \tag{1.6}$$

Since we are dealing with probabilities, it is reasonable to assume that the initial conditions add up to one (the channel is either open or closed) and therefore, by adding the equations above, we find that

$$o(t) + c(t) = 1$$

for all time. Hence the variable c in (1.5) can be replaced by $1 - o$ and the system (1.5,1.6) can be written as a scalar equation of the form

$$o'(t) = (k_{co} + k_{oc}) \left(\frac{k_{co}}{k_{co} + k_{oc}} - o\,(t) \right). \tag{1.7}$$

Here we see that

$$o = \frac{k_{co}}{k_{co} + k_{oc}}$$

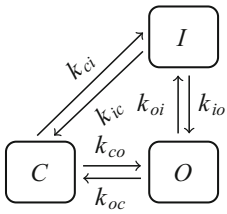

Fig. 1.5 Markov model including three possible states: open (O), closed (C), and inactivated (I)

is a stable equilibrium solution. Furthermore, if we know that the channel is closed initially, that is, $o(0) = 0$, we get the solution

$$o(t) = \frac{k_{co}}{k_{co} + k_{oc}} \left(1 - e^{-(k_{co}+k_{oc})t}\right)$$

and we notice that the equilibrium is reached more quickly as the sum of the rates $k_{co} + k_{oc}$ increases.

1.2.2 The Master Equation of a Three-State Model

The development of the master equation for the two-state model above can be carried out for any Markov model. For instance, if we consider the three-state Markov model shown in Fig. 1.5, we realize that the probabilities of the open (O), closed (C), and inactivated (I) states are governed by the following system of ordinary differential equations:

$$\begin{aligned} o' &= k_{io} i + k_{co} c - (k_{oi} + k_{oc})\, o, \\ c' &= k_{oc} o + k_{ic} i - (k_{co} + k_{ci})\, c, \\ i' &= k_{oi} o + k_{ci} c - (k_{io} + k_{ic})\, i, \end{aligned} \tag{1.8}$$

Since

$$i = 1 - (o + c)\,, \tag{1.9}$$

we have the following 2×2 system:

$$o' = k_{io} + (k_{co} - k_{io})\, c - (k_{oi} + k_{oc} + k_{io})\, o, \tag{1.10}$$

$$c' = k_{ic} + (k_{oc} - k_{ic})\, o - (k_{co} + k_{ci} + k_{ic})\, c. \tag{1.11}$$

We will now show, using a numerical computation, that the solution of the system (1.10,1.11) coincides with the average result of Monte Carlo simulations using the Markov model shown in Fig. 1.5 as the number of Monte Carlo runs goes to infinity.

1.2.3 Monte Carlo Simulations Based on the Markov Model

Before we compare the two computational schemes, let us briefly describe how the Monte Carlo simulation can be implemented. We choose a small timestep Δt and we assume that the state at time $t = t_n = n\Delta t$, where n is a non-negative integer, is either O, C, or I. For simplicity, we describe how the computation proceeds in the case of the channel being in the open (O) state at time $t = t_n$. In order to decide the state at time $t_{n+1} = t_n + \Delta t$, we divide the unit interval into three non-overlapping parts: $A_c = [0, k_{oc}\Delta t)$, $A_i = [k_{oc}\Delta t, k_{oc}\Delta t + k_{oi}\Delta t)$, $A_o = [k_{oc}\Delta t + k_{oi}\Delta t, 1]$. Then, at time $t_{n+1} = t_n + \Delta t$, we can update the state of the channel based on a random number r_n in the unit interval drawn from a uniform distribution. Specifically, if $r_n \in A_o$, the channel remains open; if $r_n \in A_c$, the state of the channel changes from open to closed; and, finally, if $r_n \in A_i$, the state of the channel changes from open to inactivated.

Similar steps are straightforward to devise for the case of the channel being in the closed or inactivated states at time $t = t_n$.

1.2.4 Comparison of Monte Carlo Simulations and Solutions of the Master Equation

In Fig. 1.6 we compare the probabilities computed by solving the master equation (1.10,1.11) (red lines) and by Monte Carlo simulations using the Markov model as described above. In the simulations we have used the initial conditions $o(0) = i(0) = 0$ and $c(0) = 1$ and the rates used in the computations are given in Table 1.1. As the number of Monte Carlo simulations increases, we see that the average approaches the solution of the continuous master equation. In these computations the master equation was solved using the function ODE15s in Matlab.

1.2.5 Equilibrium Probabilities

The equilibrium state of the reaction shown in Fig. 1.5 is characterized by the equations

$$\begin{aligned} k_{co}c &= k_{oc}o, \\ k_{oi}o &= k_{io}i, \\ k_{ic}i &= k_{ci}c, \end{aligned} \tag{1.12}$$

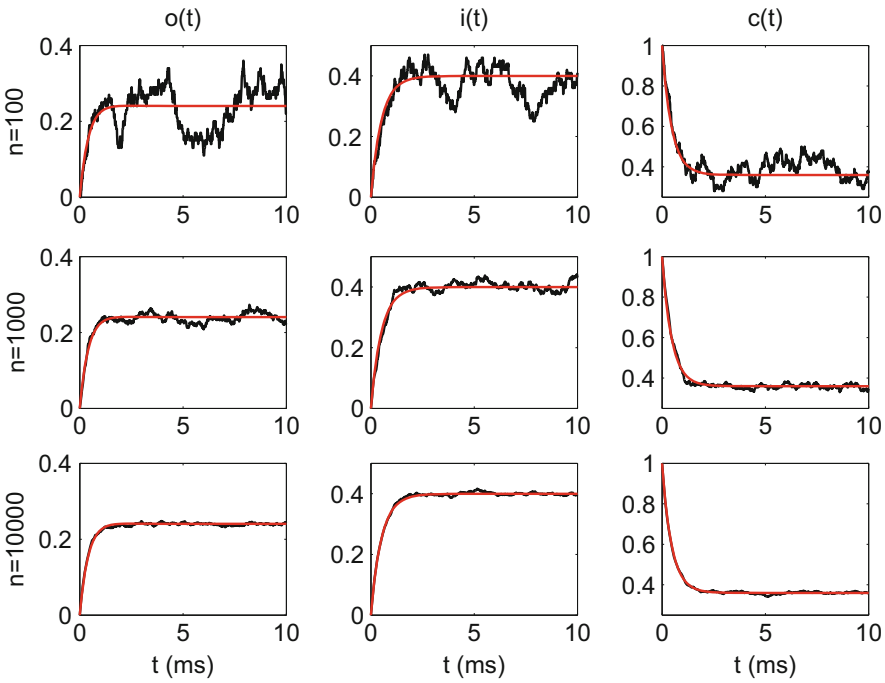

Fig. 1.6 Comparison of the solution of the master equation (1.10,1.11) (*red lines*) and the results of Monte Carlo simulations based on the Markov model given in Fig. 1.5. The time step used in the Monte-Carlo simulations was $\Delta t = 0.01$ ms in all the panels and the simulations were run for 10 ms. The number of Monte Carlo simulations increases from 100 (*top*) to 10,000 (*bottom*)

Table 1.1 Rates (in 1/ms) of the Markov model given in Fig. 1.5 used in the computations presented in Fig. 1.6

k_{oi}	k_{io}	k_{co}	k_{oc}	k_{ic}	k_{ci}
0.5	0.3	0.6	0.9	0.72	0.8

where o, c, and i denote the probabilities of the channel being open, closed, or inactivated, respectively. It follows that

$$c = \frac{k_{oc}}{k_{co}} o$$

and

$$i = \frac{k_{oi}}{k_{io}} o.$$

By using the fact that $o + c + i = 1$, we obtain

$$\left(1 + \frac{k_{oc}}{k_{co}} + \frac{k_{oi}}{k_{io}}\right) o = 1$$

and therefore

$$o = \frac{1}{1 + \frac{k_{oc}}{k_{co}} + \frac{k_{oi}}{k_{io}}},$$

$$c = \frac{\frac{k_{oc}}{k_{co}}}{1 + \frac{k_{oc}}{k_{co}} + \frac{k_{oi}}{k_{io}}},$$

$$i = \frac{\frac{k_{oi}}{k_{io}}}{1 + \frac{k_{oc}}{k_{co}} + \frac{k_{oi}}{k_{io}}}.$$

For the particular rates given in Table 1.1, we get the following equilibrium probabilities: $o = 0.24$, $c = 0.36$, and $i = 0.4$.

1.2.6 Detailed Balance

In order to compute the equilibrium solution of (1.8) above, we assumed that each of the sub-transitions of the diagram given in Fig. 1.5 was in equilibrium. More precisely, we assumed that

$$k_{co}c = k_{oc}o,\ k_{oi}o = k_{io}i,\ \text{and}\ k_{ic}i = k_{ci}c.$$

These three relations yield

$$k_{co}k_{oi}k_{ic} = k_{ci}k_{io}k_{oc}. \tag{1.13}$$

This relation is referred to as the condition of *detailed balance*. In these notes, we will always assume that Markov models satisfy this condition. More generally, the product of the rates in a loop (e.g. the I-O-C loop of Fig. 1.5) in the clockwise direction equals the product of the rates in the counterclockwise direction. Under this assumption, the equilibrium solution can always be computed by the method indicated above. We will use the same technique many times in these notes.

1.3 The Master Equation and the Equilibrium Solution

We have seen that the Markov model written in the form

$$C \underset{k_{co}}{\overset{k_{oc}}{\leftrightarrows}} O \tag{1.14}$$

leads to a master equation of the form

$$o'(t) = k_{co}c(t) - k_{oc}o(t), \tag{1.15}$$

$$c'(t) = k_{oc}o(t) - k_{co}c(t). \tag{1.16}$$

Since $o + c = 1$, we can reduce the system to the scalar equation,

$$o'(t) = (k_{co} + k_{oc})\left(\frac{k_{co}}{k_{co} + k_{oc}} - o\,(t)\right)$$

and we readily see that the equilibrium solution is given by

$$o = \frac{k_{co}}{k_{co} + k_{oc}}.$$

Exactly the same steps can be followed for the three-state Markov model illustrated in Fig. 1.5. The associated Markov model reads

$$o' = k_{io}i + k_{co}c - (k_{oi} + k_{oc})\,o$$
$$c' = k_{oc}o + k_{ic}i - (k_{co} + k_{ci})\,c$$
$$i' = k_{oi}o + k_{ci}c - (k_{io} + k_{ic})\,i$$

and since

$$i = 1 - (o + c) \tag{1.17}$$

we arrive at the following 2×2 system:

$$o' = k_{io} + (k_{co} - k_{io})\,c - (k_{oi} + k_{oc} + k_{io})\,o,$$
$$c' = k_{ic} + (k_{oc} - k_{ic})\,o - (k_{co} + k_{ci} + k_{ic})\,c.$$

The equilibrium solution is now defined by a 2×2 linear system of equations of the form

$$Bq = b, \tag{1.18}$$

where

$$B = \begin{pmatrix} k_{oi} + k_{oc} + k_{io} & k_{io} - k_{co} \\ k_{ic} - k_{oc} & k_{co} + k_{ci} + k_{ic} \end{pmatrix}, \; q = \begin{pmatrix} o \\ c \end{pmatrix}, \text{ and } b = \begin{pmatrix} k_{io} \\ k_{ic} \end{pmatrix}.$$

By solving this linear system and using (1.17), we find (as above) that

$$o = K^{-1}, \; c = \frac{k_{oc}}{k_{co}} K^{-1}, \; i = \frac{k_{oi}}{k_{io}} K^{-1},$$

where

$$K = 1 + \frac{k_{oc}}{k_{co}} + \frac{k_{oi}}{k_{io}}.$$

1.3.1 Linear Algebra Approach to Finding the Equilibrium Solution

Calculations to find the equilibrium solution will be done repeatedly in these notes. We will always use the special structure of the Markov model to derive the equilibrium solution, but it also worth noting that this can be done by solving a linear system. The master equation associated with a Markov model of the form (1.14) or of the form given in Fig. 1.4 can always be written in the form

$$p' = Ap,$$

where p is a vector containing the probabilities of occupying the different states of the Markov model. Since the sum of the probabilities adds up to one, the number of unknowns can be reduced by one and the system takes the form

$$q' = b - Bq.$$

Therefore, the equilibrium solution can be found by solving the linear system (1.18).

Instead of reducing the number of unknowns, we can also address the problem more directly by computing the eigenvector associated the eigenvalue $\lambda = 0$. For instance, using Matlab we can put $z = \text{null}(A)$ and then define

$$p = \frac{z}{\sum_i z_i}$$

where z_i denote the components of the vector z.

1.4 Stochastic Simulations and Probability Density Functions

Given the Markov model, defining a stochastic differential equation describing changes of the transmembrane potential due to the opening and closing of the channel is quite straightforward. Additionally, based on the stochastic differential equation, we will derive deterministic differential equations describing the probability density functions of the states involved in the Markov model. We thus have two ways to analyze models of ion channels: We can either run numerous Monte Carlo simulations using the stochastic differential equation or solve the deterministic differential equations defining the probability density functions. Both these methods will be used throughout the notes. Although one method is the average of the other, we will see that both provide distinct insights useful to understanding the mechanisms under consideration.

1.5 Markov Models of Calcium Release

The contraction of the heart is a collective and very well-coordinated effort achieved in a collaboration involving billions of cells. For each of these cells, the contraction depends on the release of a massive amount of calcium from internal storage. The release takes place in many thousands of release units within each cell and the state of the release process is believed to be adequately modeled using Markov models.

We will study this release in several steps and we start by assuming that the only varying concentration is in the dyad and that the reaction rates of the Markov model vary only with this single concentration. This case will be studied in great detail and we will explain how drugs can be theoretically constructed to repair mutations affecting the release mechanism. The analysis is based on a scalar stochastic differential equation representing the concentration of calcium in the dyad. The properties of this model will be analyzed using Monte Carlo simulations. Furthermore, we will derive a system of deterministic partial differential equations describing the probability density function of the states of the Markov model.

It is more common to divide the calcium concentration into two values—not only one—which leads to 2×2 stochastic differential equations to be analyzed. This model will also be analyzed using Monte Carlo simulations and by a 2D deterministic system of partial differential equations representing the probability density functions of the states of the Markov model.

Next, we shall couple the calcium concentration to the voltage-gated release of calcium through so-called L-type calcium channels. This model will allow us to study optimal drugs, combining the effect on calcium release and L-type channels. The balance of these mechanisms rules the calcium-induced calcium release that is at the crux of cardiac contraction. The calcium-induced calcium release model is stated in terms of a 2×2 model of stochastic equations where the transmembrane potential V is included as a parameter in the model. The associated model for the

probability density functions is given by a 2D system of partial differential equations where the transmembrane potential is again included as a parameter.

1.6 Markov Models of Ion Channels

After analysis of the calcium release we move on to study voltage-gated ion channels. We will immediately see that in mathematical terms the problem is very similar to the calcium release problem. For the ion channel case, however, the stochastic equation is one-dimensional and so is the associated deterministic partial differential equation. The basic Markov model is still based on the open and closed states, but we will also see that an inactivated state plays a central role. Optimal theoretical drugs will be derived and we will observe that they work nicely.

1.7 Mutations Described by Markov Models

A trademark of mutations affecting ion channels and calcium release mechanisms is that they change the open probability and possibly also the mean open time and other characteristics of the channels involved. We will show below that the equilibrium open probability of the channel described by the Markov model of the form (1.1) is given by

$$o = \frac{k_{co}}{k_{co} + k_{oc}}$$

and the mean open time is given by

$$\tau_o = \frac{1}{k_{oc}}.$$

The concept of mean open time will be discussed in Chap. 13 and the formula $\tau_o = 1/k_{oc}$ will be derived in that chapter. Given these formulas, it is straightforward to see that the effect of mutations affecting the open probability or the mean open time can be modeled by changing the parameters of the Markov model. In these notes we shall focus on rather simple changes in the model but, again, the techniques can be generalized to more intricate cases.

Two examples of the effect of mutations are given in Figs. 1.7 and 1.8. Figure 1.7 shows recordings of the open and closed states for the wild type and the V2475F mutation of the ryanodine receptor (RyR). The graphs in Fig. 1.8 show similar results for the voltage-gated sodium channel when the wild type recordings are compared with recordings from a mutant (ΔKPQ) channel.

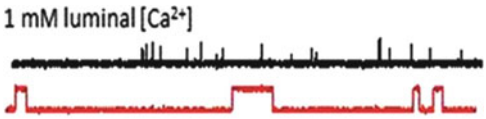

Fig. 1.7 Single-channel recordings of wild type (*black*) and mutant (*red*) cardiac RyR channels. The open probability and the mean open time are significantly increased for the mutant (V2475F) case. The graphs are from Figure 3 of Loaiza et al. [52]

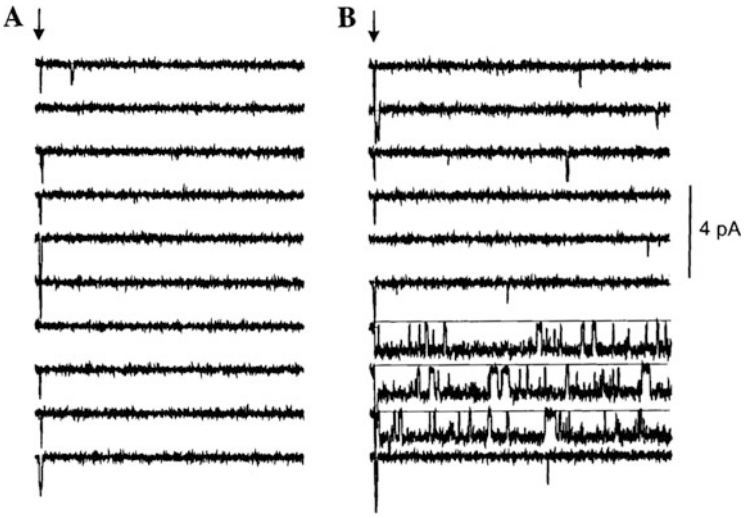

Fig. 1.8 Sodium current recordings taken from Figure 4 of Chandra et al. [13]: A represents the wild type and B represents the ΔKPQ mutant. The recordings are based on 200-ms depolarizing pulses from −100 to −40 mV

1.8 The Problem and Steps Toward Solutions

Assume that experimental data on wild type cells can be used to identify the parameters of a Markov model faithfully describing the stochastic properties of the wild type channel and that experimental data on mutant cells can be used to establish a Markov model of similar structure representing the stochastic properties of the mutant channel. Furthermore, we assume that the Markov model of the mutant can be extended to account for the effect of a theoretical drug. *The problem is then to compute the reaction rates of the drug such that, after the drug is applied, the mutant channel behaves as similarly to the wild type channel as possible.* The essence of these notes is to show how to solve this problem mathematically; we show how to compute an optimal theoretical drug. To clarify what we mean by an optimal theoretical drug, we will give a few examples that will be discussed later and then we will briefly discuss the concept of a theoretical drug more generally.

1.8.1 Markov Models for Drugs: Open State and Closed State Blockers

By using the notation of chemical reactions introduced above, we can explain the problem in a bit more detail. The reaction scheme for an open state blocker can be illustrated as follows:

$$C \underset{k_{co}}{\overset{k_{oc}}{\leftrightarrows}} O \underset{k_{ob}}{\overset{k_{bo}}{\leftrightarrows}} B. \tag{1.19}$$

For theoretical purposes, this drug is well defined, provided that we know the values of the parameters k_{ob} and k_{bo}. We will often assume that these parameters are constants. As mentioned above, one example of a problem we want to overcome is mutations leading to an increased open probability; so either the release mechanism is too prone to releasing calcium from internal storage or the ion channels are too prone to allowing current to flow through the cell membrane.

Since the problem involves too high of an open probability, it seems reasonable to try to fix the open probability by extending the reaction scheme and directly affecting this state, as illustrated in the reaction scheme above. By allowing the probability to be moved from O to B, the open probability will be reduced and thus the goal will be achieved. This reasoning seems impeccable and it seems much less intuitive to use a closed state drug of the form

$$B \underset{k_{bc}}{\overset{k_{cb}}{\leftrightarrows}} C \underset{k_{co}}{\overset{k_{oc}}{\leftrightarrows}} O. \tag{1.20}$$

We will see, however, that both open and closed state blockers may be optimal, depending on the nature of the mutation.

1.8.2 Closed to Open Mutations (CO-Mutations)

We have seen that for a Markov model written in the form

$$C \underset{k_{co}}{\overset{k_{oc}}{\leftrightarrows}} O, \tag{1.21}$$

the equilibrium open probability is given by

$$o = \frac{k_{co}}{k_{co} + k_{oc}} = \frac{1}{1 + \frac{k_{oc}}{k_{co}}}$$

and the mean open time is given by

$$\tau_o = \frac{1}{k_{oc}}.$$

A mutation leading to an increased open probability can be represented by a Markov model written in the form

$$C \underset{\mu k_{co}}{\overset{k_{oc}}{\leftrightarrows}} O, \tag{1.22}$$

where $\mu \geq 1$ will be referred to as the *mutation severity index* and we always use the convention that $\mu = 1$ refers to the wild type case. At this point, it is useful to recall the interpretation of a scheme of this form. In particular, it is useful to note that the probability of going from the closed state (C) to the open state (O) during a time step Δt is now given by $\mu \Delta t k_{co}$, compared to $\Delta t k_{co}$ for the wild type channel. It is pretty clear that increasing the mutation severity index will increase the probability of being in the open state and this is also reflected by the equilibrium open probability given by

$$o_\mu = \frac{1}{1 + \frac{k_{oc}}{\mu k_{co}}},$$

which clearly increases as a function of the mutation severity index μ. It is also interesting to observe that, for this mutation, the mean open time is unchanged. We will refer to a mutation of this form as a CO-mutation and we will show repeatedly that, for CO-mutations, closed state blockers are theoretically optimal.

1.8.3 Open to Closed Mutations (OC-Mutations)

Another way to introduce a mutation that increases the open probability is to decrease the rate from open to closed. This can be written as follows:

$$C \underset{k_{co}}{\overset{k_{oc}/\mu}{\leftrightarrows}} O, \tag{1.23}$$

where, again, $\mu \geq 1$ is the mutation severity index and $\mu = 1$ represents the wild type. The probability of leaving the open state is now reduced and this will lead to an increased open probability. In particular, the equilibrium open probability is again given by

$$o_\mu = \frac{1}{1 + \frac{k_{oc}}{\mu k_{co}}},$$

as above, but now the mean open time changes; it is given by

$$\tau_o = \frac{\mu}{k_{oc}}$$

and thus increases with the mutation severity index.

We will refer to a mutation of this form as an OC-mutation and we will show that, for such mutations, open state blockers are theoretically optimal.

1.9 Theoretical Drugs

The concept of a theoretical drug is essential in these notes. Basically, we will *refer to a theoretical drug*[2] *as a purely mathematical construction* that may or may not have a viable pharmaceutical counterpart. A mental image of how the drug may work is given in Fig. 1.9; the figure is taken from Starmer [87]. With no drug involved, the channel can take on two conformational states: the open state (O), when ions can flow freely through the channel, and the closed state (C), when there is no flow of ions through the channel. An open blocker can change the open state such that there is no flow through the channel. The reaction scheme of the situation described in the figure is given by

$$C \underset{k_{co}}{\overset{k_{oc}}{\leftrightarrows}} O \underset{k_{ob}}{\overset{k_{bo}}{\leftrightarrows}} B. \tag{1.24}$$

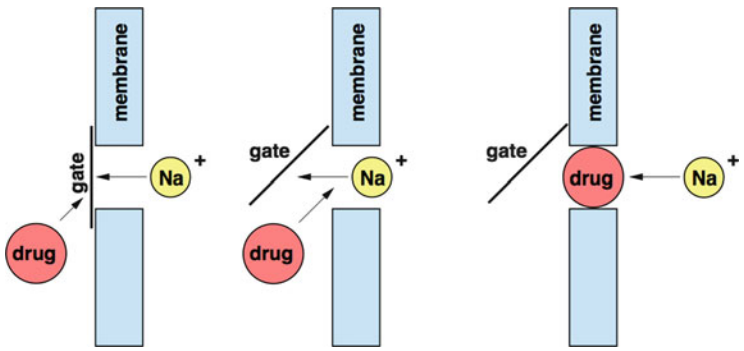

Fig. 1.9 Illustration of a blocker associated with the open state. In the leftmost case the channel is closed and no ions can pass through it. In the center case, the channel is open and ions may flow freely. In the rightmost case the channel is blocked by the drug and no ions can pass through it. The figure is taken from Starmer [87]

[2]We also use the terms *mathematical drug, numerical drug*, and so forth interchangeably with *theoretical drug*.

where we again note that the properties of the theoretical drug are solely given by the values of the rates k_{ob} and k_{bo}.

This way of describing the effect of a drug has been used for many years, see e.g. Hille [31] or Hondeghem and Katzung [34]. Our use of this notation is clearly motivated by the paper of Clancy et al. [16]. In these papers, an existing drug is characterized using a scheme of the form (1.24). That is, data obtained from experiments using a particular drug are used to characterize the rates k_{bo} and k_{ob} referred to, respectively, as the on and off rates of the drug. As mentioned above, we often view the rates as free parameters that can be optimized in order to create the best possible theoretical drug in the sense that the channel should work as much like the healthy case as possible. This way of describing a theoretically optimal drug was introduced in [99] and clearly motivated by the drug vector approach discussed in [97].

1.10 Results

Many of the models, methods, and results described in these notes are well known in the literature. All the Markov models are taken from the literature and so are the stochastic differential equations and the models describing the probability density approach. Compared to earlier published models, we will often derive simplified models, but the ideas behind them are basically the same as those used by many authors. Concerning the modeling of mutations, we aim to consistently model the effect of mutations as simply as possible and preferably only by changing a single parameter: the mutation severity index.

The novel part of these notes is that we attempt to systematically describe how to compute characterizations of drugs that are optimal in a specific sense and we do so for a number of applications. We almost exclusively address so-called gain-of-function mutations. For such mutations, the open probability of the channel or receptor is too large, which can lead to severe difficulties for the cell and, ultimately, for large collections of such cells.

1.11 Other Possible Applications

The focus in this text will be on how to compute characterizations of optimal theoretical drugs defined in terms of parameters describing the associated Markov model. The methods can, however, also be used to compare existing drugs. If Markov models are developed for two drugs, the associated probability density functions can be computed and thus a comparison of the quality of the two drugs can be computed. This approach will rely heavily on accurate representations of the function of a drug in terms of a Markov model, which is a problem beyond the scope of the present notes.

1.12 Disclaimer

These notes are written to explain in some detail how we can compute characterizations of theoretical drugs in terms of Markov models. However, we specifically avoid discussing whether it is possible to realize a certain drug given the characterization in terms of a Markov model, simply because we do not know and have been unable to find any reasonable answer to this in the literature. The applicability of our results therefore remains uncertain.

1.13 Notes

1. Several excellent introductions to Markov models of the stochastic behavior of receptors and ion channels are available (e.g., [39, 42, 79, 85]). In particular we recommend the recently published book by Bressloff [6] (see also [7]). Bressloff [6] provides a broad introduction to stochastic processes in cells and covers most of the models covered in the present text and much more. It is an excellent text that will become a standard reference in the field.
2. A comprehensive mathematical analysis of the stochastic properties of single ion channels using Markov models was initiated by Colquhoun and Hawkes (e.g., [19–21]).
3. Insight into the electrophysiology of excitable cells was fundamentally enhanced by the development of the patch clamp technique of Sakmann and Neher (see, e.g., [77, 78]). The authors received the Nobel Prize in Physiology or Medicine in 1991 for their work on single ion channels. The patch clamp technique is used to generate measurements of the form illustrated in Fig. 1.3. These data are used to determine the Markov model and are therefore of fundamental importance. As mentioned below, however, the problem of finding the Markov model based on experimental data is still an active research problem.
4. The models studied in these notes address the flow of ions through various types of channels. An excellent introduction to ion channels is given in the book by Hille [32].
5. Our discussion is focused on mechanisms of the heart but, at the level of single channels, these mechanisms are similar to channel-based mechanisms of the brain or, more specifically, the mechanisms of neurons. There are several excellent introductions to neuroscience (e.g., [22, 23, 38, 90]).
6. Given the Markov model, we have seen that it is pretty straightforward to compute what state the channel is in as a stochastic function of time. We have also seen that we can solve the master equation and find the average behavior of the channel when the rates are independent of the surroundings. Furthermore, we will show how to compute probability density functions for each state when the rates depend on the transmembrane potential. Such simulations are forward

problems: Given the model, compute the solution. The inverse problem in this setting is quite a bit harder; the problem is to compute the rates (i.e., the values of k_{oc}, k_{co} etc.) of the Markov model in order for the stochastic behavior of the model to match the measurements of the channel. The analysis of the inverse problem was started by Colquhoun and Hawkes [19], beginning in 1977, and their findings are summarized by Sakmann and Neher [78] (see also [17]). More recently the problem has been addressed in a series of papers by Sachs and his co-authors; see [59, 68, 69]. Their methods are available in the open-source QuB software package. Furthermore, Markov chain Monte Carlo (MCMC) has been used in a series of papers by Siekmann, Sneyd and his co-authors [27, 82–84]. Interestingly, their analysis shows that certain Markov models cannot be identified using standard data. The MCMC method was used for inversion of single ion channel data more than 15 years ago by Ball et al [1], and Rosales and co-authors, see [72, 73].

7. For whole cell data, the problem of identifying the parameters of Markov models is carefully studied by Fink and Noble [24].
8. The terms *CO-mutation*, *OC-mutation*, and *mutation severity index* are not standard and introduced here for convenience.
9. A thorough discussion of the principle of detailed balance can be found in the paper by Colquhoun et al. [18]. The validity of the principle for given data can be tested as shown by Song and Magleby [86] and Ullah et al. [101] (suppl. material). There are examples of Markov models that do not satisfy the principle of detailed balance (see, e.g., [6], p. 208).
10. The numerical method for handling the Markov model described on page 8 is not particularly efficient. For the case of constant rates in the Markov model, considerable acceleration can be achieved by using the method of Gillespie [26]. The Gillespie method is particularly useful for simulations involving many channels (see, e.g., [85]).
11. For comprehensive introductions to modeling the cardiac action potential, we refer to the recent overview by Rudy [74] and to Rudy and Silva [75]. For the action potential shown in Fig. 1.2, we used the model of Grandi et al. [29]. An alternative is the model of O'Hara et al. [64] and a huge collection of models is available at the CellML project (CellML.org).
12. The dynamics of cardiac electrophysiology are introduced in numerous papers and books; a recent comprehensive review is provided by Qu et al. [71]. The book by Katz [41] is a standard reference in cardiac physiology and the book by Glass et al. [28] is a standard reference in the modeling of the heart. Numerical methods for the simulation of cardiac electrophysiology are presented by Sundnes et al. [93] (see also [25, 67]).