It’s all about the targets. The targets may be membrane or cytosolic receptors, ion channels, transporters, signal transduction kinases, enzymes, or specific sequences of RNA or DNA, but the pharmacodynamic principles that govern these interactions remain the same (Table 2-1). Drugs bind to specific targets, activating (stimulating) or inactivating (blocking) their functions and altering their biological responses.

**DOSE-RESPONSE RELATIONSHIPS**

Often, the lock-and-key concept is useful to understand the way drugs work. In this analogy, the target is the lock and the drug is the key. If the key fits the lock and is able to open it (i.e., activate it), the drug is called an agonist. If the key fits the lock but can’t get the lock to open (i.e., just blocks the lock), the drug is called an antagonist.

The pharmacodynamic properties of drugs define their interactions with selective targets. Pharmaceutical companies identify and then validate, optimize, and test drugs for specific targets via rational drug design or high-throughput drug screening. Table 2-2 identifies some pharmacodynamic concepts that determine the properties of drugs.

Terms such as affinity and potency (see Table 2-2) are most appreciated in graphical form. Figure 2-1A illustrates a graded (quantitative) dose-response curve. Often, this type of curve is graphed as a semi-log plot (see Fig. 2-1B). Notice that the y-axis is depicted as a percentage of the maximal effect of the drug, and the x-axis is the dose or concentration of the drug. Several important relationships can be appreciated through graded dose-response curves:

1. **Affinity** is a measure of binding strength that a drug has for its target.
2. Affinity can be defined in terms of the $K_D$ (the dissociation constant of the drug for the target). In this instance, affinity is the inverse of the $K_D$ ($1/K_D$). The smaller the $K_D$, the greater affinity a drug has for its receptor.
3. The dose of a drug that produces 50% of the maximal effect is known as the $ED_{50}$ (effective dose to achieve 50% response). If concentrations are used, then the concentration to achieve 50% of the maximal effect is known as the $EC_{50}$.
4. When plotted on linear graph paper, the dose-response relationship for most drugs is exponential, often assuming the shape of a rectangular hyperbola.
5. By plotting response vs log dose, we can transform a graded dose-response curve into more linear (sigmoidal) relationships. This facilitates comparison of the dose-response curves for drugs that work by similar mechanisms of action. Without knowing anything about the mechanisms of opioids or aspirin, a glance at Figure 2-1C tells you that hydromorphone, morphine, and codeine work by the same mechanism, but aspirin works by a different mechanism. Often, the slope of the curves and the maximal effects are identical for drugs that work via the same
mechanism. These curves also tell you that of the three opioids, hydromorphone is the most potent. Potency is a comparative term that is used to compare two or more drugs that have different affinities for binding to the same target.

6. Below the threshold dose, there is no measurable response.

7. $E_{\text{max}}$ is a measure of maximal response or efficacy, not a dose or concentration. Once the maximal response is achieved, increasing the concentration/dose of the drug beyond the $E_{\text{max}}$ will not produce a further therapeutic effect but can lead to toxic effects.

Doesn’t the curve depicted in Figure 2-1B look familiar? The same mathematical relationships that define how a drug (ligand) interacts with a receptor to elicit or diminish a biological response also governs the ways in which substrates (ligands) interact with enzymes to generate metabolic end products. In fact, the terms $K_D$ and $E_{\text{max}}$ (ceiling effect) can easily be redefined as $K_m$ and $V_{\text{max}}$, which you recall from Michaelis-Menten enzyme kinetics.

Another useful mathematical concept is quantal (“all-or-none”) dose-response curves. These population-based dose-response curves include data from multiple patients, often plotting percentages of patients who meet a predefined criterion (e.g., a 10 mm Hg reduction in systolic blood pressure, going to sleep after taking a sleep aid) on the y-axis versus the dose of drug that produced the biological response on the x-axis (Fig. 2-2A). These curves often take the shape of a normal frequency distribution (i.e., bell shape). These all-or-none responses can easily be thought of in terms of drugs that are sleep aids. The drug either puts people to sleep or it doesn’t. There is no in-between. However, the dosage that induced sleep may vary among various people. Most folks will fall asleep with a medium-range dose, but there will be outliers—some will be very sensitive to the drug at low doses, whereas others will be relatively resistant to hypnotic effects until higher drug levels are achieved.

These data can be transformed into a cumulative frequency distribution (see Fig. 2-2B), where cumulative percent
maximal patient responses are plotted versus dose. This type of sigmoidal curve yields useful safety information when the all-or-nothing responses are defined as therapeutic maximal responses, toxic responses, or lethal responses. In this way, for a single drug, cumulative frequency distributions can be compared for therapeutic efficacy, toxicity, and lethality (see Fig. 2-2C). This type of analysis can be used to compute the therapeutic index for any drug. The therapeutic index is defined as the TD50 (the dose that results in toxicity in 50% of the population) divided by the ED50 (the dose at which 50% of the patients meet the predefined criteria). As a rule of thumb, when a drug’s therapeutic index is less than 10 (meaning that less than a tenfold increase in the therapeutic dose will lead to 50% toxicity), then the drug is defined as having a narrow therapeutic window. Examples of drugs with narrow therapeutic windows are listed in Box 2-1. Plasma concentrations are routinely assessed for drugs with narrow therapeutic windows. This is especially critical for patients whose pharmacokinetic parameters are compromised by renal or hepatic diseases.

### Box 2-1. DRUGS WITH NARROW THERAPEUTIC WINDOWS

<table>
<thead>
<tr>
<th>Drug</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theophylline</td>
<td>Digoxin</td>
</tr>
<tr>
<td>Warfarin</td>
<td>Carbamazepine</td>
</tr>
<tr>
<td>Valproate</td>
<td>Phenytoin</td>
</tr>
<tr>
<td>Lithium</td>
<td>Gentamicin</td>
</tr>
</tbody>
</table>

For some analyses, it is often advantageous to graph time to drug action versus defined response. This time-response curve (Fig. 2-3) depicts the latent period (time to onset of action), the time to peak effect, as well as the duration of action. Often the y-axis for this type of relationship is given as the plasma concentration of the drug (since plasma concentration is directly related to response). The maximal peak response should be below the toxic dose and above the minimal effective dose. If it isn’t obvious why the processes of absorption, distribution, metabolism, and excretion determine the shape of this curve, please refer back to Chapter 1.

### TIME-RESPONSE RELATIONSHIPS

For some analyses, it is often advantageous to graph time to drug action versus defined response. This time-response curve (Fig. 2-3) depicts the latent period (time to onset of action), the time to peak effect, as well as the duration of action. Often the y-axis for this type of relationship is given as the plasma concentration of the drug (since plasma concentration is directly related to response). The maximal peak response should be below the toxic dose and above the minimal effective dose. If it isn’t obvious why the processes of absorption, distribution, metabolism, and excretion determine the shape of this curve, please refer back to Chapter 1.

### DRUGS AS AGONISTS

How does a practitioner interpret two drugs that have equal affinities (binding) for a specific target but have different efficacies (degree of response) (Fig. 2-4)? In this example, even though all these drugs are agonists for the target, the drugs that elicit a maximal response are full agonists (drugs C and D), while those that do not elicit a maximal response are often referred to as partial agonists (drugs A and B in Fig. 2-4). In other words, despite occupying all of the receptors for the drug at the target site, the biological response for partial agonists is muted or lower than that of full agonists. Often the reasons for this muted or weak biological response at full receptor occupancy (saturation) is unknown. However, the key point is that partial agonists are often used clinically to competitively inhibit the responses of full agonists, and thus they can be thought of as competitive pharmacologic antagonists. Buspirone is an example of a partial agonist; buspirone exhibits full agonist properties at presynaptic
5HT_{1A} serotonin receptors but very weak agonist activity at postsynaptic 5HT_{1A} receptors. The net result of these disparate biological responses leads to classification of this drug as a partial agonist.

Continuing with Figure 2-4, there can be cases when partial agonists (drug A) display greater potency (greater effect at a lower concentration) than full agonists (drug D). Understanding these dose-response curves requires an appreciation of the two-state model for receptor activation. Receptors can be thought to undergo a dynamic conformational or structural transition between inactive and active states in the presence of ligands. This model can be useful to explain why partial agonists exhibit weak biological responses at full saturation of receptors. In this model, full agonists preferentially bind to the active form of the target with high affinity; whereas partial agonists have affinities to both the active and the inactive conformations of the target. By extending this model, drugs can be designed to stabilize the inactive form of targets. These drugs theoretically would exhibit negative efficacy, and they are called inverse agonists. For inverse agonism to be observed, there must be some level of constitutive activity in the absence of agonist. Although these issues are frequently incorporated into test questions, there are few, if any, demonstrated examples of inverse agonism in vivo.

**DRUGS AS ANTAGONISTS**

Often physicians prescribe a drug that blocks or competes with an endogenous metabolite or pathway or exogenous xenobiotic (foreign substance) or drug. These agents are antagonists in that they block (or antagonize) the natural signal. These antagonists change the shape of dose-response curves. For example, a competitive, reversible antagonist shifts the dose-response curve to the right, indicating that the agonist must now be given at a higher dose to elicit a similar response in the presence of the antagonist (Fig. 2-5A). In contrast, an irreversible antagonist shifts the dose response curve downward, indicating that the agonist can no longer exert maximal effects at any therapeutic dose (see Fig. 2-5B). There are also allosteric interactions (binding at an alternative or “distant” site), where different drugs bind to distinct sites on one target in a reversible but not competitive manner. In these cases, the action of one drug positively or negatively impacts the binding of a second drug to the target, a phenomenon known as cooperativity.

Antagonists, such as β-adrenergic receptor antagonists, (“β-blockers”) have affinity, but no efficacy, for β-adrenergic receptors. These drugs compete for and block endogenous norepinephrine or epinephrine from stimulating adrenergic receptors. Because membrane receptors may be recycled after drug binding (desensitization), may be newly transcribed, or may have amplified responses through actions at multiple effectors, the actual magnitude of antagonism corresponding to a reduced biological response may not
always be linear and, in fact, may be less than expected. The term *spare receptor* is often used to describe this phenomenon.

### SIGNALING AND RECEPTORS

The critical concepts of signal transduction pathways are amplification, redundancy, cross-talk, and integration of biological signals. From a pharmacological perspective, identification of individual signal transduction elements often uncovers potential targets for drugs to selectively disrupt the integrated circuits that control cell growth, survival, and differentiation. To fully appreciate the complexity of signaling networks requires an understanding of a “New York City subway map” of interconnected receptors, effectors, targets, and scaffold proteins. The physician should understand the critical concepts of cell signaling, as well as some of the therapeutic targets that can now be modified with drugs.

Figure 2-6 depicts several intracellular signals that are regulated via receptor activation. A major family of membrane receptors is the 7 transmembrane-spanning domain G protein–coupled receptors. These receptors couple to heterotrimeric GTP-binding proteins, which regulate downstream effectors, including adenylate cyclase. This is a critical element in the discussion of the autonomic (see Chapter 6) and central nervous systems (see Chapter 13).

As depicted in Figure 2-7, amplification of the signal occurs as one receptor interacts with multiple G proteins that remain activated even after the receptors dissociate. In a cyclical fashion, activated receptors couple to the α/βγ subunits of the inactivated G protein (bound to GDP). This interaction induces GDP dissociation, followed by GTP binding, and activation of the G protein. The activated G protein dissociates into distinct α and βγ subunits. The α subunit interacts with adenyl cyclase, the enzyme that produces cyclic AMP, the biological cofactor for protein kinase A (PKA). Hydrolysis of GTP to GDP dissociates the α subunit from adenyl cyclase and permits reassociation with the βγ subunits, resetting the cycle for subsequent activation by another receptor. Leading to further complexity is that fact that distinct α subunits couple to different and specific effectors (Fig. 2-8) as well as the fact that βγ subunits themselves can interact with other downstream effectors including phospholipases.

Examples of a receptor class that couples to Gs (“s” stands for “stimulatory” as opposed to Gi, in which the “i” stands for “inhibitory”) to activate adenylate cyclase and generate cAMP are the β-adrenergic receptors. Pharmacologic intervention with a β-agonist like isoproterenol activates β-adrenergic receptors, whereas antagonists such as propranolol, a β-blocker, prevent endogenous activation of these receptors.

Understanding the mechanisms by which these receptors undergo desensitization or internalization helps explain...
why receptor responses dissipate over prolonged activation (Fig. 2-9). Interaction of β-adrenergic receptors with epinephrine promotes phosphorylation of the receptor by β-adrenergic receptor kinases (BARKs). The hyperphosphorylated receptors interact with arrestin, a molecule that either prevents activation of G proteins by the receptor and/or induces receptor internalization. One of the critical concepts in signal transduction is that posttranslational modifications of targets by phosphorylation alter receptor function.

Besides coupling to adenylate cyclase, G protein-linked receptors can regulate lipid turnover in membranes. Another critical concept in signaling is that altered lipid metabolism generates lipid-derived second messengers that amplify primary signals. Simply put, it’s all about metabolism of a phosphorylated lipid that makes up less than 0.01% of the total lipid content of the membrane. G protein–coupled receptors, like the angiotensin II receptor, activate phospholipase C via Gq, which preferentially hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) to form two distinct lipid-derived second messengers (Fig. 2-10A): inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). IP3, being hydrophilic, leaves the membrane and interacts with calcium channels on the endoplasmic reticulum, producing an increase in intracellular free calcium. Calcium-regulated kinases impact multiple systems responsible for blood clotting, neuronal function, and proton secretion in the stomach. In contrast, DAG, being hydrophobic, remains at the plasma membrane, where it is a lipid cofactor that activates protein kinase C.

To complicate matters, growth factor receptors, such as platelet-derived growth factor, which are tyrosine kinases, also couple to phospholipases to form lipid-derived second messengers (see Fig. 2-6). Another critical concept in signaling is that dimerization and resultant autophosphorylation of tyrosine kinase receptors often leads to propagation of the signal. Many of the latest therapeutic approaches work through inhibiting these tyrosine kinase receptor activation mechanisms. In addition, these tyrosine kinase receptors also activate phosphatidylinositol-3-kinase (PI3K; Fig. 2-10B), which can form a third messenger from phosphatidylinositol 4,5-bisphosphate. The generated phosphatidylinositol 3,4,5-trisphosphate can interact with proteins containing pleckstrin homology domains, such as AKT, which are critical kinases for cell survival. Growth factor receptors are overexpressed in...
cancerous lesions. Figure 2-11 depicts several of the pro-mitogenic cascades activated by this class of receptors, as well as designated targets for therapeutic intervention.

Figure 2-12 illustrates another lipid metabolite formed from hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP₂). Phospholipase A₂ hydrolyzes fatty acids from lipids, such as PIP₂ or phosphatidylcholine. These fatty acids are often highly unsaturated, containing multiple double bonds. Fatty acids containing 20 carbons with 4 double bonds that occur starting 6 carbons from the carboxyl terminus are known as arachidonic acid. These fatty acids can be oxidized by multiple enzymes to form prostaglandins, leukotrienes, and epoxides (HETEs) by cyclooxygenase, lipoxygenase, and epoxygenases, respectively.

The onslaught of lipid-derived messengers is referred to as “arachidonophobia.” Multiple drugs, either irreversibly (aspirin) or reversibly (nonsteroidal anti-inflammatory agents [NSAIDs]) inhibit cyclooxygenase and are reviewed in Chapter 10. Inhibitors of leukotriene synthesis, such as montelukast, are effective in asthmatic patients.

Another signaling concept is that lipid-derived second messengers such as prostaglandins can themselves activate G protein-coupled receptors, again amplifying responses (Fig. 2-13). It should be noted that lipid-derived messengers can signal by creating structured membrane microdomains (also called lipid rafts), directly interacting with lipid-binding domains on proteins, or by posttranslationally modifying proteins. Examples of posttranslational modifications include proteins made hydrophobic by covalent modifications with 14-carbon (myristoylate) or 16-carbon (palmitoylate) fatty acids. A critical example of a myristoylated target protein is Ras, which is over-expressed or mutated in multiple cancers.

**CLINICAL MEDICINE**

**Why do physicians need to know Signaling 101?**

More and more drugs that alter signal transduction cascades are being validated, tested, approved, and marketed. These drugs offer the promise of specificity, selectivity, and reduced toxicity, since signaling elements are often mutated or overexpressed in disease states, including cancer and inflammation. In this way, normal tissues may not be dramatically affected by the drug, resulting in reduced side effects. Examples of some approved designer drug targets are:

<table>
<thead>
<tr>
<th>Target Signal</th>
<th>Approved Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erb-B₂ receptor</td>
<td>Breast cancer</td>
</tr>
<tr>
<td>Erb-B₂ receptor</td>
<td>Non–small cell lung cancer</td>
</tr>
<tr>
<td>Erb-B₂ receptor</td>
<td>Colorectal cancer</td>
</tr>
<tr>
<td>BCR-ABL</td>
<td>Chronic myelogenous leukemia</td>
</tr>
<tr>
<td>mTOR</td>
<td>Re-stenosis after coronary stenting</td>
</tr>
<tr>
<td>Peroxisome proliferator</td>
<td>Diabetes</td>
</tr>
</tbody>
</table>

The Her2/neu gene product, the Erb-B₂ receptor, a member of the human epidermal growth factor family of tyrosine kinases, is overexpressed in multiple cancers and is associated with a poor prognosis. Erb-B₂ forms a heterodimer with other Erb receptors that exhibit enhanced mitogenic signaling potential. Several different strategies have been used to target this receptor. Monoclonal antibodies (trastuzumab) as well as low-molecular-weight inhibitors (gefitinib) have been designed to block these actions. Additional strategies, including coupling a specific antibody to cytotoxins or ligands that activate immune cells, are being investigated.
Signal transduction cascades can interact with and dramatically impact ion channels. In fact, ligand-gated ion channels themselves serve as targets for both intracellular signal transduction cascades as well as therapeutic drugs. Pharmacologic regulation of ion channels serves as one approach to controlling cardiac (verapamil, a Ca\(^{++}\) channel blocker), renal (furosemide, an Na\(^+\)/K\(^+\)/Cl\(^{−}\) cotransporter antagonist), and neuronal (benzodiazepines, a Cl\(^{−}\) channel allosteric modulator) function. Modifying pathologic ion channel activity with therapeutics can be affected by direct interaction with the channel itself or upstream/downstream signal transduction targets of that ion channel. Ligand-gated ion channels can be regulated by Ca\(^{++}\), cAMP, lipid mediators, and tyrosine phosphorylation signal transduction mechanisms.

A detailed example of ion channel modulation with therapeutics is γ-aminobutyric acid (GABA)–activated neuronal chloride channels. Benzodiazepines are examples of drugs that work via modulation of GABA-activated chloride channels. GABA serves as the endogenous ligand for this ligand-gated ion channel (Fig. 2-14). Benzodiazepines cannot activate GABA receptors in the absence of GABA, but
benzodiazepines do facilitate the actions of GABA to alter the conformation of the receptor-ion channel, allowing the chloride channel to remain open longer than it would be otherwise. The enhanced chloride flux hyperpolarizes the membrane, diminishing neuronal transmission and inducing sedation or cessation of anxiety (anxiolytic). The target of benzodiazepines, then, is a ligand-gated ion channel. This is also an example of positive cooperativity between the GABA neurotransmitter and a drug.

TOP FIVE LIST

1. Drugs bind to targets.
2. Targets themselves can be receptors, ion channels, transporters, signaling molecules, enzymes, or specific nucleic acid sequences.
3. Interactions between drugs and targets can be agonistic or antagonistic.
4. Pharmacodynamic terms used to define drug-target interactions include affinity, potency, and efficacy.
5. Drugs with a narrow therapeutic window (therapeutic index equals toxic dose [TD50] divided by effective dose [ED50]) must be closely monitored by the practitioner.

Figure 2-11. Targeted cancer therapy. Erb-B2, a tyrosine kinase receptor; PLCγ, phospholipase C subtype that couples to tyrosine kinases; CRK/Src, another group of scaffold proteins that couple tyrosine kinases to downstream effectors; cABL, Myc, mTOR, S6 Kinase, various downstream kinases and transcriptional factors that can serve as selective “targets” for drugs.
**Figure 2-12.** Arachidono-phobia: 20 carbons, 4 double bonds, and the precursor to multiple lipid-derived second messengers (leukotrienes, prostaglandins, thromboxanes, and HETEs [hydroxyeicosatrienoic acids]) that regulate myriad physiologic responses from vasoreactivity, to bronchial constriction, to labor, to protection of the gastrointestinal tract, to inflammation, and so on. The inset prostaglandin E₂ is an example of the kinds of structures that are created.

**Figure 2-13.** Lipid-derived second messengers can interact with their own G protein-coupled receptors. PGE₂, prostaglandin E₂; cAMP, cyclic adenosine monophosphate.
Figure 2-14. Ligand-gated channels regulate the flow of ions through plasma membrane channels. This example depicts the GABAA receptor, which modulates chloride conductance.