Chapter 7

Professional Reference Shelf

R7.3 Receptor Kinetics

Just as enzymes are fundamental to life, so is the living cell's ability to receive and process signals from beyond the cell membrane. Cells receive input from receptors on or embedded in the cell membrane that elicit specific responses such as muscle contraction, secretion of growth hormones, metabolism alteration, generation of an electrical signal, and triggering of the activation of secondary reactions. *In all, these activities are generated by the signal detected by a specific receptor and converted to a cellular response.* This topic is included in the book (1) because receptors are important in directing cellular behavior, and (2) also because of the author is interest in and has done research in the pharmacokinetics of acute toxicology.

Receptors are transmembrane proteins whose ligand-binding site is found on the outer surface of the cell membrane. When a ligand such as a neurotransmitter binds to the receptor a signal is sent to the cell containing the receptor. This signal initiates physiological changes inside the cell such as activation of intracellular enzymes and entire cascades of intracellular reactions.[1] Some responses are on the order of milliseconds (action potentials or nerve inputs), and others on the order of hours such as in protein synthesis. Figure R7.3-1 shows a cell with a voltage potential across the cell membrane which has an excess of intracellular negative ions and extracellular positive ions with the ligands and receptors attached to the outside of the cell.

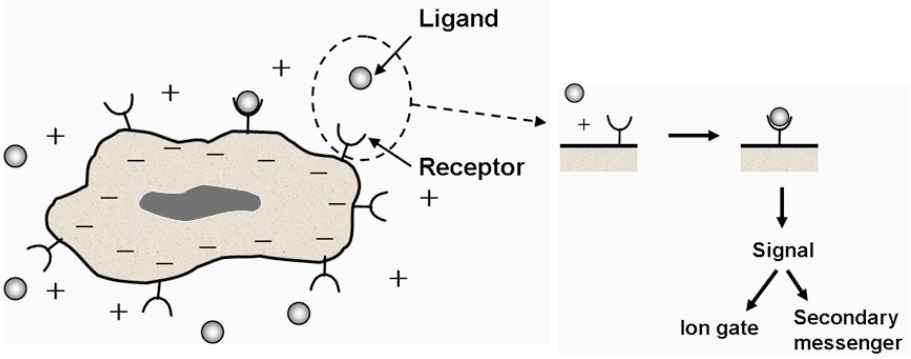


Figure R7.3-1 Schematic of negatively charged cell with receptors surrounded by a positively charged fluid containing ligands (e.g., neurotransmitters).

As shown in Figure R7.3-2, two examples of signals generated by the sent formation of the ligand-receptor complexes are alterations of gated ion channels and initiation of enzyme cascades.[2] The signal can cause a number of responses such as the contraction of muscles and the opening of ion channels, which can either cause or suppress cell depolarization. Many pharmaceuticals are ligands to cell receptors. We will apply receptor-ligand kinetics to describe two examples of the body's response to acute toxicology. The first example was featured at the World Congress

[1]D. A. Lauffenburger and J. J. Linderman, *Receptors*: *Models for Binding, Trafficking, and Signaling* (New York: Oxford University Press, 1993).

[2]D. L. Nelson and M. M. Cox, *Lehninger Principles of Biochemistry*, 3rd ed. (New York: Worth Publishers, 2000), p. 440.

of Chemical Engineering in Melbourne Australia, in 2001, because over 100,000 people die each year as a result of venomous snake bites. The second example is the interaction of alcohol with the GABA receptor in respiratory failure from alcohol overdose. In both cases, the malfunction of ligand-receptor interaction can result in death.

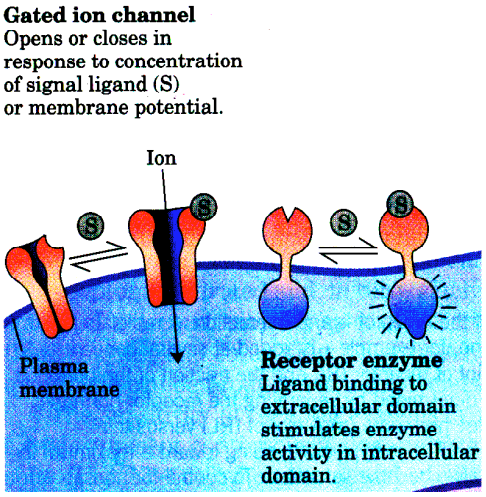


Figure R7.3-2 Examples of signals resulting from the formation of a receptor-ligand complex (Courtesy of D. L. Nelson and M. M. Cox, *Lehninger Principles of Biochemistry*, 3rd ed. (New York: Worth Publishers, 2000).

The two cases of acute toxicology both involve the malfunctioning of nerve cells. Consequently, we will focus on the transmission of signals between nerve cells, such as the one shown schematically in Figure R7.3-3.

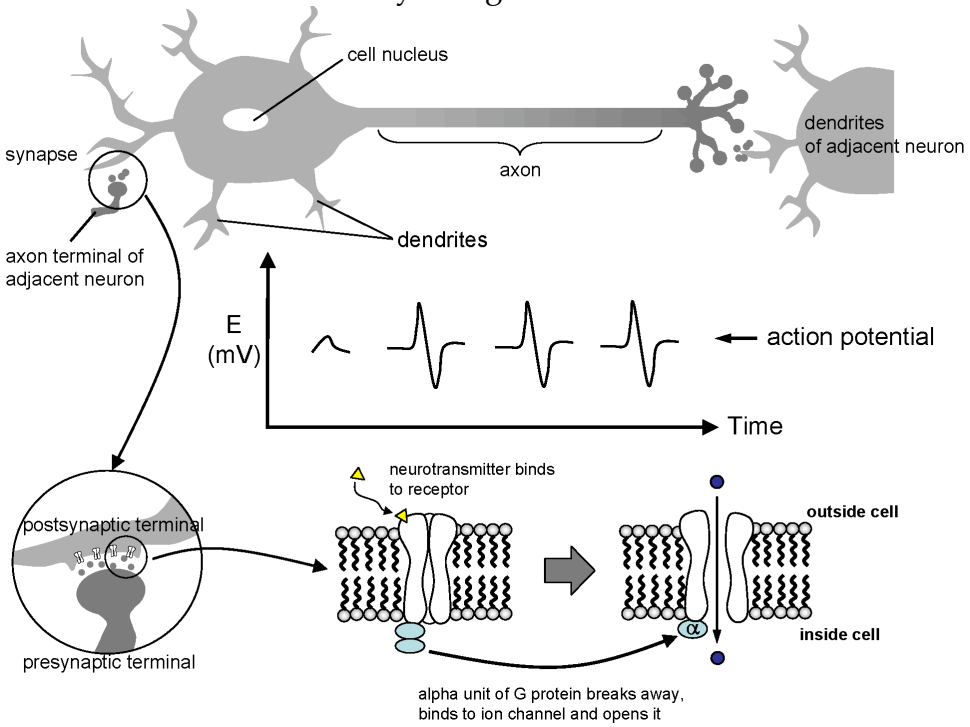


Figure R7.3-3 Nerve cell. (Courtesy of W. M. Becker, L. J. Lewis, and J. Hardin, *The World of the Cell*, 5th ed. (New York: Benjamin Cumming, 2003).

Notice the nerve cell in between two adjacent nerve cells. A signal is transmitted through from one nerve cell to another through chemical synapses from the axon terminal of one neuron to the dendrites of the adjacent neuron. (See ① in this figure). The presynaptic terminal releases neurotransmitters upon receipt of a voltage pulse called the action potential. These neurotransmitters then travel across the synaptic cleft to the postsynaptic terminal and attach to the receptors on the dendrites. Once the receptor-ligand complex is formed, a signal is sent to the cell. In the case of acetyl-choline, the signal opens an ion gate to allow sodium ions into the cell. Because the cytosol inside the cell is negatively charged, this influx of sodium ions can cause the cell to depolarize. If the depolarization is above the threshold value, the action potential travels down the nerve cell until it reaches the axon terminal (see ② in this figure) at the end of the cell where it causes the release of neurotransmitters which can then activate the next cell. These neurotransmitter molecules diffuse across the synaptic cleft (see Figure R7.3-4) and attach to the dendrites of the adjoining nerve and the process is repeated as the signal is passed on to the next cell. The process of neurotransmitter release, transport across the cleft and attachment at the receptor is called a *chemical synapse*.[3] In this section we will present the basics of ligand/receptor binding kinetics. The binding of the ligand L, ●, with the receptor R, ⅄, to form the receptor complex, C, is shown schematically in Figure R7.3-4.

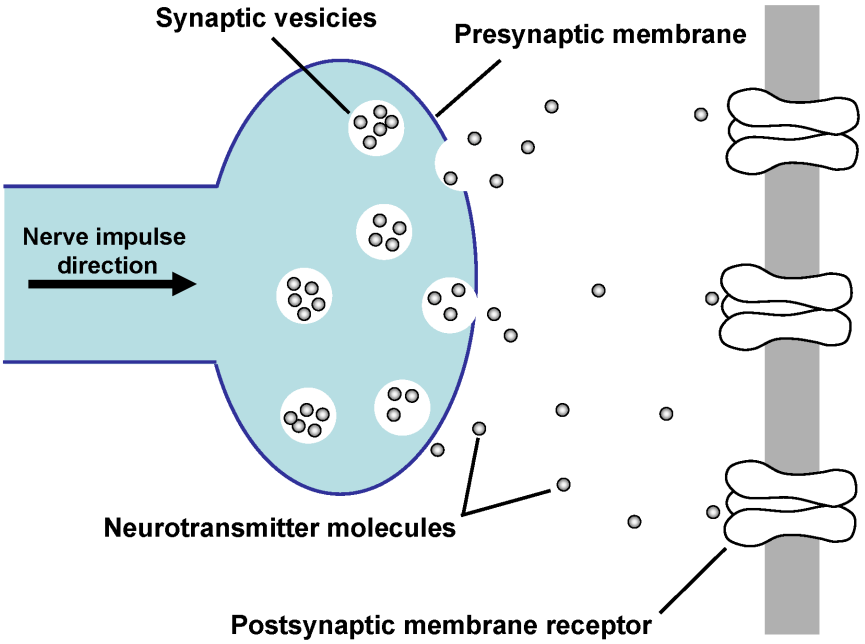
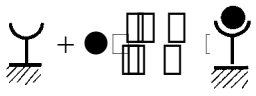


Figure R7.3-4. A chemical synapse. When the brain sends an electrical impulse through the nervous system, it depolarizes the nerve terminal, opening up the voltage gated calcium channels found in the presynaptic membrane. These channels allow for a rapid influx of calcium ions to enter the nerve terminal, enabling the release of neurotransmitters into the synaptic cleft. The neurotransmitter then diffuses across the synaptic cleft to the postsynaptic membrane, where they bind to neurotransmitter specific membrane receptor sites.

[3]D. L. Nelson and M. M. Cox, *Lehninger Principles of Biochemistry*, 3rd ed. (New York: Worth Publishers, 2000), p. 444.

We now will focus on the rate of binding between the receptor, R, and ligand, L, to form the ligand-receptor complex, C. Following Lauffenburger and Linderman, the binding is written symbolically as



$$R + L \underset{k_r}{\overset{k_f}{\rightleftharpoons}} C \qquad \text{(R7.3-1)}$$

A balance on the concentration of complexes on the surface is

$$\begin{bmatrix}\text{Rate of}\\ \text{accumulation}\\ \text{on surface}\end{bmatrix} = \begin{bmatrix}\text{Rate of}\\ \text{formation}\\ \text{on surface}\end{bmatrix} - \begin{bmatrix}\text{Rate of}\\ \text{internalization}\\ \text{by endocytosis}\end{bmatrix}$$

Neglecting endocytosis which will be discussed later, for the moment,

$$\frac{dC}{dt} = r_C - 0$$

Where r_C is the rate of formation of the complex C. The rate of binding can be written in terms that follow the kinetics of an elementary reaction

$$r_C = r_{Cf} - r_{Cr} = k_f(R)(L) - k_r(C) \qquad \text{(R7.3-2)}$$

Where R is the number of unoccupied receptors per cell. (#/cell)

L_0 is the initial ligand concentration (mol/dm^3)

L_{eq} is the equilibrium ligand concentration (mol/dm^3)

L is the free ligand concentration (mol/dm^3)

C is the number complexes per cell (#/cell)

r_{Cf} is the forward rate #/cell/s.

r_{Cr} is the reverse rate #/cell/s.

k_f is the forward rate constant M^{-1}/s

k_r is the reverse rate constant s^{-1}

Combining Equations (R7.3-1) and (R7.3-2)

$$\frac{dC}{dt} = k_f(R)(L) - k_r(C)$$

Analogous to the total enzyme concentration, the total number of receptors bound and unbound is constant neglecting endocytosis. The sum of the bound receptors, C, and unbound receptors, R, is constant at the total number of receptors, R_T

$$R_T = R + C \qquad \text{(R7.3-3)}$$

Typical values of R_T range between 10^4 and 10^6 receptors/cell (#/cell). The ligand concentration is also constant at

$$L_0 = L + \frac{n}{N_{Avo}} C \qquad \text{(R7.3-4)}$$

where n is the number of cells per unit volume and N_{Avo} is Avogadro's number.

R7.3.1 Equilibrium Analysis

We shall first consider the case where the rate of formation and rate of dissociation of complexes are equal. At equilibrium, $dC/dt = 0$, $R = R_{eq}$, and $L = L_{eq}$

$$\left(C_{eq}\right) = \frac{\left(R_e\right)\left(L_{eq}\right)}{K_D} \tag{R7.3-5}$$

where $K_r = k_r/k_f$ (mol/dm^3) is the dissociation equilibrium constant. Values of K_D range between 10^{-12} and 10^{-6} M. Because K_D is typically 10^{-9} or smaller, the receptors can detect picoconcentrations of the ligand molecule. Substituting for L and C in Equation (R7.3-5) for equilibrium conditions

$$\left(C_{eq}\right) = \frac{\left(R_T - C_{eq}\right)\left(L_{eq}\right)}{K_D} \tag{R7.3-6}$$

If the experiment is done in such a way that the ligand concentration is in excess, it is essentially constant, $L=L_0$, so we can solve Equation (R7.3-6) for the equilibrium concentration of complex to

$$C_{eq} = \frac{R_T \; L_{eq}}{K_D + L_{eq}} \tag{R7.3-7}$$

Experimental values of C_{eq} are plotted as a function of L_{eq} in Figure R7.3-5.

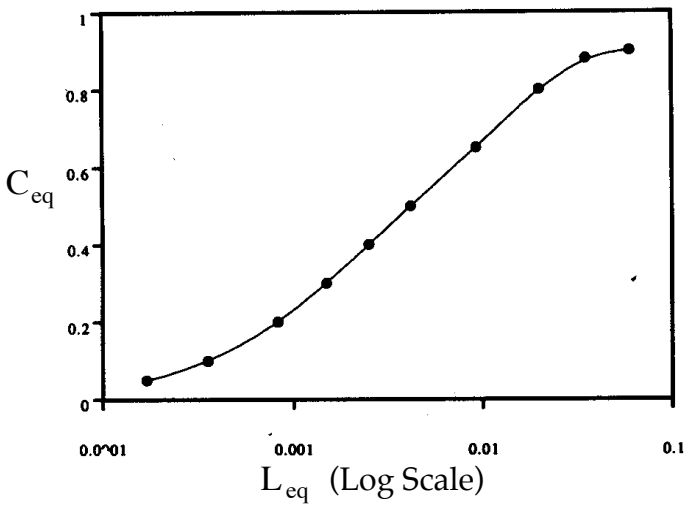


Figure R7.3-5 Equilibrium plot of complex concentration as a ligand concentration. D. A. Lauffenburger and J. J. Linderman, *Receptors: Models for Binding, Trafficking, and signaling*, (New York: Oxford University Press, 1993), p. 27.

We can rearrange Equation (R7.3-7) in the form

$$\frac{C_{eq}}{L_{eq}} = -\frac{1}{K_D} C_{eq} + \frac{R_T}{K_D} \tag{R7.3-8}$$

from which we can observe that plotting C_{eq}/L versus C_{eq}, we can determine the dissociation parameter K_D from the slope and R_T/K_D from the intercept $C_{eq}/L_{eq} = 0$. These data are plotted in Figure R7.3-6, which is referred to as a Scatchard plot.

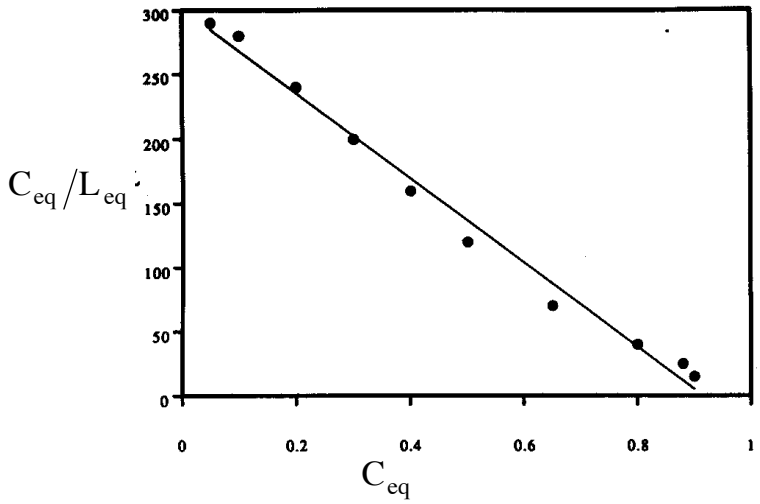


Figure R7.3-6 Scatchard plot of equilibrium concentrations. D. A. Lauffenburger and J. J. Linderman, *Receptors: Models for Binding, Trafficking, and signaling*, (New York: Oxford University Press, 1993), p. 27.

R7.3.2 Transient Analysis

We now return to the transient analysis. Neglecting endocytosis and receptor synthesis and substituting for R and L in Equation (R7.3-2), we obtain

$$\frac{dC}{dt} = k_f \overbrace{\left[R_T - C\right]}^{R} \overbrace{\left[L_0 - \frac{n}{N_{Avo}} C\right]}^{L} - k_r C \qquad \text{(R7.3-9)}$$

Typically, more neurotransmitters are released than there are receptor sites so the ligand concentration is virtually constant. For constant ligand concentration, $L = L_0$

$$\frac{dC}{dt} = L_0 k_f \left(R_T - C\right) - k_r C \qquad \text{(R7.3-10)}$$

solves to

$$C = \left[\frac{k_f R_T L_0}{k_f L_0 + k_r}\right] + \left[C_0 - \frac{k_f L_0 R_T}{k_f L_0 + k_r}\right] \exp\left[-\left(k_f L_0 + k_r\right)t\right] \qquad \text{(R7.3-11)}$$

Equilibrium is reached as $t \to \infty$,

$$C_{eq} = \frac{k_f R_T L_0}{k_f L_0 + k_r} = \frac{R_T L_0}{K_D + L_0} \qquad \text{(R7.3-12)}$$

R7.3.3 Endocytosis

Endocytosis is a mechanism for transporting components into the cell interior, while exocytosis is a mechanism of transporting components out of the cell.

A simplified schematic diagram for endocytosis is shown in Figure 7.3-7 as nine events which take components from the cell surface, internalize them, and then process them.

The first event ① shows the binding of a free ligand to a free receptor while event ② shows the reverse of this process and is described by Equation (R7.3-1). In this process, the bound receptors can migrate/diffuse to an area called a coated pit where proteins, such as clothrin and dynamin, attach to the complex to begin the internalization process by forming a vesicle around the complex.[4] These coated vesicles are then broken off from the surface and internalized in even ③ to an endosome. In an analogous manner, the free receptors are invaginated (i.e., internalized) in event ④. The terms k_{ec} and k_{eR} are the specific rate constants for the invagination of complexes and free receptors, respectively. Once in the endosome, sorting takes place, and the receptor-ligand complex dissociates into free receptors and ligands. Some of the receptors and ligands are directed to be recycled to the surface, events ⑤ and ⑥ respectively, while others undergo degradation (event ⑦). The rate constants for recycling and degredation are k_{rec} and k_{deg}, respectively. Event ⑧ represents the synthesis of new receptors at a rate V_s, and event ⑨ represents the uptake of ligands directly from the outer fluid with a specific rate k_{fP}.

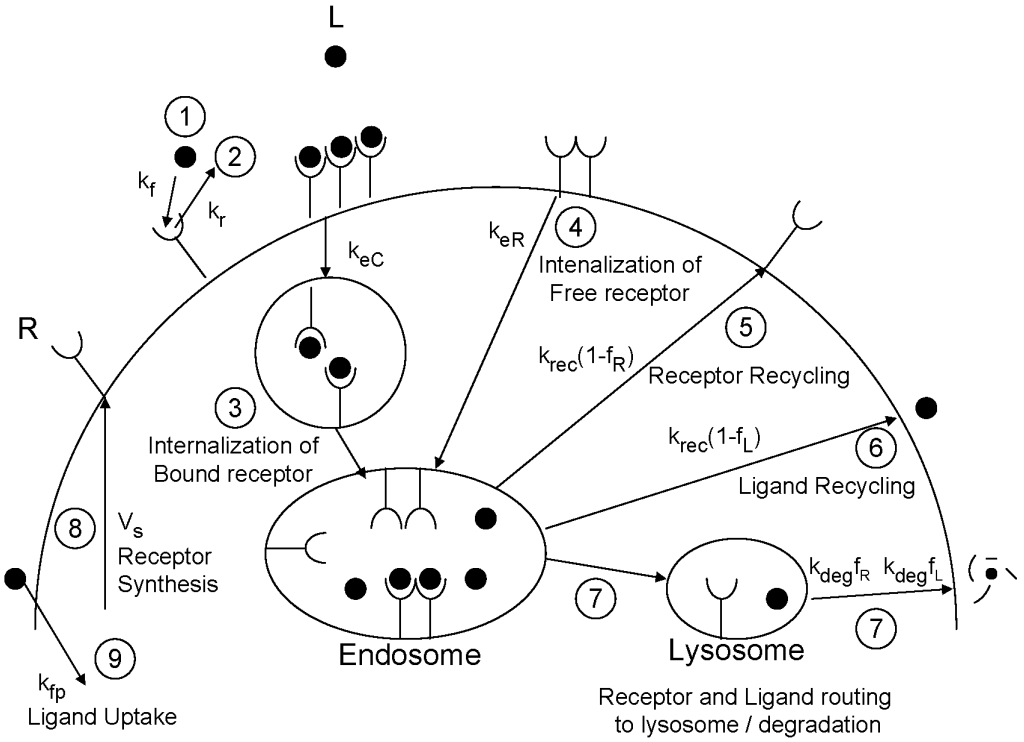


Figure R7.3-7 Endocytosis cycle.

We will first write balances on the receptors and receptor-ligand complexes on the surface, R_s and C_s, respectively. We next write balance on the total number of ligands internalized (free plus bound), R_{Ti}. Of the internalized receptors, R_{Ti}, a

[4]W. M. Becker, L. J. Lewis, and J. Hardin, *The World of the Cell*, 5th ed. (New York: Benjamin Cumming, 2003), p. 351

fraction, f_R, will be degraded as will a fraction, f_L, of the total internalized ligands, L_{Ti}.

The balance of the free receptors on the surface is

	①		②		③		⑤		⑧
Accumulation of surface receptors	=	Loss by forming complex	+	Gain by complex dissociation	+	Loss by internalization	+ Gain by recycle	+	Gain by internal synthesis

$$\frac{dR_S}{dt} = -k_f R_S \cdot L + k_r C_S - k_{er} R_S + k_{rec}(1-f_R) R_{Ti} + V_S \qquad \text{(R7.3-13)}$$

The balance of the total number of internal receptors is

	③		④		⑥		⑦
Accumulation of internal receptors	=	Gain by internalization of free feceptors	+	Gain by internalization of bound receptors	- Loss by recycling	-	Loss by degrodation

$$\frac{dR_{Ti}}{dt} = k_{er} R_S + k_{ec} C_S - k_{rec}(1-f_R) R_{Ti} - k_{deg} f_R R_{Ti} \qquad \text{(R7.3-14)}$$

Similar balances can be written on the complexes

On the surface,

$$\frac{dC_S}{dt} = k_f L R - k_r C - k_{ec} C_S \qquad \text{(R7.3-15)}$$

Internally,

$$\frac{dL_{Ti}}{dt} = k_{ec} C_S + k_{fp} L N_{avgo} - k_{rec}(1-f_L) L_{Ti} - k_{deg} f_L R_{Ti} \qquad \text{(R7.3-16)}$$

Typical values of the specific rate constants are given in Table R7.3-1 along with V_s can be found in Lauffenburger and Linderman.

TABLE R7.3-1. RECEPTOR TRAFFICKING PARAMETERS FOR EFG/EGF RECEPTORS ON B82 FIBROBLASTS

Parameter	Value
k_f	$7.2 \times 10^7\ M^{-1}\ min^{-1}$
k_r	$3.4 \times 10^{-1}\ min^{-1}$
k_{eR}	$3.0 \times 10^{-2}\ min^{-1}$
k_{eC}	3.0×10^{-2}–$3.0 \times 10^{-1}\ min^{-1}$
k_{rec}	$5.8 \times 10^{-2}\ min^{-1}$
k_{deg}	$2.2 \times 10^{-3}\ min^{-1}$
V_s	1.3×10^2 #/min
f_R	0.2–0.8
f_L	0.2–0.8

Table courtesy of D. A. Lauffenburger and J. J. Linderman, *Receptors: Models for Binding, Trafficking,*

and signaling, (New York: Oxford University Press, 1993), p. 27.

The coupled Equations (R7.3-13) through (R7.3-16) can be easily solved using Polymath (See Table R7.3-2) or some other software package. In the Polymath program, we have set $k_{fp} = 0$.

TABLE R7.3-2. POLYMATH PROGRAM FOR ENDYCYLOSIS

POLYMATH Results
Professional Reference Shelf: R7-3 Receptor Kinetics 08-24-2004, Rev5.1.232

Calculated values of the DEQ variables

Variable	initial value	minimal value	maximal value	final value
t	0	0	50	50
Rs	5.0E+04	1457.6969	5.0E+04	1457.6969
RTi	0	0	2.484E+04	1.915E+04
Cs	0	0	2.145E+04	2119.4138
LTi	0	0	2.2E+04	1.695E+04
kf	7.2E+07	7.2E+07	7.2E+07	7.2E+07
kr	0.34	0.34	0.34	0.34
keR	0.03	0.03	0.03	0.03
keC	0.165	0.165	0.165	0.165
krec	0.058	0.058	0.058	0.058
kdeg	0.0022	0.0022	0.0022	0.0022
Vs	0.013	0.013	0.013	0.013
fR	0.5	0.5	0.5	0.5
fL	0.5	0.5	0.5	0.5
L	1.0E-08	1.0E-08	1.0E-08	1.0E-08
Navgo	6.022E+23	6.022E+23	6.022E+23	6.022E+23
kfP	0	0	0	0

ODE Report (RKF45)

Differential equations as entered by the user
[1] d(Rs)/d(t) = -kf*Rs*L+kr*Cs-keC*Rs+(krec*(1-fR)*RTi)+Vs
[2] d(RTi)/d(t) = keR*Rs+keC*Cs-(krec*(1-fR)*RTi)-kdeg*fR*RTi
[3] d(Cs)/d(t) = kf*L*Rs-kr*Cs-keC*Cs
[4] d(LTi)/d(t) = keC*Cs+kfP*L*Navgo-(krec*(1-fL)*LTi)-kdeg*fR*RTi

Explicit equations as entered by the user
[1] kf = 7.2e7
[2] kr = .34
[3] keR = .03
[4] keC = .165
[5] krec = .058
[6] kdeg = .0022
[7] Vs = .013
[8] fR = .5
[9] fL = .5
[10] L = 1e-8
[11] Navgo = 6.022e23
[12] kfP = 0

The output of the polymath program is shown in Figure R7.3-8.

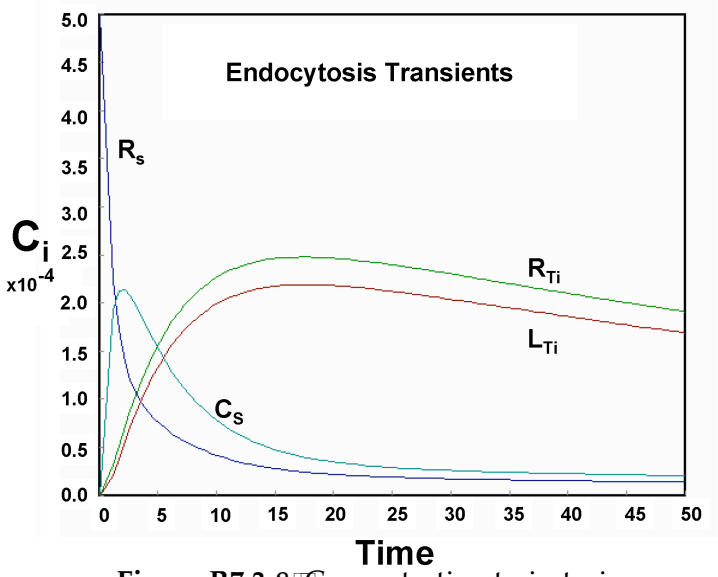
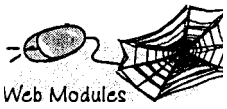


Figure R7.3-8 Concentration trajectories.



Because over 100,000 people die each year as a result of venomous snake bites, it is of interest to apply what we have learned about receptors to death caused by snake bites. As discussed in the Web Module in Chapter 6 on cobra bites, the cobra venom attaches irreversibly to the nicotine receptor sites as shown in Figure R7.3-9. As a result, the sites become blocked, and the neurotransmitter cannot adsorb on the sites and open up the ion gates to initiate the action potential. As a result, when one third of the receptor sites are blocked, the bite victim cannot send the signal for respiratory muscle contraction and so dies of suffocation.

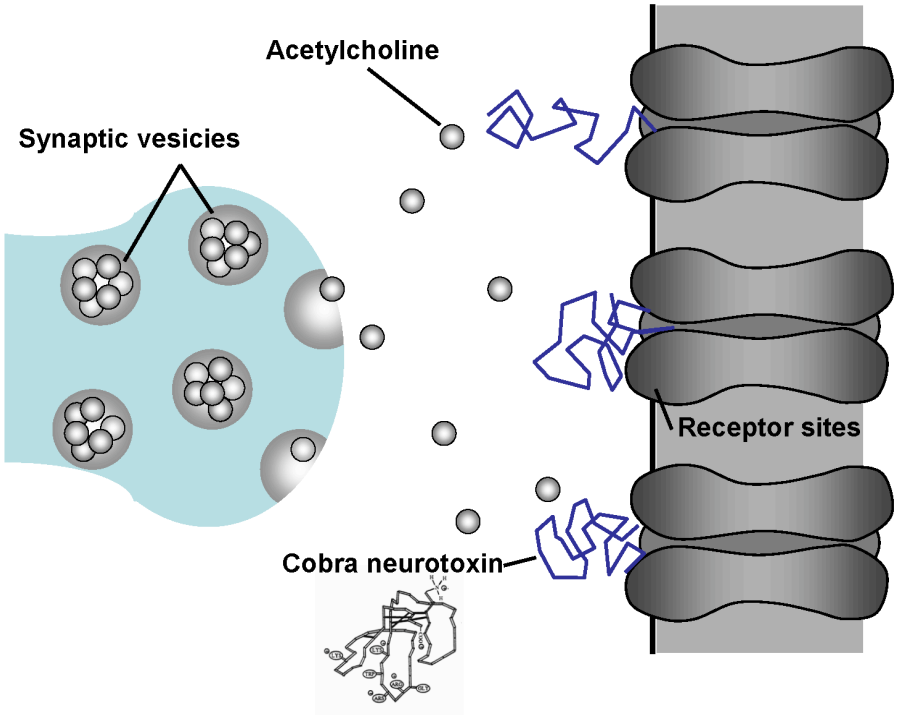


Figure R7.3-9 Cobra venom attaching to a receptor site.

Similarly, one can die of alcohol poisoning when the alcohol adsorbs on the inhibitory GABA receptors, which let in an excess of Cl^- ions. This excess results in

an insufficient potential when the excitatory inhibitors open their channels resulting in the initiation of the action potential to send the signal to breath. As a result, the victim dies of suffocation.