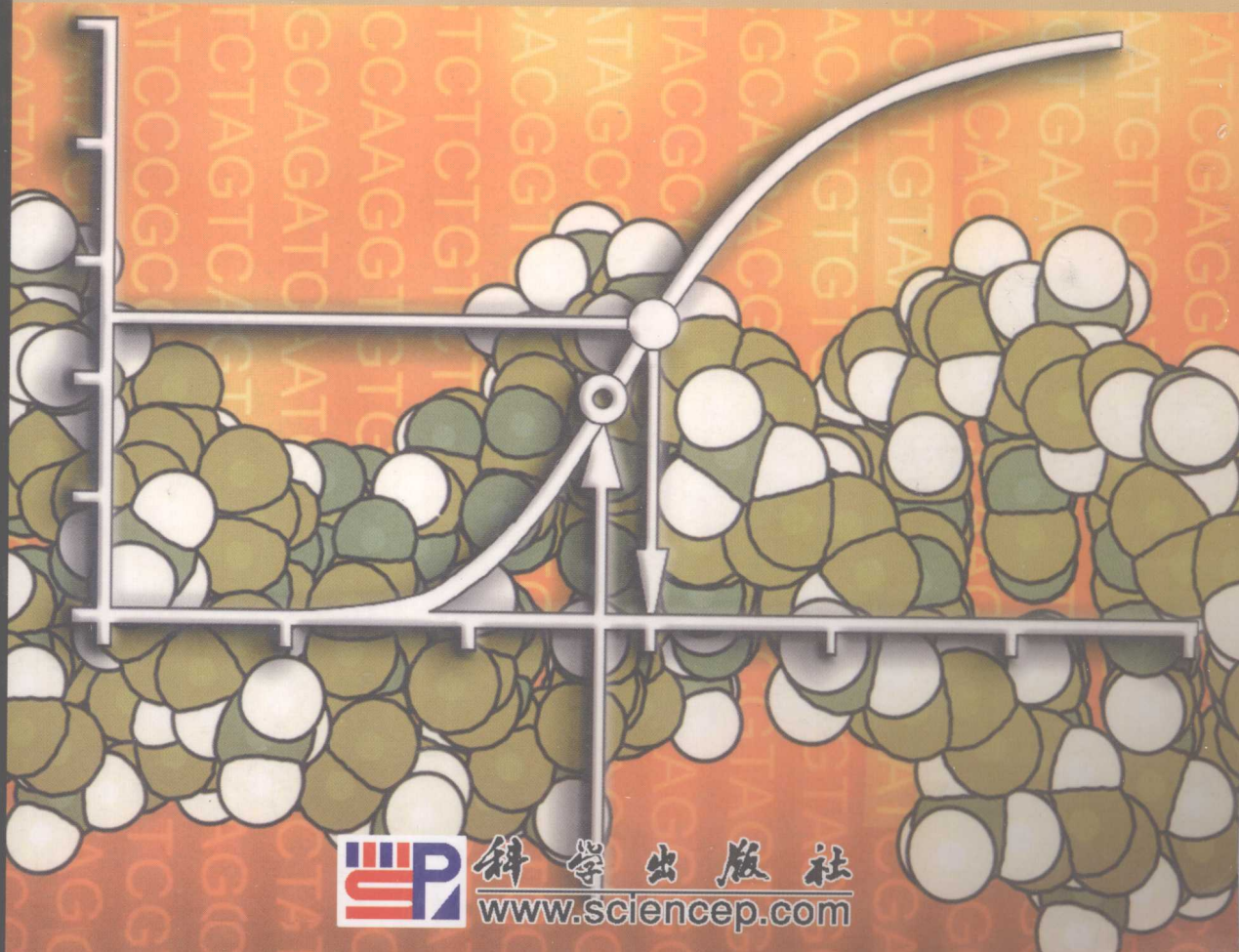
A Pharmacology Primer

Theory, Application, and Methods

药理学导论

理论、应用和方法

Terry Kenakin



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A PHARMACOLOGY PRIMER: THEORY, APPLICATION, AND METHODS

药理学导论:理论、应用和方法

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As always...for Debbie

"...more ceterum censeo is perhaps necessary in order to rouse pharmacology from its sleep. The sleep is not a natural one since pharmacology, as judged by its past accomplishments, has no reason for being tired."

Rudolph Bucheim (1820–1879)

I am indebted to GlaxoSmithKline Research and Development for support during the preparation of this book and for the means and scientific environment to make the science possible.

T.P.K.
Research Triangle Park, NC 2003

Foreword

If scientific disciplines can be said to go in and out of vogue, pharmacology is exemplary in this regard. The flourishing of receptor theory in the 1950s, the growth of biochemical binding technology in the 1970s, and the present resurgence of interest in defining cellular phenotypic sensitivity to drugs have been interspersed with troughs such as that brought on by the promise of the human genome and a belief that this genetic road map may make classical pharmacology redundant. The fallacy in this belief has been found in experimental data showing the importance of phenotype over genotype, which underscores a common finding with road maps; they are not as good as a guide who knows the way. Pharmacology is now more relevant to the drug discovery process than ever as the genome furnishes a wealth of new targets to unravel. Biological science often advances at a rate defined by the technology of its tools (i.e., scientists cannot see new things in old systems without new eyes). A veritable explosion in technology coupled with the great gift of molecular biology have definitely given pharmacologists new eyes to see.

This book initially began as a series of lectures at GlaxoSmithKline Research and Development on receptor pharmacology aimed at increasing the communication between pharmacologists and chemists. As these lectures developed it became evident that the concepts were useful to biologists, not specifically trained in pharmacology. In return, the exchange between chemists and biologists furnished new starting points from which to view the pharmacological concepts. It is hoped that this book will somewhat fill what could be a gap in present biological sciences, namely the study of dose–response relationships and how cells react to molecules.

Terry P. Kenakin
Research Triangle Park, 2003

序　言

如果以是否流行来比喻科学的发展过程，那么药理学恰好如此。受体理论兴起于 20 世纪 50 年代，而生化结合技术则于 20 世纪 70 年代发展起来，目前再次燃起人们解释细胞表型对药物敏感性的兴趣，人类基因组展现出的希望进一步推动了它，并使人相信基因图谱可能会使经典药理学成为多余。谬误在于这种信念是建立在发现细胞表型的重要性超过基因型的试验资料的基础上，它们强调与基因图谱一致的共同的发现。然而，尚无人熟知此道并加以指导。目前，就药物发现过程来说，药理学提供的新靶点的资源较之基因组学更为重要。生物科学的发展速度往往决定于其工具和技术（即科学家不可能在没有新观点的旧体系中看新事物），技术迅猛的发展和分子生物学这个最好的礼物相结合确实使药理学家能以新的视点来看问题。

本书最初是格兰素-史克研究和开发部的受体药理学的系列讲座，其目的是加强药理学家和化学家之间的信息交流。作为这些讲座的扩展，事实证明这些概念对生物学家也有用，但这并非药理学的特殊训练。作为回报，化学家和生物学家之间的交流提供了观察药理学概念的新起点。希望此书将或多或少能填补目前生物科学的空白，即量效关系的研究及细胞如何对分子起反应。

Terry P. Kenakin
Research. Triangle Park 2003

（林志彬译）

1 药理学的定义和基本概念

本章除简介本书的结构特点外，按节叙述了药理学的定义，受体学说的历史、由来和基本概念，药理学的试验系统，药物与大分子作用的亲和力，内在活性和效能，剂量－反应曲线等。由于本章的基本概念贯穿于全书，仔细阅读本章，便于进一步阅读后续章节。以下分节概括介绍其内容。

1.1 本书的特点和如何使用

本书是一本定量确定药物活性的药理学著作，它不同于一般药理学方法书，偏重于药理学的基本理论，方法学的理论、数学模型及其推导。书中各章节均是从介绍基本概念开始，进而介绍用于测定有关参数的技术方法，最后对有关公式进行数学推导。虽然，熟悉数学推导对药理学并非十分必要，但重视数学推导有利于阅读本书。

1.2 药理学的定义

药理学作为一门独立的科学已近 140 年。药理学既研究药物的吸收、代谢、分布和排泄（药物代谢动力学），也研究药物与机体的相互作用（药物效应动力学）。药物与机体之间的相互作用是药理学的基本原则，其前提是了解剂量和反应的关系，细胞如何处理药理信息等基本概念。本书特别重视定量处理药物与机体相互作用的资料。

受体药理学在药物发现中是非常重要的。受体的基因型（即从基因转录产生的受体蛋白质到其氨基酸序列的特性）是继发于受体的表型（蛋白质如何与细胞内无数成分相互作用，细胞如何特异的实现这些蛋白质的特定功能）。目前，药物治疗的靶点还不到 500 个，而潜在的药物作用的靶点为 5000～10000 个，也就是说现在已用的靶点仅占人类基因组发现的潜在靶点的 5～10%。

1.3 受体的概念

本节首先介绍了药理学的先驱者 Paul Ehrlich （1845—1915）、John Newport Langley（1852—1926）和 Alfred Joseph Clark（1885—1941）提出的有关受体的概念及其发展过程，这是药理学工作者应熟知的药理学发展史中的重要内容。

受体有许多类型，从细胞表面的蛋白质到酶、离子通道、膜转运体、DNA、细胞浆蛋白质、核受体。本书论及的概念可用于各种类型受体。由单链蛋白构成的 7 次跨膜受体（Seven Transmembrane receptors，7TM 受体）可往返跨细胞膜 7 次。由于这些受体可活化 G 蛋白而引起反应，故通常被称为 G-蛋白耦联受体（GPCRs）。在基因组中有 800～1000 个此类受体。2000 年美国约半数处方药的靶点是 7TM 受体。7TM 受体占总细胞蛋白的 1%～5%，控制多种多样的生理功能。此外，由于它们在细胞表面，药物无需进入细胞发挥作用，故易用在药物开发。GPCRs 或其他受体作为一种研究“系统”必须接受输入化学信号，并反馈输出信号。

1.4 药理的试验系统

众所周知，分子生物学改变了药理学和新药发现过程。此前新药筛选是在代替的动物组织上进行，由此产生一种比较大的基因型和表型外推差异。人和小鼠的 α-肾上腺素受体有 89%的同源性，因此，可以认为受体的氨基酸序列是非常类似的。α-受体拮抗剂 atipamezole 和育亨宾与人和小鼠的 α 受体的亲和力非常相似，然而拮抗剂哌唑嗪在人和小鼠则有 29 倍的差别，因此一个代替的试验系统不能完全预测作用于人的受体的药物活性。

分子生物学技术的发展可克隆和表达细胞中

的人受体，重组的系统可代替动物离体组织工作。用于药物发现的试验系统示于图 1.3，早期在动物组织进行的功能试验目前已让位给重组的细胞系统（人体材料工程）试验，这极大地改善了药物活性的可推断性，在新药发现中，常采用适宜的代用的宿主细胞表达人的受体（通过人 cDNA 转染），表达受体的细胞可产生生理反应，且其活性可被药理试验监控。尚无明确证据指出，代用的人细胞能比动物细胞更好地预测受体活性，特别是在病理条件下更为复杂的受体活性。受体表型支配最终的器官，受体的基因型特性和表型特性之间的精确差异可能是细胞特异性的，这仍然是药物发现过程的代替试验系统和治疗应用之间的可能的突破口。

亲和力（affinity）和效能（efficacy）分别用于表示药物对生物靶点（受体）的结合能力和相互作用的强度。在精典的受体理论中，是反映药物引起的组织反应大小的因子。

1.5 受体的性质

作者指出，除某些生物靶点如 DNA 不是蛋白质外，大多数受体均是蛋白质。受体的重要特性是它们均具三维结构。受体常由一个或多个肽链组成。图 1.4 列出了构成受体的 4 种不同的蛋白质结构，结构的改变可使受体功能改变。受体的三维结构使之能识别药物，并把外部的信息带入细胞中，一些受体与药物结合后，必须发生变构，才能产生反应。

1.6 两个重要的药理学参数——亲和力和效能

在药物发现过程中，利用各种试验体系对药物分子进行试验，并不断改变药物的分子结构，直至达到产生最适活性，在此过程中完成药物的生物活性评价。每种试验体系可用不同方式阐明药物的活性，因而可能是互相抵触的。故应用独立于这些试验体系之外的药物活性参数来推断药物的活性。亲和力和效能是两个表示药物活性的重要参数。药物干扰生理活性物质如激素或神经递质与受体结合，并阻断它们的生理活性，此类药物即为拮抗剂。如果药物与受体结合并产生作用，则为激动剂，激动剂既有亲和力，又有效能。激动剂的作用可分两个阶段，即兴奋和反应阶段，也叫做兴奋—反应机制（图 1.5）。效能是指药物分子引起生理反应的能力，不同药物引起的反应不同，准确地说应称为内在效能（注：中文亦称内在活性）。每种药的内在效能均不同（拮抗剂为 0）。图 1.6 指出 5-HT 和 5-cyanotryptamine 的内在效能不同，故后者需与两倍的 5-HT 受体结合方产生相同的反应。图 1.7 显示，经结构改造，通过增强化合物对组胺 H_2 受体的亲和力，降低其效能，合成出抑制胃酸分泌，促进溃疡愈合的 H_2 受体拮抗剂西米替丁。此工作获得了 1988 年诺贝尔生理医学奖。

1.7 亲和力的基本概念

亲和力指药物与受体相互作用的强度。药物与受体之间的化学力和距离影响药物与受体的亲和力。化学力包括静电引力、氢键、范德华引力和疏水键，这些化学力使药物结合到受体蛋白质的结合位置上。药物与受体的结合是可逆的，由于结合系统中热能的改变，药物可从受体蛋白质表面解离。这使两个药物可竞争受体蛋白上的结合位置，这种竞争是取决于药物的浓度和相互作用的强度。Langmuir 吸附等温线可用于定量研究药物分子的亲和力。

1.8 Langmuir 吸附等温线公式及其意义。

化学家 Irving Langmuir（1881—1957）根据气体对金属丝的吸附作用提出亲和力模型，即 Langmuir 吸附等温线。他根据分子有吸附到表面的特性（α）和解离特性（V_1）假定，已与分子结合的表面不可能结合其他分子，用 θ_1 表示被分子结合的表面积，而未结合的游离表面积用 $1-\theta_1$ 表示，故表面的吸附速率受介质中药物浓度（μ）控制。

公式（1.1）和（1.2）分别表示吸附到表面的速率和消除速率。吸附速率和消除速率平衡时，（1.1），（1.2）可用公式（1.3）表示，这是 Langmuir 吸附等温线的原始公式。

在药理学的术语中，它可写为公式（1.4）。

式中：[AR] 与配基结合的受体数；[R_t] 受体总数；

[A] 用摩尔浓度表示最大结合的药物浓度；平衡解离常数 K_A 是消除速率（在 Langmuir 公式中称为 V_1；在受体药理学中用 K_2 表示）和始动速率（在 Langmuir 公式中称为 α；在受体药理学中用 K_1 表示）的比值。

药物与蛋白结合、细胞活化、复杂的细胞反应等过程均可用建立在这些概念上的模型来模拟。K_A 可用来定量表示亲和力，它是 50%受体被结合时（当 [A] $=K_A$ 时）的药物浓度。K_A 较小，则亲和力较大，即亲和力是 K_A 的倒数。

本节还通过图 1.9 和 1.10 举例说明了受体结合的动力学，以及激动剂在体内（外）的剂量（浓度）—反应曲线等基本概念。

1.9 效能

效能是指药物（分子）能改变受体，并因此而使细胞产生反应的特性，其机制可能与分子和蛋白质相互作用有关。

受体蛋白质的结构并非固定不变的，它们有许多潜在的构型，受体蛋白质的构型是处于动力学状态，而非静态不变的。与受体蛋白相互作用时，药物分子可改变蛋白质构型，而使受体蛋白活化。受体蛋白构型有两种形式，即活化态（R_a）和未活化态（R_i）。公式 1.5 说明这两种构型的转换关系，式中 K_a 和 αK_a 分别代表非活化态和活化态的平衡解离常数；L（即 [R_a] / [R_i]）为立体异构常数；[A] 为配体。

本节还通过公式推导和图解（图 1.11）叙述了配体与活化型受体或未活化型受体结合时的亲和力的变化。本节讲述的内容实际上是配体受体结合学说中的二态模型（two state model）。

1.10 用图解方式叙述剂量反应曲线及其意义。

剂量反应曲线描述在细胞或亚细胞体系中，激动剂的浓度与其反应的关系，特别以对数浓度作图时的反应。其中三个参数即浓度轴、斜度和最大渐进曲线（最大反应）能反映受体的结合、活化与剂量的关系（图 1.12）。此外，从剂量反应曲线的浓度轴上还能确定激动剂的效价强度（potency），它是产生一个反应所需的摩尔浓度（图 1.15）。通常用半数有效浓度（EC_{50}）来测定效价强度，即激动剂产生 50%，最大反应时的摩尔浓度。激动剂的效价强度在药物受体药理学中是一非常重要的参数。由于 EC_{50} 值是采用对数（浓度）座标求出的，其对数 EC_{50} 的分布并非 EC_{50} 值本身的正常分布，转换为 EC_{50} 时会产生偏移（图 1.16 表 1.1），故可用 P 梯度表示效价强度（pEC_{50}），并用于统计分析。

1.11 本章小结

- 讨论了某些药理学起源和相关的观点和生物学的“受体”概念。
- 现有的药物仅针对存在于人基因组药物靶点的一部分。
- 纵然重组系统很大地改善了药物发现过程，但病理现象仍然影响此过程。
- 在药物发现中，亲和力和效能可用作系统一不依赖的药物活性测量。
- 亲和力是药物结合到受体上的强度，可用平衡解离常数来量度。
- 亲和力可用 Langmuir 吸附等温线来描述和定量。
- 效能是被测定的相对术语，用来测定分子在受体上引起变化的能力。
- 剂量反应曲线测量药物活性，其最大渐进线完全依赖于效能，但效价强度则是亲和力和效能的混合物。
- 测定效价强度对数常态分布，仅 pEC_{50} 值用于统计学试验。

1.12 公式推导

1.12.1 作为效能的机制的构象选择（公式 1.8，1.9）

（林志彬编译）

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1

What Is Pharmacology

I would in particular draw the attention to physiologists to this type of physiological analysis of organic systems which can be done with the aid of toxic agents.

—CLAUDE BERNARD (1813–1878)

1.1 About This Book

Essentially this is a book about the methods and tools used in pharmacology to quantify drug activity. Receptor pharmacology is based on the comparison of experimental data to simple mathematical models with a resulting inference of drug behavior to the molecular properties of drugs. From this standpoint, a certain understanding of the mathematics involved in the models is useful, but it is not imperative. This book is structured such that each chapter begins with the basic concepts, then moves on to the techniques used to estimate drug parameters, and finally, for those so inclined, the mathematical derivations of the models used. Understanding the derivation is not a prerequisite to understanding the application of the methods or the resulting conclusion; these are included for completeness and are for readers who wish to pursue exploration of the models. In general, facility with mathematical equations is definitely not required for pharmacology; the derivations can be ignored to no detriment to the use of this book.

Second, the symbols used in the models and derivations, on occasion, duplicate each other (e.g., α is an extremely popular symbol). However, the use of these multiple symbols has been retained because this preserves the context of where these models were first described and used. Also, changing these to make them unique would cause confusion if these methods are to be used beyond the framework of this book. Therefore, care should be taken to consider the actual nomenclature of each chapter.

Third, an effort has been made to minimize the need to cross-reference different parts of the book; when a particular model is described, the basics are reiterated somewhat to minimize the need to read the relevant but different part of the book where the model is initially described. Although this leads to a small amount of repeated description, it is believed that this will allow for a more uninterrupted flow of reading and use of the book.

1.2 What Is Pharmacology?

Pharmacology as a separate science is approximately 120–140 years old. The relationship between chemical structure and biological activity began to be studied systematically in the 1860s [1]. It began when physiologists, using chemicals to probe physiological systems, became more interested in the chemical probes than the systems they were probing. *Pharmacology* can be an all encompassing term for the study of drugs on living systems in relation to their therapeutic value. Within this discipline is the study of absorption, metabolism, distribution, and excretion of drugs (*pharmacokinetics*) and the study of the interaction of drugs with living systems (*pharmacodynamics*). A differentiation of physiology and pharmacology was given by the pharmacologist Sir William Paton in 1986 [2]:

> If physiology is concerned with the function, anatomy with the structure, and biochemistry with the chemistry of the living body, then pharmacology is concerned with the changes in function, structure, and chemical properties of the body brought about by chemical substances.

Many works about pharmacology essentially deal in therapeutics associated with different organ systems in the body. Thus, in many pharmacology texts, chapters are entitled drugs in the cardiovascular system, the effect of drugs on the gastrointestinal system, central nervous system, and so on. However, the underlying principles for all of these is the same, namely the pharmacodynamic interaction between the drug and the biological recognition system for that drug. Therefore, a prerequisite to all of pharmacology is an understanding of the basic concepts of dose–response and how living cells process pharmacological information. This generally is given the term *receptor pharmacology*, where receptor is a term referring to any biological recognition unit for drugs (e.g., membrane receptors, enzymes, DNA).

With such knowledge in hand, readers will be able to apply these principles to any branch of therapeutics effectively. This book treats dose–response data generically and demonstrate methods by which drug activity can be quantified across all biological systems irrespective of the nature of the biological target.

The human *genome* is now widely available for drug discovery research. Far from being a simple blueprint of how drugs should be targeted, it has shown biologists that receptor *genotypes* (i.e., properties of proteins resulting from genetic transcription to their amino acid sequence) are secondary to receptor *phenotypes* (how the protein interacts with the myriad of cellular components and how cells tailor the makeup and functions of these proteins to their individual needs). Since the arrival of the human genome, receptor pharmacology as a science is more relevant than ever in drug discovery. Current drug therapy is based on less than 500 molecular targets, yet estimates utilizing the number of genes involved in multifactorial diseases suggest that the number of potential drug targets range from 5000 to 10,000 [3]. Thus, current therapy is using only 5–10% of the potential trove of targets available in the human genome.

A meaningful dialogue between chemists and pharmacologists is the single most important element of the drug discovery process. The necessary link between medicinal chemistry and pharmacology has been elucidated by Paton [2]:

> For pharmacology there results a particularly close relationship with chemistry, and the work may lead quite naturally, with no special stress on practicality, to therapeutic application, or (in the case of adverse reactions) to toxicology.

Chemists and biologists reside in different worlds from the standpoint of the type of data they deal with. Chemistry is an exact science with physical scales that are not subject to system variance. Thus, the scales of measurement are transferrable. Biology deals with the vagaries of complex systems that are not completely understood. Within this scenario, scales of measurement are much less constant and much more subject to system conditions. Given this, a gap can exist between chemists and biologists in terms of understanding and also in terms of the best method to progress forward. In the worst circumstance, it is a gap of credibility emanating from a failure of the biologist to make the chemist understand the limits of the data. Usually, however, credibility is not the issue and the gap exists due to a lack of common experience. This book was written in an attempt to limit or, hopefully, eliminate this gap.

1.3 The Receptor Concept

One of the most important concepts emerging from early pharmacological studies is the concept of the *receptor*. Pharmacologists knew that minute amounts of certain chemicals had profound effects on physiological systems. They also knew that very small changes in the chemical composition of these substances could lead to huge differences in activity. This led to the notion that something on or in the cell must specifically read the chemical information contained in these substances and translate it into a physiological effect. This something was conceptually referred to as the "receptor" for that substance. Pioneers such as Paul Ehrlich (1854–1915; Fig. 1.1A) proposed the existence of "chemoreceptors" (actually he proposed a collection of "amboceptors," "triceptors," "polyceptors") on cells for dyes. He also postulated that the chemoreceptors on parasites, cancer cells, and microorganisms were different from a healthy host and thus could be exploited therapeutically. The physiologist turned pharmacologist John Newport Langley (1852–1926; Fig. 1.1B), during his studies with the drug jaborandi (which contains the alkaloid pilocarpine) and atropine, introduced the concept that receptors were switches that received and generated signals and that these switches could be activated or blocked by specific molecules. The originator of quantitative receptor theory, the Edinburgh pharmacologist Alfred Joseph Clark (1885–1941; Fig. 1.1C) was the first to suggest that the data, compiled from his studies of the interactions of acetylcholine and atropine, resulted from the unimolecular interaction of the drug and a substance on the cell surface. He articulated these ideas in the classic work "The mode of action of drugs" on cells [4] later revised as the "Handbook of Experimental Pharmacology" [5]. As put by Clark in 1937:

> It appears to the writer that the most important fact shown by a study of drug antagonisms is that it is impossible to explain the remarkable effects observed except by assuming that drugs unite with receptors of a highly specific pattern . . . No other explanation will, however, explain a tithe of the facts observed.

Clark's next step formed the basis of receptor theory by applying chemical laws to systems of "infinitely greater complexity" [4]. It is interesting to note the scientific atmosphere in which Clark published these ideas. The dominant ideas between 1895 and 1930 were based on theories such as the law of phasic variation essentially stating that "certain phenomena occur frequently." Homeopathic theories such as the Arndt–Schulz law and Weber–Fechner law were based on loose ideas around surface tension of the cell membrane but there was little physicochemical basis to these ideas [6]. In this vein, prominent pharmacologists of the day such as Walter Straub (1874–1944) suggested that a general theory of chemical binding between drugs and cells utilizing receptors was "going too far . . . and . . . not admissible" [6]. The impact of Clark's thinking against these concepts cannot be overemphasized to modern pharmacology.

Drug receptors can exist in many forms from cell surface proteins to enzymes, ion channels, membrane transporters, DNA, and cytosolic proteins (see Fig. 1.2). There are examples of important drugs for all of these. This book deals with general concepts that can be applied to a range of receptor types, but most of the principles are illustrated with the most tractable receptor class known in the human genome, namely *seven transmembrane* **(7 TM)** *receptors*. These receptors are named for their characteristic structure, which consists of a single protein chain that traverses

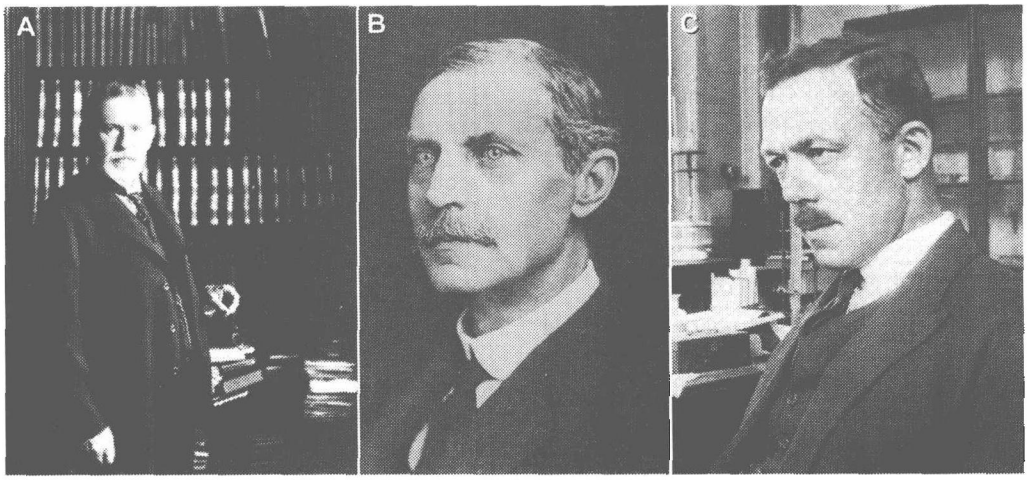

FIGURE 1.1 Pioneers of pharmacology. (A) Paul Ehrlich (1854–1915). Born in Silesia, Ehrlich graduated from Leipzig University to go on to a distinguished career as head of institutes in Berlin and Frankfurt. His studies with dyes and bacteria formed the basis of early ideas regarding the recognition of biological substances by chemicals. (B) John Newport Langley (1852–1926). Although he began reading mathematics and history in Cambridge in 1871, Langley soon took to physiology. He succeeded the great physiologist M. Foster to the chair of physiology in Cambridge in 1903 and branched out into pharmacological studies of the autonomic nervous system. These pursuits led to germinal theories of receptors. (C) Alfred J. Clark (1885–1941). Beginning as a demonstrator in pharmacology in King's College (London), Clark went on to become professor of pharmacology at University College, London. From there he took the chair of pharmacology in Edinburgh. Known as the originator of the modern receptor theory, Clark applied chemical laws to biological phenomena. His books on receptor theory formed the basis of modern pharmacology.

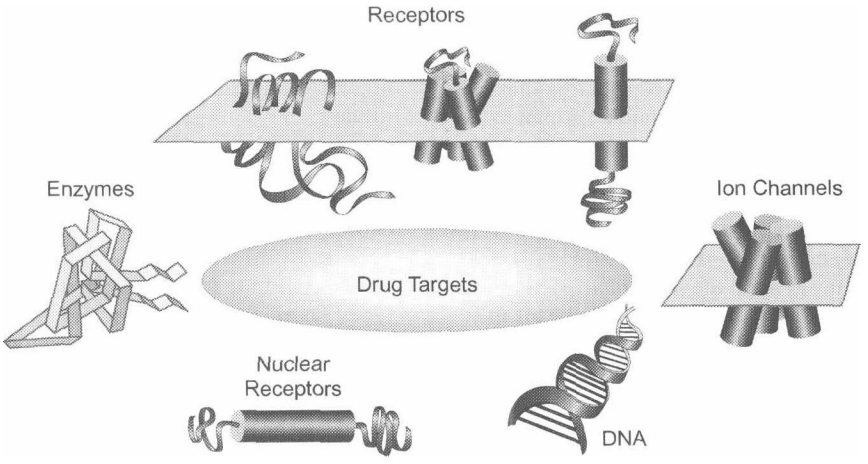

FIGURE 1.2 Schematic diagram of potential drug targets. Molecules can affect the function of numerous cellular components both in the cytosol and on the membrane surface. There are many families of receptors that traverse the cellular membrane and allow chemicals to communicate with the interior of the cell.

the cell membrane seven times to produce extracellular and intracellular loops. Because these receptors activate G-proteins to elicit response, they are also commonly referred to as *G-protein–coupled receptors* **(GPCRs)**. There are between 800 and 1000 [7] of these in the genome (the genome sequence predicts 650 GPCR genes of which approximately 190 [on the order of 1% of the genome of superior organisms] are categorized as known GPCRs [8] activated by some 70 ligands). In the United States in the year 2000, nearly half of all prescription drugs were targeted toward 7TM receptors [3]. These receptors, accounting for 1–5% of the total cell protein, control a myriad of

physiological activities. Because they are on the cell surface, they are tractable for drug discovery; therefore, drugs do not need to penetrate the cell to produce effect. In the study of biological targets such as GPCRs and other receptors, a "system" must be employed that accepts chemical input and returns biological output. It is worth discussing such receptor systems in general terms before their specific uses are considered.

1.4 Pharmacological Test Systems

Molecular biology has transformed pharmacology and the drug discovery process. As recently as 10 years ago, screening for new drug entities was carried out in surrogate animal tissues. This necessitated a rather large extrapolation spanning differences in genotype and phenotype. The belief that the gap could be bridged came from the notion that the chemicals recognized by these receptors in both humans and animals were the same (see later). Receptors are unique proteins with characteristic amino acid sequences. While *polymorphisms* (spontaneous alterations in amino acid sequence; see later) of receptors exist in the same species, in general, the amino acid sequence of a natural ligand binding domain for a given receptor type largely may be conserved. There are obvious pitfalls of using surrogate species receptors for the prediction of human drug activity, and it never can be known for certain whether an agreement for estimates of activity for a given set of drugs ensures accurate prediction for all drugs; the agreement is very much drug and receptor dependent. For example, the human and mouse α_2-adrenoceptor are 89% homologous and thus are considered very similar from the standpoint of an amino acid sequence. Furthermore, the affinities of the α_2-adrenoceptor antagonists atipamezole and yohimbine are nearly indistinguishable (atipamezole human α_2-C10 $K_i = 2.9 \pm 0.4$ n*M*, mouse α_2-4H $K_i = 1.6 \pm 0.2$ n*M*; yohimbine human α_2-C10 $K_i = 3.4 \pm 0.1$ n*M*, mouse α_2-4H $K_i = 3.8 \pm 0.8$ n*M*). However, there is a 20.9-fold difference for the antagonist prazosin (human α_2-C10 $K_i = 2034 \pm 350$ n*M*, mouse α_2-4H $K_i = 97.3 \pm 0.7$ n*M*) [9]. Such data highlight a general theme in pharmacological research, namely that a hypothesis, such as one proposing two receptors to be identical with respect to their sensitivity to drugs are the same, cannot be proved, only disproved. Although a considerable number of drugs could be tested on the two receptors (thus supporting the hypothesis that their sensitivity to all drugs is the same), this hypothesis is immediately disproved by the first drug that shows differential potency on the two receptors. The fact that a series of drugs tested shows identical potencies may only mean that the wrong sample of drugs has been chosen to unveil the difference. Thus, no general statements can be made that any one surrogate system is completely predictive of activity on the target human receptor; this will always be a drug-specific phenomenon.

The link between animal and human receptors is the fact that both proteins recognize the endogenous transmitter (e.g., acetylcholine, norepinephrine), and, therefore, the hope is that this link will carry over into other drugs that recognize the animal receptor. This imperfect system formed the basis of drug discovery until human *cDNA* for human receptors could be used to make cells express human receptors. These engineered (recombinant) systems now are used as surrogate human receptor systems and the leap of faith from animal receptor sequences to human receptor sequences is not required (i.e., the problem of differences in genotype has been overcome). However, cellular signaling is an extremely complex process, and cells tailor their receipt of chemical signals in numerous ways. Therefore, the way a given receptor gene behaves in a particular cell can differ in response to the surroundings in which that receptor finds itself. These differences in phenotype (i.e., properties of a receptor produced by interaction with its environment) can result in differences in both the quantity and the quality of a signal produced by a concentration of a given drug in different cells. Therefore, there is still a certain, although somewhat lesser, leap of faith taken in predicting therapeutic effects in human tissues under pathological control from surrogate recombinant or even surrogate natural human receptor systems. For this reason, it is a primary requisite of pharmacology to derive system-independent estimates of drug activity that can be used to predict therapeutic effects in other systems.

A schematic diagram of the various systems used in drug discovery, in order of how appropriate they are to therapeutic drug treatment, is shown in Fig. 1.3. As discussed earlier, early functional experiments in animal tissue have now largely given way to testing in recombinant cell systems engineered with human receptor material. This huge technological step greatly improved the predictability of drug activity in humans, but it should be noted that there are still many factors that intervene between the genetically engineered drug testing system and the pathology of human disease.

A frequently used strategy in drug discovery is to express human receptors (through *transfection* with human cDNA) in convenient surrogate host cells. These host cells are chosen mainly for their technical properties (i.e., robustness, growth rate, stability) and not with any knowledge of verisimilitude to the therapeutically targeted human cell type. There are various factors relevant to the choice of surrogate host cell, such as a very low background activity (i.e., a cell cannot be used that already contains a related animal receptor for fear of cross-reactivity to molecules targeted for the human receptor). Human receptors often are expressed in animal surrogate cells; the main idea here is that the cell is a receptacle for the receptor, allowing it to produce physiological responses, and that activity can be monitored in pharmacological experiments. In this sense, human receptors expressed in animal cells are still a theoretical step distanced from the human receptor in a human cell type. However, even if a human surrogate is used (and there are such cells available), there is no definitive evidence that a surrogate human cell is any more predictive of a natural receptor activity than an animal cell when compared with the complex receptor behavior in its natural host cell type expressed under pathological conditions. The receptor phenotype dominates in the end organ, and the exact

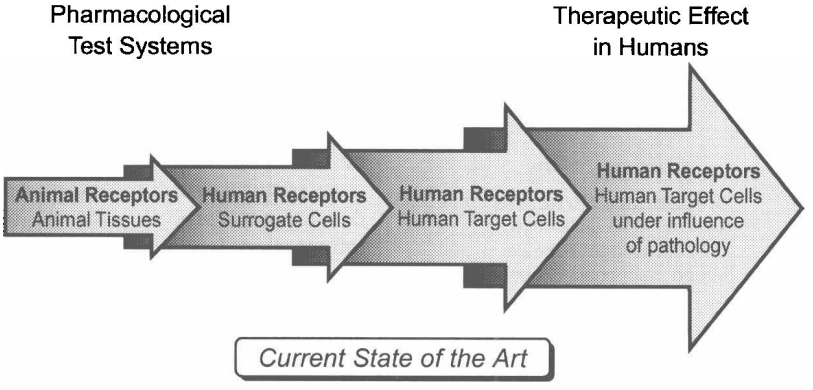

FIGURE 1.3 A history of the drug discovery process. Originally, the only biological material available for drug research was animal tissue. With the advent of molecular biological techniques to clone and express human receptors in cells, recombinant systems supplanted animal isolated tissue work. It should be noted that these recombinant systems still fall short of yielding drug response in the target human tissue under the influence of pathological processes.

differences between the genotypic behavior of the receptor (resulting from the genetic makeup of the receptor) and the phenotypic behavior of the receptor (due to the interaction of the genetic product with the rest of the cell) may be cell specific. Therefore, there is still a possible gap between the surrogate systems used in the drug discovery process and the therapeutic application. Moreover, most drug discovery systems utilize receptors as switching mechanisms and quantify whether drugs turn on or turn off the switch. The pathological processes that we strive to modify may be subtler. As put by pharmacologist Sir James Black [10]:

> angiogenesis, apoptosis, inflammation, commitment of marrow stem cells, and immune responses. The cellular reactions subsumed in these processes are switch like in their behavior ... biochemically we are learning that in all these processes many chemical regulators seem to be involved. From the literature on synergistic interactions, a control model can be built in which no single agent is effective. If a number of chemical messengers each bring information from a different source and each deliver only a subthreshold stimulus but together mutually potentiate each other, then the desired information-rich switching can be achieved with minimum risk of miscuing.

Such complex end points are difficult to predict from any one of the component processes, leading to yet another leap of faith in the drug discovery process.

Even when an active drug molecule is found and activity is verified in the therapeutic arena, there are factors that can lead to gaps in its therapeutic profile. When drugs are exposed to huge populations, genetic variations in this population can lead to the discovery of *alleles* that code for mutations of the target (isogenes), which can lead to a variation in the drug response. Such polymorphisms can lead to resistant populations (e.g., resistance of some asthmatics to β-adrenoceptor bronchodilators) [11]. In the absence of genetic knowledge, these therapeutic failures for a drug could not be averted easily, because they in essence resulted from the presence of new biological targets not considered originally in the drug discovery process. However, with new epidemiological information becoming available, these polymorphisms can now be incorporated into the drug discovery process.

Two theoretical and practical scales can be used to make system-independent measures of drug activity on biological systems. The first is a measure of the attraction of a drug for a biological target, namely its *affinity* for receptors. Drugs must interact with receptors to produce an effect, and *affinity* is a chemical term used to quantify the strength of that interaction. The second is much less straightforward and is used to quantify the degree of effect imparted to the biological system after the drug binds to the receptor; this is termed *efficacy*. This property was named by R. P. Stephenson [12] within classical receptor theory as a proportionality factor for tissue response produced by a drug. There is no absolute scale for efficacy; rather it is dealt with in relative terms (i.e., the ratio of the efficacy of two different drugs on a particular biological system can be estimated and, under ideal circumstances, will transcend the system and be applicable to other systems as well). It is the foremost task of pharmacology to use the translations of a drug effect obtained from cells to provide system-independent estimates of affinity and efficacy. Before a specific discussion of affinity and efficacy, it is worth considering the molecular nature of biological targets.

1.5 The Nature of Drug Receptors

Although some biological targets such as DNA are not protein in nature, most receptors are. It is useful to consider the properties of receptor proteins to provide a context for the interaction of small molecule drugs with them. An important property of receptors is that they have a three-dimensional structure. Proteins usually are composed of one or more peptide chains; the composition of these