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Review – Part of the Special Issue – Pharmacology in 21st Century Biomedical Research

The fall and rise of pharmacology – (Re-)defining the discipline?

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ABSTRACT

Pharmacology is an integrative discipline that originated from activities, now nearly 7000 years old, to identify therapeutics from natural product sources. Research in the 19th Century that focused on the Law of Mass Action (LMA) demonstrated that compound effects were dose-/concentration-dependent eventually leading to the receptor concept, now a century old, that remains the key to understanding disease causality and drug action. As pharmacology evolved in the 20th Century through successive biochemical, molecular and genomic eras, the precision in understanding receptor function at the molecular level increased and while providing important insights, led to an overtly reductionistic emphasis. This resulted in the generation of data lacking physiological context that ignored the LMA and was not integrated at the tissue/whole organism level. As reductionism became a primary focus in biomedical research, it led to the *fall* of pharmacology. However, concerns regarding the disconnect between basic research efforts and the approval of new drugs to treat 21st Century disease tsunamis, e.g., neurodegeneration, metabolic syndrome, etc. has led to the reemergence of pharmacology, its *rise*, often in the semantic guise of systems biology. Against a background of limited training in pharmacology, this has resulted in issues in experimental replication with a bioinformatics emphasis that often has a limited relationship to reality. The integration of newer technologies within a pharmacological context where research is driven by testable hypotheses rather than technology, together with renewed efforts in teaching pharmacology, is anticipated to improve the focus and relevance of biomedical research and lead to novel therapeutics that will contain health care costs.

Contents

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1. Introduction

In critically assessing the relevance of a legacy scientific discipline to current science, it is useful to review its past contributions in order to gain an historical perspective and to assess its evolution in the dynamic context of new concepts, ancillary disciplines and enabling technologies. This assessment should also consider the intellectual and cultural environment in which the discipline is currently practiced together with its technological attributes that in the case of pharmacology can provide a realistic vision for future contributions to basic biomedical research and drug discovery.

As a seminal scientific discipline, pharmacology evolved from activities, now nearly 7000 years old, to identify therapeutics from natural product sources. It became formalized as a distinct discipline based on physiological studies in the early-mid-19th Century [1–4] that used compounds, both drugs and natural products, as research tools to study their effects on tissue and organ function in order to understand disease causality. Since that time pharmacology has undergone continuous modification as the technologies used to interrogate biological system function at the cellular, tissue and animal levels have increased in precision and degree of detail, and also in their capability to generate increasing amounts of data. While the latter ability is generally viewed as a "key basis of competition, productivity growth. . .[and]. . . innovation" [5], irrespective of its conception, execution, quality, reproducibility and usability with Brenner describing much of the current focus on data generation in biomedical research as "low input, high throughput, no output science" [6]. With the advent of the personal computer and the high throughput robotics systems that the former has enabled, more data can now be generated in the space of a year using currently available research tools than could be generated in the full century following the founding of pharmacology. While now an ingrained feature of 21st Century biomedical research, especially with high throughput screening (HTS), GWAS (genome wide association studies) and NGS (next generation sequencing), the ability to productively interrogate and integrate this information has become extremely challenging and requires a far more critical, objective and context-relevant approach where the data can be used to both inform and refine hypotheses related to basic cell function and disease causality.

Thus the re-emergence of interest in the integrative, hierarchical approach that is the core of pharmacology as a discipline represents a major contribution in productively dealing with this information overload especially as it pertains to improving the intrinsic value of the archived data and its physiological relevance.

2. Pharmacology – its fall and rise

Despite its key role as an integrative discipline focused on evaluating disease hypotheses and compound properties, as contrasted to finding uses for technology platforms, the central role of pharmacology in biomedical research has diminished over the past 40 years. This has occurred as a result of: (a) a reductionistic approach to biological systems research where the explosion in funding and training in the discipline of molecular biology has predominated to the exclusion of other disciplines; and (b) the introduction of high throughput platform technologies, biological and chemical, that have tended to reduce the intellectual component of research efforts – prioritizing data quantity over its quality [6].

Events over this time frame – the mid1980s through 2010 – have appropriately been referred to as the *fall* of pharmacology, the full negative impact of which is only now being felt, despite concerns, raised both in the early 1990s [7] and 2000s [8–10], regarding the questionable value of reductionism in biomedical research in the absence of context. More recently, pharmacology has necessarily remerged – *its rise* – in a number of novel guises, most of which from a pharmacological perspective are semantic rather than scientifically substantive in nature [11]. These include translational research [12,13] and a number of "systems-related" disciplines including: systems biology [14,15]; systems pharmacology [16,17]; chemical biology [18] network biology [19,20], molecular networks [21], network medicine [22] and Quantitative and Systems Pharmacology (QSP [23,24]) that conceptually reflect classical pharmacology within a bioinformatics context.

Pharmacology has certainly gained a significant benefit from the reductionist approaches of molecular biology with these having, according to James Black, "proven to be our most successful analytical tool" [25]. This is especially, and perhaps only true when these approaches are hierarchically integrated within a framework that also uses tissue, whole animal and human models to provide context. The contribution of the high throughput sciences, e.g., combinatorial/parallel chemistry and compound screening and

bioinformatics, becomes less useful in the absence of an integrative approach and can actually become dilutive rather than providing a substantive and synergistic influence negatively impact progress [26]. This has led to the concept of "confusing technical success with progress" [10] making context-free data, to a major extent, archival with minimal utility in understanding basic cell function and the search for novel therapeutics [27–29]. A critical point is that *high content* assays [30] not be confused with HTS, especially with the availability of new generations of high throughput functional assays which are focused on increasing data content (Table 1).

2.1. In the beginning...

From prehistory through to the end of the 19th Century, the search for medications to treat human disease states was focused on natural product sources. These activities were first documented approximately 4600 years ago [31] in the form of the Chinese materia medica – *Traditional Chinese Medicine*. This in turn evolved as part of Japanese Kampo and Indian Ayurvedic medicine approaches. The study of medicinal plants was also a major component of Greek, Roman and Arabic pharmaceutical traditions [1,3,31–33] that involved major efforts in isolating active ingredients and experimenting with their combinations. Subsequent European, together with emerging US research efforts in the 19th Century were focused on the understanding of drug actions on tissue, animal and human function and set the stage for pharmacology to become a distinct research-based discipline in the mid 19th Century [3,4]. In approximately the same time frame, the commercialization of vaccines, serum antitoxins and the synthetic compounds that emerged from the German chemical industry led apothecaries in Europe, Japan and the US to begin their evolution to become pharmaceutical companies [4,34–36]. This began a symbiotic relationship between the pharmaceutical industry and academic pharmacologists [37–41] that while mutually expedient has frequently led to differing viewpoints as to which of the two: (a) has been the primary source of new drug candidates and actual drugs [38,42] and (b) does the more innovative, credible and reproducible science [43–45].

It is noteworthy that Traditional Chinese Medicine has undergone a renaissance in the 21st Century as China has emerged as a major player in biomedical research, using contemporary drug discovery technologies and new knowledge regarding disease targets to reexamine the therapeutic value of natural products in terms of defined compound entities and mechanisms [46–48]. Distinct from Traditional Chinese Medicine, natural products – plants, marine organisms, microorganisms, soil samples, reptiles and sea snails – have remained a major source of new drugs or drug leads [31,49]. These include a variety of novel immunosuppressants, e.g., rapamycin; anticancer agents, e.g., doxorubicin, taxane, combretastatin [50]; new generations of antibiotics [51], novel analgesics, e.g., the frog alkaloid toxin, epibatidine [52] and synthetic cone snail venom, ziconotide [53]; and the anticoagulant, desmoteplase, isolated from the saliva of the vampire bat [54].

2.2. Pharmacology as a distinct discipline

The research contributions of many distinguished – and often legendary – scientists – biologists and chemists – established the founding principles of modern day pharmacology. From the pre-19th Century period, Galen, Avicena and Paracelsus; the 19th Century, Buchheim, Schmiedeberg, Bernard, Pasteur, Ehrlich, Domagk, Elliott, Fischer, Langley and Abel; and in the 20th Century, Dale, Clark, Ahlquist, Schild, Ariens, Paton, Stephenson, Coloqhoun, Mackay, Waud, Rang, Black, Lefkowitz, Kobilka and Kenakin among many others [2,55; Table 1]. Collectively these scientists were responsible for initiating and refining – via experimental trial and error – an important paradigm shift in the characterization of therapeutics, namely a shift from empirical descriptors of the activities observed with natural products, e.g., heating, cooling, drying, moistening, emetic, poisonous, etc., to the concept of defined therapeutic entities producing dose/concentration-dependent effects via interactions with receptive elements with differing topographies and functionality in animal and human tissue. This concept was captured in Ehrlich's now famous principle, "*corpora non agunt nisi fixata*" ("agents only work when they are bound" [56]) – the lock and key concept of drug action that was – and remains – the basis of receptor theory [57–61].

2.3. The receptor concept

The concept of the dose response as a therapeutic principle has been ascribed to "the father of toxicology", Paracelsus (~1500s) who noted that "nothing is without poison; only the dose permits something not to be poisonous". The extension of the concept of dose-dependence by Withering (1785) in studies on the therapeutic use of digitalis led Berthollet [62] and Guldberg [63] and Wagge [64] to describe the *Law of Mass Action* [LMA] – the core concept of pharmacology. With the development of the bioassay, a basic physiological technique, the ability to measure the relative potencies of plant extracts and their quantitative, e.g., concentration-/dose–dependent effects [65], was critical to the evolving receptor concept and has provided the context [61,66] for current efforts in drug discovery and the exploration of new concepts in drug actions and drug targets that include residence time [67], constitutive receptor activity [68,69], allosterism [66,70,71], signaling bias [72] and intracellular drug targets [73,74] that include DNA, RNA and mitochondria [75].

The receptor concept evolved throughout the 20th Century based on the extensive work not only of pharmacologists like Clark [76], Gaddum [77], Schild [78], Ariens [79], Ahlquist, [80], Stephenson [81], Mackay [82], Waud [83], Black [84], Paton [85], Lefkowitz, Caron and Kobilka [86,87], Colquhoun [88] and Kenakin [60,89] but also by enzymologists, Michaelis and Menten [90] and the biochemists Monod, Wyman and Changeux [91] and Koshland, Némethy and Filmer [92] (Table 1). The studies of the latter on enzyme theory and induced-fit/cooperative interactions in multimeric protein complexes were instrumental in providing a basis for the concepts of allosterism [70] and ternary receptor theory [93,94]. These activities led to a constant evolution in the conceptualization of receptor properties and function that added to their complexity and to the types of experimentation required to objectively assess and refine hypotheses.

2.3.1. Receptors as drug targets

The term receptor was originally used to describe the receptive substance for an endogenous mediator, compound or drug on the cell surface. Its usage has gradually been expanded to encompass *all* types of drug target [95,96] including: (i) the 7-transmembrane, heptahelical *GPCRs* (G-protein-coupled receptors); (ii) *transmembrane ion channels* including ligand (*LGICs*) and voltage-gated (*VGICs*) ion channels; (iii) *catalytic receptors* that include cytokine, pattern recognition, natriuretic peptide, GDNF (glial cell-derived neurotrophic factor) receptors and the receptor tyrosine kinase and phosphatases (RTPs), receptor serine/threonine kinase (RSTK) and TNF (Tumor necrosis factor) receptor families; (iv) *enzymes* e.g., the Cytochrome P450 and serine/threonine kinase super families, apoptotic and necrotic protein cascades and constituent proteins of the mitochondrial electron transport chain; (v) nuclear hormone receptors (retinoic acid, steroid and orphan) and; (vi) transporters including the solute carriers (SLCs), ATP-binding cassette proteins and various ATPases. Emerging drug targets

Table 1
The fall and rise of pharmacology.

Era	Classical	Biochemical	Molecular	Genomic	Systems
Timeframe	Mid 1800s–1940s	1948–1970	1970–1986	1987–present	2003–present
Receptor/drug target concepts/theories	Receptor/drug target "Lock and key" Law of Mass Action (LMA)	Intrinsic activity Efficacy Receptor reserve/spare receptors Rate theory Allosterism Desensitization/ tachyphylaxis/ tolerance	Protein ensembles Ternary complex model Oligo-/Di-merization Receptor trafficking	Constitutive receptor activity Target residence time	Pluridimensional efficacy Biased signaling
Major figures	Clark [76] Dale [159] Ehrlich [56,57] Gaddum [77] Langley [97] Michaelis and Menten [89]	Ahlquist [80,162] Ariens [79] Coloqhoun [88] Cuatrecasas [102,170,178] Hollenberg [169] Koshland [92] Lefkowitz/Caron/ Kobilka/DeLean [86,93,138] Mackay [82] Monod [91] Pastan/Roth [176,177] Paton [85] Rang [60] Schild [78] Stephenson [81] Waud [83]	Black/Leff [25,84,173,308] Changeux [91,165] Snyder [180]	Bond [69] Bouvier [118,136] Civelli [244,245] Christopoulos [70,72,101] Copeland [67] Costa [68,149] Kenakin [72,89,98,100,138] Hall [151] Milligan [104,119,155] Roth [197,210]	Hood [14] Wehling [13]
Concepts	Receptors Null hypothesis	Receptor isolation Receptor subtypes LGICs	GPCRs Drug receptors Transporters Preclinical safety assessment Reductionism	Positive (PAM) and negative (NAM) allosteric modulators Orphan receptors	Omics – proteomics, epigenomics, metabolomics, etc. Pathway analysis Drug repurposing Data replication Translational medicine Molecular networks
Techniques	Tissue baths Animal models	Enzymology Biochemistry Electrophysiology Animal models of disease Immortalized cell lines	Radioligand binding assays Recombinant systems	FRET, BRET Electrophysiology X-ray crystallography Imaging Genetically engineered disease models (mice, rats, zebrafish)	Receptor function DREADDs
Enabling technologies	Medicinal chemistry [2,3] Statistics [329]	Affinity labeling [167,170] Radioimmunoassays [175–178]	Personal Computer [303] Genetics – Cloning, expression, target mutation [236,248] CAMD [260]	HTS [223] Combinatorial/parallel chemistry/compound libraries [222] Human Genome Map [269] ENCODE [303,327,328] GWAS/NGS [270] Biomarkers [330] Bioinformatics	Animal models of human disease [307] High content screening [30] Phenotypic screening [228,325] Public Database Interrogation [299]

Abbreviations: BRET, bioluminescence resonance energy transfer; CAMD, computer aided molecular design; FRET, fluorescence resonance energy transfer; DREADDs, designer receptors exclusively activated by designer drugs; ENCODE, encyclopedia of DNA elements; GPCRs, G-protein coupled receptors; GWAS, genome wide association study; LGIC, ligand gated ion channel; NGS, next generation sequencing.

include a variety of nucleic acids including non-coding functional elements in DNA and ribosomal, messenger, micro and short interfering RNAs.

2.4. Evolution of the receptor concept

As noted, the seminal concept that all classes of therapeutic agents produce their effects by acting as "magic bullets" at discrete molecular targets comes from the work of Ehrlich [56,57] and Langley [97] who independently generated the experimental data that led to the seminal "lock and key" hypothesis for drug action. This involved a ligand (L) – a drug, new chemical entity (NCE), natural product etc. – interacting with a receptive substance (R; drug target) in a reversible manner to form a receptor-ligand complex, R/L, the functional consequences of which are to modulate cell function to maintain and/or restore tissue homeostasis.

As the 20th Century progressed, research in pharmacology focused on an improved characterization and understanding of receptor function with much of this effort focused on the evolution of the *occupancy theory* originally proposed by Clark [76]. With the increased sophistication of the various technologies available to study receptor properties – and their function, research methodologies progressed from the use of smoke drums to technologies like FRET (fluorescence resonance energy transfer). Parallel efforts led to receptor isolation and crystallization with the routine use of recombinant receptor proteins in drug discovery, especially in conjunction with HTS and chemical synthesis technologies. While

this created a transformative means by which to interrogate receptor systems, it also moved research toward a more reductionistic approach. Thus data from studies on proteins produced by transfecting cDNA into cell lines whose heritage derived from human tumors, that lacked any semblance of the natural milieu of the native drug target [98] were complemented by in silico computer assisted molecular design (CAMD) activities, to predict protein behaviors in the intact animal and human with somewhat mixed results that were usually hindsight in nature. The evolution of the receptor concept is dealt with briefly below with the reader directed to more comprehensive reviews for additional detail [59–61,69,70,72,98–100,102].

2.4.1. Occupancy theory

The basic feature of the occupancy theory proposed by Clark [76] was that an agonist-induced tissue response was a function of the number of receptors occupied by the agonist that in turn was related to the agonist concentration used. This assumed that: (i) the RL complex formation was reversible; (ii) the association of the receptor with the ligand to form the RL complex was a bimolecular event with dissociation being a monomolecular process; (iii) all the receptors in a given biological system were equivalent to each other and able to bind the ligand independently of one another; (iv) formation of the RL complex did not alter the free (F) concentration of the ligand or the affinity of the receptor for the ligand; (v) the response elicited due to receptor occupancy was directly proportional to the number of receptors occupied; and (vi) the biological response was dependent on an equilibrium being obtained between R and L. Occupation of the receptor/drug target by an antagonist, a ligand with zero efficacy [65,89] which could block the functional response of an agonist could be overcome by increasing the agonist concentration if its actions were competitive in nature. Non-competitive antagonists acting at allosteric sites or that bound irreversibly to the agonist (orthosteric) site could be differentiated from a competitive antagonist using a Schild analysis [78].

From the effects of a series of cholinergic agonists in skeletal muscle not all of which were able to elicit a maximal response even at supramaximal concentration, Ariens modified occupancy theory to include the concept of the *intrinsic activity* (IA) of a ligand [79] where a full agonist had an IA value of 1.0 and an antagonist an IA of zero. Compounds that bound to the receptor and were only able to produce a portion of the response seen with a full agonist were defined as *partial agonists* that, by definition, were also *partial antagonists*. Agonists have also been identified that can produce a response greater than that of a "gold standard" full agonist and have been termed "super agonists" [101].

The partial agonist concept was modified further by Stephenson [81] in introducing the concept of *efficacy*, ε. This differed from IA where the latter was defined as a fraction of the maximal response while efficacy related to situations where a maximal agonist response occurred in a tissue when only a small portion of the total receptor number were occupied. This phenomenon has accordingly been described in terms of *spare receptors* and *receptor reserve* and could be measured in situations where receptors were inactivated by selective alkylating agents revealing a non-linear occupancy/efficacy relationship. The concept of spare receptors while experimentally demonstrated is a difficult concept to appreciate in everyday use. For instance, how are spare receptors practically addressed from a medicinal chemistry perspective? Thus the common day usage of the term efficacy, has become rightly or wrongly, the magnitude of a response relative to other compounds, be these agonists, partial agonists or super agonists.

Additional data showing the persistence of antagonist-mediated responses and agonist "fade" where transient maximal responses were followed by lesser responses of longer duration and agonist-mediated blockade of agonist effects, led Paton [85] to add a chemically-based rate term to the occupancy concept. In *rate theory*, the response was determined not only the number of receptors occupied by a ligand, but also the *rate* of RL formation. The latter was described in quantal terms in terms of discrete "all or none" changes for receptor-mediated events with the RL formation rate being the primary factor delineating occupancy and the dissociation rate constant representing the residence time [67,103]. Per occupancy theory, ligand-mediated responses in a tissue can be described in terms of six parameters: (i) receptor density; (ii) bias/pleiotropy in the receptor interaction with and multiple cellular signaling proteins [71]; (iii) efficiency of the transduction process; (iv) the equilibrium dissociation constant of the RL complex; (i) the IA or efficacy of the ligand at the receptor and (vi) in vivo, the PK profile of the ligand including its residence time [67,103].

2.4.2. The ternary complex model (TCM)

The ternary complex model (TCM) of receptor function, the foundation of GPCR-based research [93,94], reflects concepts from studies of induced-fit/cooperative interactions in multimeric protein complexes including hemoglobin and enzymes [90–92]. The hydrolysis of guanine nucleotides altered agonist binding to GPCRs and led to the conceptualization of the three-component TCM model. The latter has as its basis the establishment of an equilibrium on the one hand between the ligand-bound receptor and free G-protein, and on the other the receptor, ligand, G-protein complex. While originating from agonist binding/guanine nucleotide interactions, the TCM concept has been extended to allosteric interactions between orthosteric and allosteric sites present on a single protein monomer and to other two-state interactions involving sites on adjacent proteins [70,100].

2.4.3. Constitutive receptor activity

As noted, the basic concept in receptor theory is that a ligand binds to and activates a receptor, the effect produced by the ligand being proportional to the concentration of the ligand, following the LMA. Receptors can also spontaneously form active complexes as a result of interactions with other proteins in the absence of any ligand, an event termed *constitutive activity* [68,69] that occurs with both GPCRs [104] and ion channels [105]. At one level, constitutive activity can be manifest when receptor cDNA is *overexpressed* in a transfected cell system such that the relative abundance of receptor protein is in excess of that normally occurring in the native state. It can then associate with proteins that reflect the atypical milieu in which the receptor is expressed, rather than the interactions that occur for a receptor in its natural environment, or with proteins that in their natural stochiometry to the receptor would not interact with it [98]. More importantly, this phenomenon can also occur in intact, native tissues and can reflect basal activity in a normally quiescent system as well as that resulting from the homeostatic dysfunction occurring with disease and aging. This suggests that receptors are normally in a constitutively active state and that this activity, under normal homeostatic conditions, e.g., in the absence of disease, trauma or aging, is attenuated by other cellular factors. This may explain why the majority of drugs acting via receptors function as antagonists. Constitutive receptor activation involves *allosteric transition* [69,106] that occurs in the context of *protein ensemble* theory [107–109] where changes in receptor conformation can occur through random thermal events [108] that lead to spontaneous activation in a "non-ligand bound" receptor.

2.4.4. Regulation of receptor function

Receptors are highly dynamic entities that are, under normal conditions, subject to a variety of regulatory processes that involve the location of the receptor, its association with other proteins, including other receptors, and the degree of coupling occuring with

multiple signaling pathways. While much of the existing literature reflects studies on GPCRs, there are similar themes emerging with other drug targets including receptor tyrosine kinases (RTKs), LGICs and transporters that suggest, to the extent which it has been determined, what has been observed with GPCRs may be applicable to all drug targets. This is an area of intense research with considerable and evolving complexity. Accordingly, the reader is referred to additional reviews for more detail [72,100,110–114].

2.4.4.1. Receptor desensitization/tachyphylaxis/tolerance. Agonist activation of a receptor can be endogenously regulated by desensitization of the receptor, a phenomenon variously known as tachyphylaxis, desensitization or tolerance where cellular changes involving both alterations in the receptor and/or its associated signaling pathways can attenuate the magnitude and duration of signaling causing the cell to enter a refractory, unresponsive state as a result of sustained stimulation thus preventing the cell from over-responding to an agonist. A classical example of this phenomenon is morphine-induced tolerance where the analgesic response to the opioid in both animals and humans undergoes a progressive reduction with repeated exposure thus necessitating an increase in dose (and side effects) to achieve its desired effect. Evaluating the occurrence of tolerance is a critical event in assessing the attractiveness of novel agonist NCEs for the treatment of pain [115].

The mechanisms for tolerance involve receptor internalization and recycling, the precise mechanism(s) of which has been the subject of controversy for many years [116]. This can involve receptor dimerization [117], the β-arrestin-GPCR kinase (GRK) pathway [111,115] and other kinase-mediated mechanisms [115]. Receptor desensitization thus reflects a situation where ligand efficacy is a direct function of the state of the receptor and of its prior history in terms of exposure to agonists, a state-dependent situation.

2.4.4.2. Dimerization/oligomerization. Oligomerization is a fundamental regulatory mechanism for GPCRs [118–120], receptor protein-tyrosine kinases (RPTKs) [121–124] and enzymes [125]. It can involve both homo- (the same proteins) and hetero-oligomeric interactions, the latter involving different proteins from the same drug target/receptor family or different receptor classes, proteins, chaperones, etc. and can be ligand-dependent, allosteric or occur in the absence of ligand [126,127], the latter representing a constitutive response. The functional consequences of receptor oligomerization, its stochiometry and equilbrium [128], is varied and can involve activation, modulation, biogenesis and translocation of the target protein.

2.4.4.3. Receptor endocytosis. The presence of receptors or other drug targets, whether on the cell surface or in discrete intracellular organelles (e.g., mitochondria), allows a cell to respond to its milieu in order to maintain homeostasis. The trafficking of a receptor, either on its own or occupied/activated by a ligand, away from the cell surface or from its normal organelle location can obviously alter its responsiveness and function. For instance, binding of insulin to its receptor results in the receptor–hormone complex being rapidly internalized into the cell, a phosphorylation-dependent event that results in proteolysis of the complex leading to receptor down-regulation and a reduced responsiveness of the tissue to the hormone [129]. The latter appears to be a generic event [130] common to GPCRs and involves GRKs, arrestins and other cellular proteins [131–133]. Internalization of the insulin receptor can also result in the activated receptor interacting with intracellular proteins to pleiotropically modulate cellular signaling events [133] acting in this particular instance as a mitogen [134]. In addition to the GPCRs and RTKs, LGICs can also undergo extensive trafficking that alters neuronal function [135,136].

2.4.4.4. Pluridimensional efficacy. Recent evidence for biased G-protein/β-arrestin ligands with "pluridimensional" efficacy [137,138] has altered the basic concept of ligand efficacy [72,137] and of ligand characterization with the latter being viewed as dependent on the receptor/G-protein, GRK/arrestin repertoire present within a cell, as well as the pathway used to experimentally measure efficacy and the prior events to which a receptor was exposed. Receptors can thus be promiscuous in their pathway and ancillary protein interactions with ligands having dual, and even opposite (e.g., agonist and antagonist), efficacy effects depending on the conformation of the receptor stabilized by a particular ligand [137] and its associated pathway events.

This concept has major ramifications in compound assessment in the drug discovery setting where the efficacy resulting from the ligand receptor interaction may be disease-dependent. Assessing the effects of a ligand in "normal" tissue may therefore lead to incorrect and misleading information. GPCRs can engage both G-protein dependent [113] and independent, e.g., MAPK (mitogen-activated protein kinase) and arrestin cascades, pathways leading to phenomena such as ligand directed trafficking of receptor signaling (LDTRS), the latter a function of the duration of agonist action [137].

The efficacy bias factor [113] of a ligand has the potential to explain unexpected clinical results [138,139] and also adds an additional confound in facilitating the translational process [140].

2.4.4.5. Signal transduction-β-arrestin-GPCR kinase (GRK) modulation. The arrestins are adaptor proteins that regulate GPCR function and represent an alternate pathway to the G-protein-dependent pathway [113]. Of the four mammalian arrestins, 1–4 [133], 1 and 4 are localized in the retina and act to quench phototransduction. Arrestins 2 and 3 are ubiquitously distributed and modulate GPCR trafficking and signaling. The GRKs, of which seven (GRK 1–7) have been identified [112] are involved in attenuating agonist-induced GPCR activation and in mediating receptor desensitization and trafficking. Mechanistically, GRK-mediated phosphorylation of a GPCR facilitates arrestin binding to block agonist-initiated events and mediate receptor desensitization [113,114].

β-Arrestins can also function as scaffold proteins [141] interacting with a variety of other proteins involved in signaling events which are both G-protein-dependent and -independent. These proteins include the G proteins, Gαγ and Gβγ, the non-receptor proteins, RKIP (Raf kinase inhibitor protein), PI3K (Phosphatidylinositol 3-kinase), GIT 1 and 2 (GRK interactors 1 and 2), that contain multiple domains that can interact with the GTPases like ARF, Rac and cdc42, MEK and PAK kinases, the Rho family GEF PIX, and the focal adhesion protein, paxillin Akt (Protein Kinase B), MEK (MAP kinase kinase), the guanine-nucleotide-exchange factor (GEF) EPAC (exchange protein directly activated by cAMP), AP-2/clathrin and caveolin as well as tubulin, α- and β-synuclein, Smad and the GPCR, Smoothened (Smo), the latter of which is involved in the hedgehog signaling pathway [139]. This has led to the suggestion of the possible existence of a GRK "interactome" [142]. Other proteins regulated via the GPCR/arrestin/G-protein/GRK axis include the MAP kinases, ERK (Extracellular signal-regulated protein kinase), JNK (c-Jun N-terminal kinases), SAPK (Stress-Activated Protein Kinase) and p38, regulators of G-protein signaling (RGS [143]) and G-protein signaling modulators (GPSMs)/activators of G-protein signaling (AGS [144,145]).

Ligands have been identified that differentiate between the G-protein- and β-arrestin-dependent pathways [113] with the β-blocker, carvediol being the seminal biased ligand that engages the β-arrestin-dependent pathway [146]. The latter appears explain its improved efficacy in the treatment of heart failure [147]. A number of studies, the majority involving knockout mice, have established a role for GRK-arrestin mechanisms in lung disease,

analgesia, autoimmune diseases, lipid and bone mineral homeostasis, cancer metastasis, cognition, cell motility and proliferation, chemotaxis, cell survival and autophagy [138,140] and in regulating chemokine-mediated inflammatory processes [148].

2.4.5. Receptor complexes and allosteric modulation

Classical receptor theory generally assumed that affinity and efficacy were independent parameters with no consistent or necessary relationship between the affinity of a ligand and its ability to elicit a full response [81,89]. Thus a ligand with relatively low affinity, e.g. K_i = 1 μM, could still act as a full agonist as a result of its intrinsic activity or efficacy. It has however been argued that a lack of a consistent relationship between potency/occupancy and efficacy is more reflective of an inability to measure receptor-mediated activity than a potency disconnect related to a compound [98,100] with all ligands capable of demonstrating some type of efficacy if tested in an appropriate system that takes into account the possible presence of allosterism, constitutive activity and pluridimensional bias [70,71,100,137,138].

2.4.5.1. Allosterism. Interactions between allosteric and orthosteric binding sites either on a single protein or on adjacent proteins can be mediated via a cooperative, conformational change in the binding protein for the second ligand from a site adjacent to the ligand recognition site building off of the allosteric models of Koshland, Nemethy and Filmer (*sequential or induced fit* [92]) and Monod, Wyman and Changeux (*concerted model* [91]). For receptors, the effect of an allosteric ligand on the affinity of its cognate orthosteric ligand involves the allosteric TCM model [93,94,149] and incorporates a cooperativity factor (α) acting as a multiplier to modify the dissociation constant of the ligand at the orthosteric site. For α values less than 1, the allosteric ligand decreases the affinity for the orthosteric ligand and is thus an *allosteric inhibitor* or negative *allosteric modulator (NAM)*. When α is greater than 1, the allosteric ligand produces an increase in affinity for the orthosteric ligand, and thus is termed an *allosteric potentiator* or *positive allosteric modulator (PAM)*. If α = 1, there is no effect of the allosteric ligand on the affinity for the orthosteric ligand and the compound is termed a *neutral allosteric ligand*. Some forms of neutral allosteric ligand, e.g. for mGluR5 [150] while neither activating or inactivating the GPCR in the presence or absence of the orthosteric agonist can block the activity of both PAMs and NAMs by occupying the allosteric site – a phenomenon described as *neutral cooperativity* or *pharmacological silence*.

An allosteric two-state model has been proposed that incorporates an additional cooperativity factor governing the transition of the receptor between active and inactive states in the presence of an allosteric ligand [106,151]. Serendipity has played a major role in the identification of allosteric ligands with either positive or negative effects on the function of the orthosteric site. These modulators have three potential advantages over drugs that act via orthosteric sites in that their effects are:

(i) *saturable* – with a ceiling effect to activity that can provide a good margin of safety for human use;
(ii) *selective* – as their binding sites are distinct from the orthosteric site and their effects depend on the degree of cooperativity between the allosteric and orthosteric sites.
(iii) "*use-dependent*" – with the actions of an allosteric modulator occurring *only* when the endogenous orthosteric ligand is present. In the absence of the latter, an allosteric modulator is theoretically quiescent and may thus represent an ideal prophylactic treatment for disease states associated with sporadic or chronotropic receptor-mediated signaling dysfunction.

The first drug identified as an allosteric modulator was the benzodiazepine, diazepam that produces its therapeutic effects by facilitating the actions of GABA at the $GABA_A$ receptor [152]. This allosteric modulator, unlike directly acting $GABA_A$ receptor agonists like gaboxadol [153] or indirect $GABA_A$ receptor agonists like the GABA uptake inhibitor, tiagabine [154], has a relatively benign side effect profile.

2.4.5.2. Receptor multimers. As receptor theory evolved, it was thought that a receptor-mediated response was a predictable, linear process that involved ligand-induced activation of a protein monomer and its signal transduction pathway independently, or with minimal influence, from other membrane proteins. It has become increasingly evident that receptors can physically interact both with one another and with other membrane proteins (see Sections 2.4.4.2 and 2.4.4.4) with numerous examples existing of receptor co-expression and interactions [155,156]. These interactions, especially those in cells of the immune system, were often necessary to produce functional receptors on the cell surface and also, via protein partner interactions, to modulate the function of the entire signaling complex related to those receptors. The functional integration of the effects of multiple signal transduction pathways can affect receptor function in a cell-specific manner (e.g., receptor cross talk) adding considerably to the complexity of the downstream signaling events associated with receptor activation. Examples of the complexity of receptor signaling at the postsynaptic level include the NMDA receptor where some 70 proteins other than the receptor are potentially involved in the function of the receptor complex [157] and the ATP-sensitive $P2X_7$ LGIC (ligand gated ion channel) receptor, with a signaling complex comprising some 11 proteins that include laminin β-3, supervillin, integrin 2, β-actin, MAGuK (membrane-associated guanylate kinase), various heat shock proteins, phosphatidylinositol 4-kinase and the receptor protein tyrosine phosphatase (RPTP) [158].

As more is learnt regarding receptor function, the more one can appreciate the prescience of the 19th Century pioneers in their conception of receptive substances and evolution of receptor theory.

3. The biochemical era in pharmacology

As receptor theories percolated through the 20th Century into the 21st, the technologies used to characterize receptor function and efforts to reduce the concept to practice led to the biochemical era of pharmacology, the question being was the elegance of receptor theory supported by the existence of a tangible target – the actual receptor?

3.1. Receptor isolation

In the early days of "receptorology" at the beginning of the 20th Century, the distinguished pharmacologist, H.H. Dale expressed considerable skepticism regarding the existence of specific receptive substances or receptors, on target tissues. Instead, he suggested that observed drug/compound actions might be due to "distributive phenomena" with compound selectivity being a function of the ease with which such substances reached their site of action [159]. The existence of receptors, defined as a "a cellular macromolecule, or an assembly of macromolecules, that is concerned directly and specifically in chemical signaling between and within cells" [160], as tangible entities remained elusive for the next 60 years. This prompted both wistfulness, as expressed by De Jongh – "To most of the modern pharmacologists the receptor is like a beautiful but remote lady. He has written her many a letter and quite often she has answered the letters. From these answers the pharmacologist has built himself an image of this fair lady. He

cannot, however, truly claim ever to have seen her, although one day he may do so" [161] – and continuing skepticism. Thus another distinguished pharmacologist, Ahlquist, in 1973 made the following comment on the physical existence of receptors following his seminal work on classifying α and β adrenoceptors (see Section 3.2) – "This would be true if I were so presumptuous as to believe that α and β receptors really did exist. There are those that think so and even propose to describe their intimate structure. To me they are an abstract concept conceived to explain observed responses of tissues produced by chemicals of various structure" [162]. Nonetheless, others were more convinced that tangible entities did indeed exist with Sutherland, Robinson and Butcher proposing that the β-adrenoceptor and the enzyme responsible for cAMP production, adenylyl cyclase, were one and the same entity [163].

As the debate on the actual existence of receptors continued, studies that had been initiated in the mid 1930s [164,165] led to the functional identification of cholinergic receptors in mouse diaphragm using [^{14}C]-curarine [166] with the isolation of nicotinic acetylcholine receptors (nAChRs) from the electric end organs of *Electrophorus* and *Torpedo* occurring in the early 1970s using receptor-selective toxins as affinity labels [167] – "a landmark in the history of pharmacology" [168] – as well as the isolation of the seminal GPCR, rhodopsin [169].

With the proven utility of affinity labeling as a means to isolate receptors [170], the next receptor to be isolated was the β-adrenoceptor [171] followed by other members of the adrenoceptor family [172] and, numerous other receptors, a task that while instrumental in revolutionizing the study of receptor function did not always follow a predictable route to a successful outcome. However, the debate regarding the existence of receptors had now moved from the hypothetical to the substantive with the next steps being the development of receptor binding assays (see Section 3.3), receptor cloning, expression and mutagenesis (see Section 4.3).

3.2. Receptor subtypes

The landmark publication in 1948 from Ahlquist [80] provided a robust dataset to support the existence of pharmacologically distinct α- and β-adrenoceptors that led to the cardiac β-adrenoceptor being identified as being involved in cardiac excitation. While the hypothesis presented in Ahlquist's paper has been widely acknowledged as being responsible for the development of the β-adrenoceptor blocker, propranolol k [173], it was rejected for publication in the pharmacological literature in 1948 [174] and instead published in a leading physiological journal and then subsequently ignored. This was attributed by Black to receptor theory at that time being "too esoteric" as well as to an "absence pharmacological taxonomy" [173]. While Ahlquist's hypothesis was generated using classical pharmacological techniques, it signaled the beginning of the biochemical era of pharmacology with receptors not only being viewed as unique entities modulating cellular homeostasis but also as primary targets for drug discovery.

3.3. Receptor binding assays

The use of affinity ligands to isolate the nAChR [164] had been preceded by the development of binding assays for receptors using radioactive ligands. This was a technique developed for the immunoassay of insulin [175] by Yalow in 1960 that was extended to the study of receptors for TSH [176], ACTH [177], insulin [178], the β-adrenoceptor [179] and the opioid receptor [180,181], the latter of which was facilitated by the simultaneous efforts of several groups [182,183]. While the technology behind measuring receptor presence using radioligand binding appeared obvious in retrospect, this was not always the case [182] and necessitated both an appreciation of "tricks of the trade" as well as the validation of the experimental findings using a series of required benchmarks that had been developed with the success of the insulin and opioid binding assays [102]. These followed from the basic concepts of receptor theory (see Section 2) and include [184]:

(i) *Saturability* – as there are a finite number of receptors on the cell surface, a concentration-response curve for ligand binding should be saturable.
(ii) *High affinity* – the radioligand, usually a drug should bind with an affinity (K_d) in the subnanomolar – 100 nM range.
(iii) *Specificity* – binding of the radioligand to the receptor or drug target site (*specific binding*) should be 60–70% or greater of the total radioactivity bound in an experiment to avoid complications from the binding that occurs non-specifically, e.g. absorption to membrane proteins or to assay components. The identification of methods like vacuum filtration, to rapidly isolate the bound fraction of the radioactivity [178,180,181], were a major advance in being able to increase the radioactive "signal to noise" ratio that then allowed the use of lower radioligand concentrations to specifically label the receptor. Early studies that attempted to label opioid receptor(s) resulted in specific binding of only 2% with 98% of the radioactivity bound being non-specific, making the assay technically unworkable [182] but setting the stage for subsequent work [183].
(iv) *Reversibility* – since the RL complex formation by definition is reversible, binding of the ligand should also be reversible as assessed in washout experiments using a high concentration of unlabeled ligand.
(v) *Pharmacologically relevant* – for a given type of receptor, binding should be displaceable by known agonists and antagonists of the receptor. For the nAChR, binding of a specific ligand, e.g. [^{3}H]-epibatidine [185] should be displaceable by the nAChR agonists, cytisine, anatoxin-a, and ABT-418 and by the antagonists, mecamylamine, dihydro-β-erythroidine, methylcaconitine and α-conotoxin but not by pilocarpine (muscarinic receptor agonist), GABA (GABA receptor agonist), diazepam (GABA receptor modulator), propranolol (β-receptor antagonist), caffeine (adenosine receptor antagonist), fluoxetine (5-HT uptake inhibitor) and so on. Binding should also be stereoselective with the component enantiomers of a receptor selective ligand showing differences in binding affinity. As an example, using [^{3}H]-CPP to label N-methyl-D-aspartate receptors, the L-isomer of glutamate has 49-fold greater affinity for the NMDA receptor (IC_{50} = 145 nM) than its D-isomer (IC_{50} = 7110 nM) [186].

The successful development of the receptor binding technique [102,178] in addition to allowing for the facile biochemical characterization of receptors resulted in an explosion in research to investigate receptors for different neurotransmitters and their subtypes. This included an ability to map receptor location/density in tissues using autoradiographical techniques, to measure receptor trafficking, expression, internalization, and receptor engagement. Additionally, receptors that had not been physically isolated could be identified and characterized in tissues enriched in the receptor, to search for potential endogenous receptor ligands for drug receptors, and to rapidly screen small amounts (5–20 mg) of both known drugs and NCEs to establish their receptor binding profiles.

3.3.1. Neurotransmitter binding assays

Following from the success in developing the opioid receptor binding assay [180,181], Snyder's group then established binding assays for dopamine [187], GABA [188], glycine [189] muscarinic cholinergic [190], 5-HT [191,192] and adrenergic receptors [193], the latter concomitantly with studies ongoing in Lefkowitz's group [194].

The approach taken by the Snyder group, in addition to providing a detailed characterization of each receptor, was uniquely focused on the utility of binding studies to better understand the actions of known drugs. This approach, which demonstrated the utility of using binding assays to answer key pharmacological questions, was instrumental in attracting the interest of medicinal chemists to their potential in enabling the drug discovery process where the structure activity relationship (SAR) for a series of ligands could be rapidly derived (days instead of weeks) independent of the ADME (absorption, distribution, metabolism, and excretion) issues occurring in vivo and with a fraction of the amount of compound required for tissue and in vivo studies.

As one example, a dopamine binding assay that used both agonist ([^{3}H]-dopamine) and antagonist ([^{3}H] haloperidol) ligands [187] demonstrated that the binding affinity for 25 clinically approved antipsychotic drugs (neuroleptics) correlated better with binding of the antagonist than the agonist ligand. This discrepancy eventually aided in the identification of five discrete members of the dopamine receptor family, D_1–D_5, with the clinical effect of the antipsychotics being correlated with affinity for the D_2 receptor among others [195].

While the initial binding assays were focused on GPCRs, a receptor superfamily that now numbers in the region of 800 distinct members [95], the basic technological approach was applicable to the majority of drug targets, e.g., ion channels, enzymes, transporters, etc. where a suitable radioactive probe that had high affinity and was selective for the target could be used to label either orthosteric and/or allosteric sites. Newer technologies have been developed using more efficient, real time detection systems that involve whole cell assays using less or no radioactivity (e.g., label free), e.g., scintillation proximity, fluorescence (FRET) and bioluminescence (BRET) resonance energy transfer assays [196] as well as in vivo opsin-based optical approaches [197,198].

Despite the methodology being relatively straightforward, having a radiolabeled ligand and a suitable tissue source was no guarantee that a receptor-binding assay would work, let alone be suitable for the pharmacological characterization of receptors. Assays for some ligands for reasons unknown, could not be reduced to practice. Thus ligands that could bind with high affinity (e.g., nanomolar) in an established binding assay, when radiolabeled themselves demonstrated minimal specific binding making them unusable. Few of these outcomes see light of day in the peer-reviewed literature, as there is little incentive to complete studies that are inconclusive and often unpublishable. One example of a problematic binding assay is the use of 2-chloro [^{3}H]-adenosine ([^{3}H]-2-CADO) as a ligand for the P1-adenosine receptor [199]. The "trick of the trade" to make this agonist ligand-based assay work was the removal of the considerable amounts of endogenous adenosine present in brain homogenates that obscured receptor binding [200]. Nonetheless, after generating the published data [199], the assay became less and less robust until it proved impossible to detect useful specific binding – for reasons unknown (E.A. Rilsey and M. Williams, unpublished data).

3.3.2. Autoradiographical techniques

With the radioligand binding approach, the presence of receptors and their relative density could be measured in intact tissues at both the cell and tissue level using intact, labeled tissue sections exposed to energy sensitive film [201] leading to binding studies that measured the density of multiple receptors in postmortem human brains from patients with diverse CNS disease states including depression/suicide [202], schizophrenia [203], Alzheimer's disease [204], etc., Disease-related alterations in receptor binding have also been reported in cardiac and vascular disease [205,206] and diabetes [207]. While the impact of many of these studies proved to be both intellectually and visually interesting and/or data rich, they were less useful than anticipated due to many conflicting reports that failed to replicate the initial findings. For the CNS studies, this may have been a reflection of the source, age and state of deterioration of the human brain tissue used (shrinking of brain tissue being a hallmark of the aging process), an inaccurate/inappropriate diagnosis for inclusion in a designated disease database, disease co-morbidities, prior prescription drug treatment and how "normal" were the control tissues used to compare the diseased samples.

3.3.3. Drug mechanism(s) of action; drug receptors

Receptor binding assays also provided a facile means to explore the mechanism of action of known drugs, the majority of which had entered into human usage via serendipitous observations of phenotypic activity both in animal models and in the clinic. Thus multiple papers were published documenting the interaction of drug X at receptor R being taken as definitive proof for the mechanism of action of compound X, to be followed by additional reports that suggested that the mechanism of action of compound X was due to its interactions at receptors X, or Y, or Z. It became difficult, assuming these interactions could be replicated, to determine whether the activity of a compound could be ascribed to a single receptor target or was the result of functional synergies between different receptors, related to the potential for side effects or due to an artifact, e.g., talc, silica, etc. [208]. In the absence of a rigorous kinetic and pharmacological evaluation, not all documented binding sites proved to be biologically relevant.

3.3.3.1. Clozapine. Clozapine is the seminal atypical antipsychotic that despite significant side effects, e.g., agranulocytosis, remains the most effective of the compounds in its therapeutic class, being used for the treatment of resistant schizophrenics or those intolerant of conventional antipsychotic medications [209]. For the better part of 50 years efforts to find a safer version of clozapine have been unsuccessful. Knowledge of a defined mechanism of action for clozapine would clearly aid in this process. However, despite knowledge that its therapeutic efficacy involves antagonism of both D_2 and 5-HT_2 receptors, the molecular causes for both its superiority to other antipsychotics and its unique side effect profile remain unknown and may involve effects on a combination of targets making clozapine the ultimate "magic shotgun" [210], the properties of which have been difficult to replicate in a new molecule despite considerable efforts. Clozapine is also a histamine H4 receptor agonist [211], which may lead to immunomodulatory effects [212] that contribute to its ability to produce agranulocytosis.

3.3.3.2. Diazepam. The benzodiazepines (BZs) represent the main class of anti-anxiety (anxiolytic) agents that were introduced into clinical use with no known mechanism of action. With the development of binding assays, [^{3}H]-diazepam was used to identify the BZ receptor, a modulatory site on the $GABA_A$ ion channel as the site of action of BZs [152,213]. Despite considerable efforts, convincing evidence for the existence of an endogenous ligand for the BZ, a natural anxiolytic, has remained elusive making the BZ receptor like the majority of allosteric binding sites, by definition, an orphan drug target (see Section 4.2).

3.3.3.3. Receptor binding profile. Using a panel of binding assays (100+) in vitro, a drug or NCE can be assessed for their putative mechanism of action or target interaction properties, respectively [214,215]. This can provide information on the target profile of the drug or NCE, information that can be used for the additional profiling of the compound for efficacy and its potential safety liability [216,217]; e.g. binding to the hERG channel prior to more expensive electrophysiological studies [218]. These binding profiles are conducted most cost effectively in laboratories dedicated to high through screening of multiple receptors/drug targets, e.g., contract research organizations like Cerep and Eurofins Panlabs, the NIMH Psychoactive Drug Screening Program or the NIH Biomolecular Screening and Profiling effort at the NIH Chemical Genomics Center [219,220]. The results of these broad-based screening profiles are routinely included in the peer-reviewed literature and in IND (Investigational New Drug) or CTA (Clinical Trial Application) applications to regulatory authorities for potential drug candidates. Comparing a receptor binding profile for an NCE with those already derived for other drugs or known compounds e.g., using a BioPrint profile http://www.cerep.fr/cerep/users/pages/productsservices/bioprintservices.asp,can provide a preliminary assessment of the potential functional activity and safety of an NCE.

3.3.4. Compound screening

With the development of binding assays and iterations in the detection technologies, the throughput in compound evaluation increased exponentially. Where grams of an NCE were required when compounds could only be tested in whole animals or tissue baths, now 5–20 mg of compound, became sufficient [221]. Similarly, while testing a compound in vivo could take 4–6 weeks with the expense of using many animals, binding assays provided replicate data in days with minimal animal usage especially when using transfected cell lines.

At the inception of compound screening approaches in the late 1970–1980s, individual experiments involved some 200–500 test tubes or "reactions" that could be used to assess the activity of 10–20 NCEs/compounds at 5 concentrations in triplicate (with appropriate controls) in a day. With the advent of combinatorial/parallel synthesis chemistries [222,223], where millions of compounds were synthesized in low quantities as combinatorial or parallel chemical libraries, enhancements in automation and detection systems were required. These high throughput screens then allowed tens of thousands to over 100,000 compounds to be assayed per day [224] using techniques like *ultra* high throughput screening (uHTS) and gel permeation/sheet screening [224,225]. A more recent iteration of a uHTS approach, in this instance studying enzyme mutants rather than compounds, oil droplets were used in place of microtiter plate vessels resulting in an assay where 100 million reactions could be run in 10 h "with a 1000-fold increase in speed and a 1-million-fold reduction in cost" [226]. The practical utility of such screens, especially in the area of drug discovery, became debatable as the data output easily overwhelmed the ability to productively analyze it such that HTS became widely viewed as "anti-intellectual and irrational" [223]. Additionally, many of the compounds amenable to synthesis using combinatorial/parallel technologies were far from drug-like in their physical properties [227] leading to an increased focus on "biology oriented synthesis" [222] with quality belatedly replacing quantity.

There is subtle but important distinction between HTS and *high content screening* (HCS). HTS is viewed as a high throughput, low content science while retains aspects of the high throughput component but generally tends to gather more detailed information [30]. The latter is usually associated with functional phenotypic- [228], fluorescence-microscopy [227], ion channel-based electrophysiology [229] and fluorescent protein [230] technologies.

3.4. Biochemical pharmacology – the first circle of reductionism

While binding assays greatly facilitated the characterization of receptors and other drug targets as well as drug/compound properties using biochemical approaches that had been used to study enzymes and develop immunoassays, they also contributed to the first wave of overt reductionism in pharmacology.

Thus to many scientists, a compound that bound with high affinity to a drug target was viewed as being 70% of the way to becoming a drug, while others viewed a compound that was specifically designed and synthesized to be active at a given target being, by definition, selective, with data from the single assay used fulfilling that expectation. There was often little appreciation of the many other aspects of preclinical compound evaluation like efficacy and ADME. Both groups were then surprised when subsequent testing of "selective binding hits" resulted in a demonstrated lack of selectivity/efficacy/drug-like properties in these compounds with many being inherently unstable, rapidly metabolized, having poor bioavailability and half-lives, and potentially toxicities.

As receptor binding became the major interface between medicinal chemistry efforts and biological testing, representing the initial step of compound evaluation, with the ability to rapidly develop SARs independent of in vivo confounds, there was greater interest and priority in using receptor-binding and receptor-mediated signaling events such that classical in vivo animal testing became increasingly relegated to later – or often the penultimate – stages of the preclinical evaluation schema. As a result, both the skill sets necessary to run these animal models of putative efficacy and their capacity were diminished and/or in limited supply [7].

4. The molecular phase of pharmacology

With the ability to isolate receptors in milligram quantities and the subsequent demonstration of activity and specific pharmacology following reconstitution in phospholipid membranes in the presence of ancillary proteins (e.g., G-proteins and adenylyl cyclase for GPCRs) [231], the next step, given the newly available microsequencing tools of molecular biology was to clone and express receptors in cell lines. This provided greater amounts of protein for additional study. Cloning was conducted using protein purification based on the primary peptide sequences from isolated receptors and was complimented by developments in homology cloning involving screening with oligonucleotides, DNA fragments or polymerase chain reaction (PCR) products and by expression cloning based on function, ligand binding, antibody recognition and differential display techniques [232,233], an approach sometimes referred to as *reverse pharmacology*.

4.1. Cloning

With the ability to clone receptors as well as other drug targets, pharmacologists focused these new technologies on the cloning of receptor/drug target families that reflected their ongoing research interests such that certain receptor classes became associated with specific research groups, e.g. Lefkowitz and Kolbika were awarded the 2012 Nobel prize for their work primarily on adrenoceptors [86]. Beginning in the early 1980s, subunits of the nAChR [234], rhodopsin [235] and the GPCR β-adrenoceptor [236] were cloned. For the next two decades, cloning efforts continued unabated and, via the use of homology screening approaches, e.g., low-stringency hybridization and degenerate PCR, resulted in the identification of a large number of receptors and drug targets that in turn have led to the discovery of many new subtypes and also a number of orphan receptors – "receptors for which no ligand is known" [237]. Often, cloning showed a lack of sequence homology between

species which often correlated with differences in the pharmacology of a given receptor between species, e.g., rat and human, leading to cloning and expression of the human receptor in cell lines and transgenic mice for both basic research and drug discovery activities.

Despite the enormous literature on receptor cloning, the technology, including receptor expression and like binding assays, was often not always as straightforward as it may have seemed. There were major challenges in finding the right conditions and tools to clone a receptor. As an example, the identification and cloning of the sigma receptor, a binding site for certain opioid analogs and antipsychotics that could be pharmacologically divided into σ_1 and σ_2 receptors proved difficult. The σ_1 receptor was eventually cloned from guinea pig [238] and rat brain [239] and found to have a rather unusual structure with a single putative transmembrane domain (as contrasted to the 7-transmembrane (7-TM) motif of the GPCRs). It was subsequently determined to be a ligand-regulated molecular chaperone present in the endoplasmic reticulum [240]. The σ_2 receptor has yet to be cloned although its binding domain has been identified as part of the PGRMC1 (progesterone receptor membrane component 1) protein complex, a tumor biomarker [241].

Finding the ideal conditions for a receptor protein to be expressed can also involve considerable trial and error efforts especially if the functional receptor requires posttranslational modification, e.g., phosphorylation, glycosylation, etc. that can provide the necessary conditions to enable the correct folding of the native protein to form its active state and permit its transport and expression, where appropriate, on the cell surface. These considerations became exponentially more challenging as the structural complexity of the target protein(s) increased. As an example, it took well over 2 years to identify the conditions necessary for the stable, functional expression of subunits for the pentameric $\alpha_4\beta_2$ nAChR [242].

4.2. Orphan receptors

Orphan receptors identified by homology screening and bioinformatic analyses and subsequently cloned have been used as baits to identify their cognate ligands, e.g., novel neuromodulators which had been previously unsuspected, e.g., hydoxycarboxylic acid, free fatty acids, oxolglutarate, resolvin, chemerin, etc. [243]. These pairings provided not only additional insights into the complexities of tissue function and homeostasis [244] but potential new drug targets.

The orphan receptors identified to date are primarily GPCRs [244,245] and nuclear receptors [237], the former being the larger group of the two ($\sim$ 130 members) with the latter comprising approximately 30 members. As a group, GPCRs were originally classified into 6 classes based on sequence homology and functional similarity. These were Class A – Rhodopsin-like; Class B – secretin; Class C – metabotropic glutamate; Class D – Fungal mating pheromone; Class E – Cyclic AMP; and Class F – Frizzled/Smoothened. Group A currently contains 19 subclasses while Group B includes the adhesion GPCRs, many of which are orphans [243]. The current nomenclature [246,247] comprises 5 classes that are termed GRAFS (Glutamate, Rhodopsin, Adhesion, Frizzled/Taste, Secretin). Given the definition of orphan status, the lack of an endogenous ligand, as noted the majority of allosteric binding sites, can be designated as orphan receptors/drug targets .

4.3. Mutagenesis

An additional outcome of receptor cloning has been the ability to introduce point mutations into a receptor/drug target gene to change key amino acids in the expressed protein [248]. This provides a facile means to understand interactions between the target and its cognate ligand, drugs and NCEs at the molecular level to better understand structure–activity and structure–efficacy relationships. Mutagenesis approaches also allow the creation of mutant receptors, constitutively active as well as chimeric, the latter being comprised of different types of receptor (e.g., α_2/β_2-adrenoceptor) with rat/human and mouse/human chimeras representing important research tools. For instance, genetically splicing segments of the α_2-adrenoceptor, activation of which *inhibits* adenylyl cyclase activity, with those of the β_2-adrenoceptor, activation of which *stimulates* adenylyl cyclase activity, were performed to better understand what structural properties determined the effect of receptor activation on adenylyl cyclase activity [249]. A more recent iteration on receptor mutagenesis is that of "designer receptors exclusively activated by designer drugs" (DREADDs [197,250]). The latter are receptors, to date GPCRs, that have been mutated to interact only with synthetic agonists – not their natural agonist – that can be used in both cells and in vivo to characterize the role of specific receptor subtypes in neuronal circuits, to identify novel signaling pathways [250].

4.4. Receptor crystallization

The next logical progression from mutagenesis was the crystallization of receptor/drug target proteins to determine their native structure using X-ray crystallography [251]. The first drug target protein to be crystallized was the potassium channel [252] followed by the prototypic Class A GPCR, rhodopsin [253]. Since then many other receptors including the ubiquitous β_2-adrenoceptor [254] have moved from proteins to crystal status. By 2012, the crystalline structures of all three opioid receptors μ, δ and γ, had been solved [180] with the information derived hopefully providing sufficient insights into differences in the structure of the three opioid receptors to facilitate the development of more selective agonists. Functionally these would conceptually have a reduced liability for the tolerance, constipation, respiratory depression and addiction associated with classical opioids. The latter is the "holy grail" of pain research that has proved unsuccessful to date [255].

With the availability of the 3D structure of a receptor/drug target and their co-crystallization with ligands and ancillary proteins [256–259], technologies like computer-aided molecular modeling/design (CAMM/CAMD) [260] and SAR by NMR (nuclear magnetic resonance [261]) can be used to aid in the design of compounds in silico. Similarly fragment-based compound design [262] used NMR-based co-crystallization techniques to predict combinations of compound fragments that could be combined to produce novel, tight binding NCEs as potential "hits" for chemistry optimization.

4.5. Molecular pharmacology – cloning and expression – the second circle of reductionism

Without exception, the tools of molecular biology that became widely available at the end of the 20th Century have added immeasurably to the scope of research efforts in pharmacology and drug discovery [25]. And while the discussion above has inevitably been GPCR-centric, a reflection of the efforts and progress in this particular area coupled, at least initially, with the relative structural simplicity of the 7TM receptor compared with other multicomponent drug targets, this has not precluded these techniques being applied to all types of drug targets as each target class increased in size, some exponentially, as different tissues were used for homology cloning, and new targets were identified.

There are however several key technical issues in using cloned and expressed drug targets for research that added significantly to

the reductionism intrinsic in their use. These mainly involved the expression of receptors/drug targets that were both functional and appropriately located in their natural environment, e.g., on the cell surface. In many instances, receptor cDNA was transfected into cell lines like HEK297 and COS that may or may not have had the necessary ancillary receptor/drug targets present in the natural milieu of the native receptor. This resulted in the ability to express functional, signaling pathway-coupled receptors that resulted in reports of novel and unexpected signaling pathways/proteins for receptors that were the consequence of the receptor being present in an atypical environment or being overexpressed. The latter increased the likelihood of a promiscuous signal coupling event that was unrelated to the downstream pathways(s) of the receptor in its native state. In other instances, receptor cDNA was expressed in cell lines in which the endogenous receptor repertoire was not fully considered.

A GPCR cloned from human erythroleukemia cells transfected into COS7 cells was designated as the p2y7 receptor based on its ATP-like pharmacology [263]. However, when transfected into 1321N1 cells, cDNA for the putative p2y7 receptor failed to demonstrate any functional response to ATP [264]. It was subsequently determined that COS7 cells endogenously expressed another ATP receptor, the $P2Y_2$ that was responsible for the effects seen in response to ATP. In contrast, 1321N1 cells did not express any type of ATP-sensitive receptor. The p2y7 receptor was subsequently identified as a GPCR for leukotriene B4 [265]. Findings such as these emphasize the paramount need to advance compounds identified in recombinant cell systems into native receptor systems where the degree of receptor expression and the intrinsic receptor milieu were "normal" [98].

Crystallization studies are also subject to similar caveats to those for an expressed receptor where the milieu for crystallization often fails to appropriately reflect the normal architecture/environment of the cell/membrane. The derived structure then represents a static "snap shot" of one of the many conformations that the receptor can assume, especially when these conformations are frozen at a thermodynamic minimum using 3D structural algorithms or as a result of the constraints imposed within the crystallization matrix [251].

While receptor expression was highly useful as a research tool in that it markedly reduced the use of animal tissues as a receptor source, it also had limitations when evaluating NCEs. In addition to the frequently atypical milieu of the cells in which expression took place, the use of a single expressed target system reduced compound evaluation to a highly constrained format where the ligand being examined was evaluated in a system that only expressed the receptor/target of interest. This led to a *fait accompli* where a ligand was synthesized to be selective for drug target/receptor X based on molecular modeling parameters and tested in a transfected cell system where drug target/receptor X was the predominately expressed/over-expressed leading to an inevitable and obvious outcome. This resulted in a false confidence in assigning compound selectivity based on the constraints of the system. While this approach is the major premise for engineering DREADDs [197,250], it can often lead to surprises when an NCE is subsequently tested in native receptor systems.

4.5.1. Patenting novel drug targets and their use

Another issue, albeit scientifically tangential to the reductionism that emerged with the molecular era of pharmacology was the fact that many newly cloned receptors and their use, were frequently viewed as intellectual property. Very often, biotechnology companies were established on the basis of patents for a particular drug target(s) based on research in academia. These could only be legally used by other researchers with the permission of the holders of the rights to those patents. In addition to limiting access to the receptor and/or drug target, this often led to key details on the science behind the cloning and expression of new targets being deliberately omitted from the peer reviewed literature, reflecting an unfortunate intrusion of intellectual property into basic biomedical research with the inability to use patented clones to advance basic research. One of the most restrictive of these was US Patent 5,401,629 [266], often referred to as '629', that was issued in 1995 for methods for "identifying compounds which interact with cell surface proteins such as receptors and ion channels". By nature of its claims, it became a major constraint in the freedom to operate for all researchers conducting biological experiments after it was licensed to a biotechnology company who initiated various legal proceedings until '629' was finally invalidated due to obviousness [267].

4.5.2. Reductionism in signaling pathways

An additional point related to the reductionism associated with the molecular phase of pharmacology was a trend, still continuing, to study compound effects on ubiquitous signaling pathways, members of which are typically proteins like Akt, ERK, MAPK, NFκB, the caspases, Bax, SMAC/Diablo, Bcl_{XL}, etc. Engineered cell-lines bearing little resemblance to either their primary source or the original immortalized cell-line, that contain various members of these pathways are used together with a variety of selective antibodies for these proteins to derive a priori yet speculative interpretations regarding the role of these pathways in receptor function and cellular dysfunction. Data derived in these in vitro systems are often highly subjective and typically lack any evidence of either a null hypothesis approach or for any unique, specific functions of pathway members. A pathway is selected, constructed, transfected and interrogated in a totally qualitative manner with one or another of its members, the latter usually well known from other studies, then being postulated as a novel drug target for a particular disease condition. The compound used to perturb the engineered system is more often than not used at a single concentration (often in the micromolar range (just to be on the safe side to ensure it produces an effect) where its concentration many be many fold higher than that at which its selectivity was initially determined [65]) coupled with messenger RNA levels being inappropriately used as surrogate markers of protein expression. Selected pathway members are then promulgated as key modulators of specific disease mechanisms and as drug targets without little in the way of additional assessment to ascertain whether the effects observed in response to the compound are: selectively mediated by the receptor/target; statistically significant; replicable; concentration-dependent; or can occur in the milieu of the native cells. This makes the engineered cell line little more than a test tube with a cell membrane substituted for glass or polypropylene.

Finally, the segue from isolated, recombinantly expressed and crystallized receptors to the dynamics and complexity of signaling molecules and pathways [72,138,140] has had mixed outcomes. On the one hand, an expansion of the number (and type) of potential drug targets which, if validated and successfully used as the basis for drug discovery, may lead to a new generation of safer and more effective drugs. On the other is a realization that drugs currently in use, e.g., carvediol [146] – and many of the compounds already screened and likely relegated to library status – may have interesting biased signaling properties that have not yet been measured [140] questioning the value of using existing data sets to construct disease networks and as the basis for prioritizing compounds for "repurposing" efforts [268].

5. Genomic pharmacology

With the final sequencing of the human genome in 2004 [269], there were major expectations that the human genome map would

lead, via the use of genome-wide association studies (GWAS) and next generation sequencing (NGS – high-throughput sequencing; [270]) to the identification of disease-related genes, the products of which would represent novel targets to both diagnose [271,272] and treat [273] human disease states. This led to a bewildering plethora of "omes" in addition to the genome and proteome and included the epigenome, the transcriptome, the metabolome, the lipidome, the phosphoproteome, the interactome, e.g., the GRK interactome [142], the receptorome, the "diseaseome" and the "drugome" [274] – the latter two no doubt conjuring up visions of the final circles of Dante's Inferno for the experienced researcher.

The optimism for the impact of genome-based medicine [275] in improving health care proved premature [276] in addition to being wildly hyped leading to the subsequent invocation of the apocryphal First Law of Technology (ascribed to Crovitz) "that genomics obeys the First Law of Technology: we invariably overestimate the short-term impacts of new technologies and underestimate their longer-term effects" [277]. Nonetheless, the retrospective recognition of the over-optimism for the prospects of a novel technology – irrespective of its source or potential – has done little to dampen enthusiasm or suggest caution in setting expectations for the next "enabling" technology as being the answer to all that ails progress in biomedical research. The core issue is unfortunately a binary, exclusionary culture in biomedical research where a new technology – the more complex and expensive the better – becomes the single most important tool in ensuring success frequently to the exclusion of all others – however valuable the latter may have been in the past and remain so (e.g., pharmacology) – with its proponents ignoring logical context as they lurch from one intriguing "de jour" technology to the next oblivious to the fact that all that glitters may not in fact be gold.

While GWAS has consumed enormous resources in research and led to the association of thousands of loci for disease-related risk and causal genes in diseased populations (e.g., more than 120 associated with AD and growing on a monthly basis), the majority of these have added little to the elucidation of the key mechanisms responsible for disease susceptibility. As many of these associations would not have been predicted based on current knowledge regarding disease etiology, they have had minimal impact in informing existing research hypotheses, with investigator bias and dogma generally undermining much of the potential value of the new data [140]. There are however exceptions, one involving the significant efforts ongoing to identify causal mutations in cancer [278], with the harvesting of thousands of tumor samples and analysis of millions of mutations. These have led to the identification of susceptibility loci in epithelial ovarian cancer [279]. Similarly in IBD (Inflammatory Bowel Disease), GWASs have had a major impact in the understanding of the underlying disease pathophysiology and the identification of susceptibility genes [280] that have resulted in novel disease hypotheses incorporating NOD (nucleotide-binding oligomerization domain) – and autophagy-associated signaling processes [281,282]. In this instance, genetic loci that now total over 160 will necessitate the integration of functional data associated with the variants in order to achieve a therapeutic approach based on the results of these genetic studies.

There are however, caveats related to the complexity and reproducibility of the GWAS outcomes in cancer. In two studies [283,284] in pancreatic and brain cancer, cancerous tumors were found to harbor multiple mutations, an average of 63 in pancreatic cancer [283] with 47 DNA mutations in the brain cancer, *glioblastoma multiforme* [284], indicating that it is unlikely that specific cancers can be treated or cured by therapeutics that target just one or only a few genes, especially when the proteins identified, e.g. kinases, can undergo mutation as the result of drug treatment, thus acquiring resistance to the drug [285,286]. Additional confounds in cancer GWAS reflect: (a) an increase in the number of candidate genes as a function of increasing sample size and replication that results in an increase in false-positives – leading to the "misinterpret...[ation of]... artifacts as biologically important results" [287] that can obscure "true driver events" [288] and (b) intratumor heterogeneity where sequencing/profiling of adjacent biopsies from the same tumor can lead to divergent results including gene expression signatures for good/poor prognosis as well as allelic composition with the inconsistencies that result from repeat sampling increasing the possibility of false-positives [289].

5.1. Pharmacology post the human genome map

While the delivery date(s) for many of the proposed outcomes of the genomic revolution is not anticipated before 2020, efforts are ongoing to more effectively interpret and utilize the outcomes of ongoing GWAS/NGS activities. These are based on a holistic (as opposed to reductionistic) approach focused on the concepts of molecular networks/network biology and systems biology [14–22,274] where the function of a cell, tissue or whole organism is analyzed as a whole, rather than on the basis of the impact of a single gene or single protein at a time and where the interactions between cell proteins can be determined.

While a simple premise based on the collection, integration and analysis of complex data sets from multiple experimental sources using interdisciplinary tools, network approaches appear both rational and a paradigm that is arguably the basis of both pharmacology and physiology [11,290]. However, the extrapolation of systems biology into networks-based analysis of normal and diseased tissues as well as responses to drug treatment and the environment [14–22,291,292] in order to derive protein interaction networks (PINs) has taken this concept to a new level of complexity and, perhaps, abstract absurdity. In this regard, the active generation of biological data may be perceived to have taken a backseat to abstract, data-rich informatics approaches [20,21] like the human "interactome" that involves some 130,000–640,000 potential protein interactions [20] that can form 4620 discrete modules [21].

However, while both the quantity and complexity of the data that can now be gathered indicates that computationally based systems may be the only way in which to understand what goes on in biological systems, this can only be useful *provided* the quality and the meaning of the data can be assured. For instance during mitosis, more than 35,000 post-translational covalent modifications (PTMs) occur within a cell more than half of which, 20,443, involve phosphorylation [293,294], the function of which remains to be determined.

Another example of systems complexity involves a bioinformatics analysis of the targets through which approved drugs produce their effects [291]. In the latter it was found that 989 unique drugs produced their effects via 435 "effect-mediating drug targets", the majority of which were GPCRs, and which involved 2242 drug-target interactions. These data were then used to construct a "drug target network" that identified clusters of connected drug targets that formed sub-networks of which a "giant component" or node contained 489 drugs and 131 targets, all but one of the latter being GPCRs or LGICs. It was noteworthy that the targets within this giant node had a longer history in being interrogated in the research environment than the others making the results somewhat historically biased [140] and thus a questionable dataset.

The generation of target networks, in addition to further emphasizing the polypharmic interactions of drugs and drug candidates [210], may have the potential to guide current efforts in

drug "rescue" and [268,295] by predicting potential therapeutic activity [19,21] in the context of individual responses [20].

Ongoing activities in network/systems biology involve: defining disease as a function of network rewiring [20]; the use of multiple networks in target identification and drug design [21]; and the combination of systems biology and pharmacology as systems pharmacology [15,16], which has been further elaborated in the form of cellular regulatory networks as enhanced PD (pharmacodynamic; ePD) models that when integrating genomic, epigenomic and post-translational data may have the potential to assess individual patient responses to drug treatment; and the continued evolution of chemical biology [18,296,297], a discipline that many find indistinguishable in intent and content from that of pharmacology.

5.2. Genomic pharmacology – genome-based targets – the third circle of reductionism

Genomic pharmacology represents the current iteration of pharmacology. This has become far more closely aligned with the drug discovery and development process than the biochemical and molecular eras of the 20th Century, a reflection of the societal need for efficacious, cost effective therapeutics to aid in containing health care costs [26–29,42,298].

Following from the biochemical and molecular eras, the reductionism associated with genomic pharmacology remains a pervasive force in the basic research endeavor, as outlined above, and has not been nearly as useful as its advocates would argue. The controversial dearth in new drug approvals, both small molecules and biologics [26–29], has driven a reconsideration of the effectiveness of technology-driven approaches that are used in a vacuum in biomedical research with a view to return to a holistic view of pharmacology in the form of systems/network pharmacology/biology/medicine [12–24].

While some aspects of this realigment may auger well for the future focus and success of biomedical research, there are concerns that the pendulum – as tends to happen – has swung from a reductionistic focus lacking in content and context to a newly integrated, hierarchal biology (aka pharmacology) approach where overenthusiastic computer-driven data mining has, to a very major extent, replaced hypotheses, intellect and logic in providing value. It is noteworthy that much of the enthusiasm for "systems"-based research has emanated from academic sources where there is little in the way of practical experience (or success) in the drug discovery process [15,20,21]. As a result much of the information used to assemble the plethora of network omes must be viewed as highly suspect, from the many GWAS studies that have either not been replicated or have been refuted, to the putative genomic targets that have not been adequately validated, to the absence of validated biomarkers, to the (lack) of publically available PK/PD databases (the data in which may not all be of equal value or consistency), to emerging concepts like biased efficacy, which suggest that much of the historical data on compounds may need to be extended using newer assay systems.

Extensive curating of existing data sets will also be required to assess the quality of the data to avoid a GIGO (garbage in, garbage out) situation. Given these concerns and the need to avoid the attendant distractions of the next over-hyped technological breakthrough, it will be critical to integrate the bioinformatics aspects of the networks approach – as pharmacology had integrated biochemistry and molecular biology – and not 'oversell' the technology in the absence of its appropriate validation and transparent application. Thus the use of network approaches; *biological* as represented by target identification/selection; GWAS/NGS; compound screening; biomarker evaluation and validation; PK/PD relationships; "ePD" and mutated targets: and *chemical* in terms of target crystallization; synthetic strategies; chemical libraries; the identification of hits; hit to lead and lead optimization activities; metabolic profiling, represent rational *components* of an evolving, data-driven and integrated drug discovery strategy. However their potential use in qualifying/validating targets and biomarkers, creating logical disease networks as part of a putative drug-/disease-ome, and in enabling allosteric drug design and the development of what have been whimsically termed "edgetic" versus "nodally" targeted-drugs [21], is at best premature.

Another disconnect in the utility of the networks based approach is an emerging trend for research based on re- and meta-analysis of existing databases, including GWAS, NGS [299] as well as clinical trial outcomes, the latter represented by the Cochrane Library databases [300]. While database-based research can have significant value in assessing trends across multiple data sets [299], it lends itself to subjective data selection [300] and the creation of careers based solely on regurgitating "other people's" research (with "other people's" money) via data mining in the absence of any particular effort, insight, ownership or responsibility for the data.

6. The return to holistic, hierarchical pharmacology – reductionism redux

Despite the many caveats above and the dubious semantics, agendas and aims of systems/network biology approaches, biomedical research has begun to recognize the need to return to a more holistic appreciation of cell, tissue and organism, animal and human, *a rise* in the discipline of pharmacology.

While reductionism in its many forms has represented a provocative and much-needed challenge to traditional approaches in understanding disease causality [25], in isolation it has often lacked value, representing a deconstructed – and at times irrelevant annex of the research enterprise – that eventually becomes "humbled by nature's complexity" [301].

In a frequently cited paper [302], the hypothetical outcomes of reductionism in biology have been metaphorically compared to reassembling a functional radio receiver from its component parts without knowing which parts were critical to function. While some may argue that everything within a cell is necessary, including "junk" or non-coding DNA [303], extrapolation of the hypothetical radio "function from component parts" concept to the equivalent of using a parts list for a Boeing 747 to understand precisely what minimum of parts is necessary for the plane to fly, e.g., cell to function [304] leaves open whether the entertainment systems on the latter are essential and what the equivalent non essential parts are present within the cell.. From this types of consideration it was argued that reductionism in biomedical research required a more formal, systematic framework comparable to that which was common in biochemistry, specifically enzyme kinetics, up until the mid-90s, e.g., a systems-based approach, and further asked the questions – "Do we know what to measure to understand a signal transduction pathway? "and" Are we even convinced that we need to measure something?". The latter is a far easier activity than contemplating its relevance and, as Black has noted [25], "Reductionism in biology merely replaces one type of complexity by a different kind of complexity. No one level is more reliably informative than any other".

An overarching theme in the evolution of the reductionistic approach to pharmacology, temporal as well as technical, has been the seductive rise of the personal computer accompanied by a "turn on the computer, turn off the brain" culture [305] that has led to a generation of scientists who appear incapable of independent thought, being unable to function beyond interrogating spreadsheets and datasets, a phenomenon fully consistent with the disconnect with the patient-based realities of the biomedical research enterprise [8], in understanding and preventing disease. The result of this infatuation with data in the abstract context led

Shaywitz and Taleb to note that "spreadsheets are easy; science is hard" [306]. This latter comment highlights yet another facet of reductionism, the distancing of the researcher from the data with its inevitable consequences of diminished ownership and responsibility and, over time, personal time commitment and intellectual disengagement - certainly major sin a career devoted to data mining [299,300].

In harnessing the findings of biomedical research, the intended practical outcome is the integration of the information obtained and its application to create and evaluate a novel hypothesis using whatever tools are available or need to be created; hence the renewed interest in improving, to the extent possible, the predictive value of animal [307] and phenotypic [228] models. This is in marked contrast to the endless search for incremental iterations on, and uses for, brute force technologies (combinatorial/parallel chemistry, HTS, omics; Table 1) that rather than adding value to the process tends to increase costs and diffuse focus.

7. Applied pharmacology and drug hunters

As noted in Section 5.2, the timeframe of the molecular and genomic eras of pharmacology coincided with a major realignment of biomedical research with a renewed effort to develop drug therapies that was motivated by both the scientific and financial aspects of the biotechnology revolution that occurred in the latter part of the 20th Century [308,309] such that the disciplines of applied pharmacology and drug discovery became synonymous. This alignment had however been presaged by the work of individuals like Black [310,311]. Cuatrecasas [312], Vagelos [313] and others, whose primary research interests were the application of basic research findings to drug discovery, successfully bridging industry and academia.

Despite claims that new drugs are primarily the result of research from academia with the assertion that public-sector research institutions contributed in whole or *in part* to the discovery of 9–21% of approved drugs in the 18-year period from 1990 to 2007 [38], an independent assessment of the origins of 35 approved drugs/drug classes concluded that interactions between scientists in academia and private-sector research were "crucial...[to]...the development of new and improved medicines" with *both parties* (italics added) making their "highly complimentary" contribution [314] rather than "intellectual parasitism" [315].

As both a pharmacologist and a drug hunter, Black's interest in applied pharmacology had a major impact on receptor theory [84] and drug discovery [propranolol, cimetidine; 310,311] such that along with Elion and Hitchings he received the Nobel Prize in 1988. The concept of a drug hunter as exemplified by Black and by Janssen, under whose leadership, Janssen Pharmaceutica discovered and introduced approximately 80 drugs to the market [316–318] is the antithesis of a "turn on the computer, turn off the brain" culture [305].

7.1. The drug hunter – anachronism or enabler?

In their lifetimes, drug hunters like the Black and Janssen were widely considered as enthusiastic, inquisitive, innovative and focused individuals focused being driven in achieving their goal – the "desire to discover a drug" [319]. Both individuals evidenced a clear understanding of the integrative aspects of pharmacology (and of medicinal chemistry) and both were recognized – first and foremost – as scientists, inspiring and challenging their colleagues [320]. In a posthumous appreciation of Janssen published in 2005, Black acknowledged the iterative nature of science and its necessarily long learning curve within the context of a "slower rhythm" but also commented on what he perceived as a lack of focused commitment in 21st research with researchers giving up on difficult problems – "research people get tired and want to quit when the breaks are not coming" – instead of transferring their energies from one unsolved problem to another [316]. Similarly, Kubinyi [305] noted that drug researchers in the 21st Century lacked intellectual commitment "behav[ing] like lemmings in the fog, running behind every new concept or method whether it is validated or not ... [relying] ... on artificial in vitro systems hoping that the information from bits and pieces holds true for the whole system" the radio analogy.

Historically, successful drug discovery has been associated with individuals with an overwhelming passion for their research, who would change jobs rather than give up on an idea for a drug they thought worthwhile, consistent with "ideas [being] steadfastly championed by passionate believers" [321], while successes in drug discovery tend to have many contributors especially those "closet" advocates in the ranks of scientific management who cannily "hide" their support until success becomes inevitable and could be shared in with a minimum of personal risk.

Unfortunately, little has been written about individual drug hunters, many of whom remain unknown outside their immediate work environment, but are legendary in the pharma industry where their ability to turn risk into opportunity is viewed as key to improving productivity [322] leading to the comment that "the drug hunter's freedom to roam, and find innovative translational opportunities wherever they may lie is an essential part of success in drug research" [323].

Despite these noteworthy quotes, the majority produced in hindsight, often in a flurry of university/corporate public relations activities, a major question is where the drug hunters of the future will come from and how they will be nurtured. This is an open question, the answer to which is hopefully not reflected in the type of the financially self-interested Amazon/Google- infested nodal world of science fiction envisaged for 2037 [324].

8. Future considerations

The fall and rise of pharmacology, or more correctly, the re-emergence of an integrative, hierarchal, biological discipline from a 25-year period of unfettered reductionism that is focused on understanding disease causality and finding safe and effective therapeutics treat diseases, is a cause for celebration although one that needs to be viewed cautiously.

Many new technologies have appeared over the past 30–40 years, the judicious incorporation of which have reinvigorated and facilitated pharmacological research. However, bringing together these related technologies under the aegis of systems/chemical biology [14] or the paradigm of translational research [12,140], in the absence of: (a) an integrative framework and; (b) an experienced drug hunter-type culture [319], where the question to be answered rather than the technology to be used predominates, will have limited impact in either changing the fortunes of the biopharmaceutical industry or improving societal well-being. Similarly, current efforts focused on the integration and possible interrogation of vast amounts of disparate data of dubious provenance and value in the milieu of bioinformatics-based systems biology (see Section 5.2) is an effort that might justifiably be termed "systems reductionism". The latter thus represents a questionable approach, reductionism in a context of a disengaged and intellectually suspect mindset ostensibly driving an holistic, integrative approach in an environment devoid of any need to actually understand or generate new data.

8.1. Imponderables, complication, unknowns and necessary context – it all depends

The major emphasis in the current article has been on the renaissance of the integrative, hierarchical discipline of

pharmacology and the ability of this seminal discipline – now close to two centuries old – to provide the necessary focus and context for the relevance and success of the biomedical research endeavor. With a spreadsheet-type mentality [306], underpinned by networks-based data mining approach [21,299,300], data can tend to assume a life of its own, becoming independent of the nuances of the methodology that produced it or of the existence of any conflicting data that necessitates that data be re-assessed and prioritized.

Since not all data is of equal value and the reductionistic approach appears to have minimal patience with inconsistencies, nonetheless these exist and need to be objectively dealt with. This is best exemplified in the IND submission in drug discovery where a comprehensive package of data, originating from many different disciplines is assembled and presented to a regulatory authority to support advancement of an NCE to human testing.

In assembling this package, data often has to be reconciled as it not always as clear cut as a spreadsheet approach would make it appear, a reflection of "nature's complexity" [301]. For example, there are often data sets that disagree with one another and lead to confounds in the interpretation of the properties of an NCE. Examples of these include: the compound series that two years and 500 compounds later had still to show the necessary targeted improvement in bioavailability and where, in a final act of frustration/despiration, a candidate with 4% bioavailability in rat, was advanced to human studies and found to have 60% bioavailability; the NCE that showed side effect liabilities in tissue and animal models that could not be observed in humans; and the compound, carvedilol, that had unexplained efficacy in heart failure [146] the reasons for which were identified long after approval for human use [147].

No spreadsheet or computer can provide guidelines to resolve issues such as these, or assume the calculated risk based on insights that an experienced pharmacologist can, or be able to answer questions that include: which preclinical species is predictive of human ADME?; what concentration of an NCE is required at its postulated target to produce optimal efficacy with an acceptable therapeutic index?; why does an NCE with subnanomolar potency require dosing at 5 mg/kg to produce an effect?; is 10-, or 50-, or 100-fold acceptable as a therapeutic index?; why did the side effect seen in human not occur in any of the animal models used to determine efficacy and toxicity?; is the therapeutic efficacy seen with an NCE the result of its interaction with its intended target (a targephilic, "magic bullet" outcome [325]) or the consequence of a "magic shotgun" effect reflecting synergistic interactions with several targets [210]?; and how would convincing evidence be generated to differentiate between the two?

To the pharmacologist with a drug discovery background, the answer to many of these questions would be a definitive "it all depends", necessitating additional experiments that would specifically address the issue and ensure the generation of data to support or refute the initial data, with the latter outcome often leading to loose ends that may never be resolved. While it may be uncomfortable to a biologist heavily invested in a reductionistic view of research, the mechanisms behind disease causality, however convincing, are always subject to revision. Witness the indisputable role of pH in the etiology of gastric and duodenal ulcers that could be treated with histamine H_2 antagonists and proton pump inhibitors the optimal treatment for which b were antibiotics to eradicate *Helicobacter pylori* in the gut [326].

8.2. Emerging trends

Along with the litany of challenges to an effective re-emergence of pharmacology that are highlighted throughout this article (not the least of which being its semantic misappropriation under the rubric of systems biology) are new priorities and additions to research activities for the second decade of the 21st Century. These include familiar topics like the renewed interest in phenotypic screening [228,325] and animal models [307], ever more diffuse and esoteric omics approaches [274], advances in high content screening methodologies [30], NGS [270] as well as the resolution of issues in data replication [43,44], trends in database interrogation as a discipline of its own [21,299] and additional complications in genome mapping around the controversial findings reported by the ENCODE (encyclopedia of DNA elements) Consortium [303,327] that 80% of the human genome is biochemically functional including the regulatory regions that lie outside those coding for proteins. At face value, these regulatory regions represent another "treasure trove" of potential drug targets to add to the long list already generated and not validated using GWAS. The conclusions of the ENCODE Consortium have led to a heated debate [328] regarding: (i) the definition of genomic function, (ii) the contributions of population genetics and mutation rates to selection; and (iii) the questionably productive role of "Big Science" in ongoing research activities.

With less adherence to molecular dogma, an increase in holistic experimentation, and a more pragmatic acceptance of unexpected results within a systems-type approach, biomedical researchers may be more ready to accept the arc of the biomedical research pendulum settling somewhere near center rather than it being continually driven it to its extremes as each new technology *replaces* rather than compliments the proven technologies and disciplines (and in some instances cultures) that preceded it. This would avoid a situation where the proverbial baby is routinely thrown out with the bathwater at disappointingly regular intervals to the detriment of measurable progress in understanding disease causality, instead facilitating the development of novel therapeutics based on a systematic prioritization of information to healthcare.

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