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Biochemical Pharmacology 70 (2005) 1707–1716
Commentary
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Systems and integrative biology as alternative guises for pharmacology: Prime time for an *iPharm* concept?

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Abstract

Understanding the molecular basis of human disease pathophysiology is critical to accurate disease diagnosis, defining disease progression and to identifying new drugs that more specifically address target diseases. Advances in the understanding of tissue function (including draft maps of the human genome) coupled with industrial-scale, 'enabling' technologies like high throughput screening, combinatorial chemistry, proteomics, etc., have generated data on a scale never before possible. Despite this, there continues to be a dearth of new drug approvals ascribed to: (i) the challenges of working with novel, often non-validated disease targets; (ii) targeting diseases (stroke, Alzheimer's) with limited (if any) treatment and ill-defined clinical trial endpoints; (iii) enhanced regulatory hurdles for drug approval; (iv) insufficient time for the new knowledge and enabling technologies to have reached a productive level.

An alternate viewpoint is that unfettered access to such technologies, where exclusion rather than integration has been the hallmark, has markedly reduced the intellectual competent of the biomedical research endeavor, with perceived technological 'quick fixes' displacing the integrative, hierarchical approach of pharmacology, that with medicinal chemistry, represents the core of the drug discovery process. After two decades of profound neglect, pharmacology has re-emerged as the key discipline in providing context to the drug discovery process, facilitating more timely, context-relevant and data-driven outcomes in the search for new drugs. Rather than viewing the future of drug discovery in terms of the 'new' biologies, systems and integrative, a rubric along the lines of *iPharm*, integrating both established and new technologies, is required.

Keywords: Pharmacology; Drug discovery; Integrative biology; Null hypothesis; iPharm

1. Introduction

Pharmacology, defined as ''the science of drug action on biological systems'' [1] and ''the study of the mechanism of action of drugs'' [2], is generally viewed as synonymous with the drug discovery process [3], a result of the increased focus on practical societal outcomes for federally funded basic biomedical research activities [4].

A drug is defined by the WHO as ''any substance or product that is used or intended to be used to modify or explore physiological systems or pathological states for the benefit of the recipient'' and as ''an active substance that interacts with the body ... [having] ... a curative or preventive effect ... prolong[ing] ... life span and improv[ing] its quality'' [5].

The use of drugs for the treatment of human disease dates back to the traditions of Greek and Ayruvedic Medicine where products derived from natural sources evolved on a trial and error basis as safe and effective medicines [6]. The rich history of the natural product pharmacopoeia, products of which, like morphine and aspirin, remain widely used in the 21st century, resulted in research in the mid to late 19th century that extended the seminal physiological studies of Bertrand and co-workers [7] to research on the effects of compounds on tissue and animal function evolving to the formal discipline of pharmacology. Now over a century and half old [8,9], pharmacology is based on three basic concepts: (i) the existence of specific molecular drug targets both on, and within, the cell; (ii) receptor theory [10,11], based on the Law of Mass Action (LMA) that defines the pharmacodynamic outcome of the ligand interaction with its cognate receptor(s)/target(s) as being dose-/concentration-dependent, reversible and selective; and (iii) a null hypothesis-based integrative approach to experimentation [12].

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doi:10.1016/j.bcp.2005.08.019

Together, these principles provided an integrative, hierarchical approach to understanding both pharmacodynamic and pharmacokinetic aspects of compound action at the cellular and molecular levels, allowing the selection and application of appropriate technologies to generate data for hypothesis testing.

Until the mid 1980s, various iterations on these themes, using traditional receptor targets like 7TM/GPCRs and ion channels [13], in conjunction with the evaluation of new chemical entities (NCEs) from natural product sources and classical medicinal chemistry approaches [14,15], led to the successful discovery of many novel and successful drugs for the treatment of a variety of human disease states [6]. These included compounds like cisplatin and taxol (cancer therapy), cyclosporine (immunosuppressant), propranolol (β-blocker; hypertension), salbutamol (bronchodilator), cimetidine and rantidine (H_2-blockers; gastric ulcers), omeprazole and lanzoprazole (proton pump inhibitors; gastric ulcers), captopril and enalapril (ACE-inhibitors; hypertension), diazepam (benzodiazepine-$GABA_A$ receptor allosteric modulator, anxiety), fluoxetine, paroxetine and citalopram (selective serotonin reuptake inhibitors, SSRIs; depression), ritonavir, sequonavir and indinavir (HIV protease inhibitors; AIDS), erythropoietin (bone marrow stimulant) and the various statins (HMG CoA reductase inhibitors; hypercholesteremia). Concomitantly, as a result of evolving knowledge related to the elucidation of cellular events at the molecular level, the classical receptor concept, hitherto confined to the cell surface, was conceptually extended to include both extra- and intra-cellular enzymes (kinases, phosphatases, cytochromes, topoisomerases, cytochrome *c*, caspases, etc.), transporters (serotonin, GABA, norepinephrine, glycine, dopamine, *p*-glycoprotein) as well as a multitude of intra-cellular drug recognition sites (e.g., STATs, HREs, bcl_2) [13], that participate in signal transduction and the modulation of gene expression (e.g., CREB).

While the current pharmacopoeia is based on approximately 500 distinct drug targets [16], biomedical research is now focused on a far greater number of potential drug targets. As an example, the protein kinases and phosphatases that modulate cellular protein phosphorylation number 518 [17] and in excess of 1300, respectively, conceptually quadrupling the existing number of drug targets with representatives from only two of the enzyme families present in the 'drugable genome'.

With the discovery, development and marketing of such drugs, the pharmaceutical industry evolved from a relatively successful, science-based and -driven business in the 1970s with R&D budgets in the millions of dollars to a blockbuster/market-driven, global business [18] that in 2005 had R&D budgets in the region of US$ 70 bn with single drug classes like the cholesterol lowering statins, Lipitor and Zocor, having combined sales of nearly US$ 18 bn in 2004 [19].

2. Drug discovery and pharmacology

Despite the many glossy publications dedicated to drug discovery that have appeared over the past decade and the many seductive stories in the popular press outlining how new technologies have/will aid in redefining the process, drug discovery remains a very imprecise science with much, if not all, of its activities being centered around the seminal ''lock and key' hypothesis of drug action. This concept has been ascribed to the insights from both Ehrlich and Langley at the turn of the 20th Century [10,11] leading to: Ehrlich's discovery of Salvasan [20] for the treatment of syphilis; the foundations of medicinal chemistry [9,20]; and the evolution of the modern pharmaceutical industry [7–9].

The majority of human disease states are thought to result from disruption of cellular homeostasis leading to tissue dysfunction that can be initiated by: (i) a genetic predisposition towards a disease state, (ii) random tissue trauma/environmental insults, (iii) the aging process or (iv) all of the above. Drugs specifically interact with receptors on or within target tissues to alter tissue function, restoring tissue homeostasis and thus alleviate the disease phenotype. In the century since the 'lock and key' hypothesis was proposed, there have been few, if any, significant changes to this basic concept, despite major advances in knowledge related to receptor structure, receptor-initiated signal transduction and to disease-associated alterations in receptor function [21].

The drug discovery process may be considered to have evolved through four distinct technological/philosophical phases since the industry emerged from the German dyestuffs industry in the latter part of the 19th century (Table 1): the empirical/physiological (1885–1948), biochemical (1948–1987), biotechnical (1987–2001) and genomic (2001–present).

3. The biotechnology revolution

Following from the Nobel prize winning work of Jerne, Kohler and Milstein on cloning and the subsequent work of Boyer and Cohen, the realization of the commercial application of recombinant DNA technologies led to the formation of the first biotechnology company, Genentech, in California in 1982 and began what has been termed the biotechnology revolution [23]. A key aspect of this revolution was a new source of funding for the application and commercialization of basic biomedical research from the venture capital (VC) community that derived from the financial model that had led to the evolution of the computer and software businesses in California's Silicon Valley (IBM, Apple, Sun, HP, Oracle, etc.) and elsewhere (Microsoft). The VC community provided the funding that led to the formation of many entrepreneurial ''biotech'' companies in addition to Genentech. Among these were

Table 1
The phases of drug discovery

(i)	*Empirical/physiological* (1885–1948)—complex mixtures and NCEs derived from natural products, with a long history of human use, or derived from the products of the chemical industry [6], used to evaluate for: (a) effects in killing microorganisms and parasites and (b) in intact tissue preparations or in animals to assess phenotypic changes and outcomes thought to be reflective of the disease state This period included Clarke's seminal contributions to receptor theory [11,12]
(ii)	*Biochemical* (1948–1987)—beginning with the seminal work of Alquist (1948) on adrenoceptor classification [11] with the empirical approach being complimented by more precise in vitro biochemical approaches at the cellular and tissue levels to study both the mechanism of action of new compounds thought to have therapeutic potential and the molecular causes of disease. This approach, has been reincarnated under the fuzzy rubric of 'chemical genomics' or 'chemigenomics' [86] using compounds to characterize receptors and their subtypes and in turn to characterize NCEs in vitro for their target efficacy and selectivity to prioritize their advancement into more complex in vivo disease models
(ii)	*Biotechnical* (1987–2001)—resulting from the biotechnology revolution that encompassed three separate themes: (a) A *scientific* approach that focused on examining drug targets/disease processes using the tools of molecular biology (target cloning, recombinant protein expression, target mutation) (b) the addition of more high throughput, industrial-scale *technology-driven* approaches to compound evaluation (c) *Computational-based/bioinformatics* approaches that while critical, frequently assumed that knowledge, freely accessible in databases and on the Internet, could be productively interpreted in the absence of appropriate insight, training and experience [22]
(iv)	*Genomic* (2001–present)—conceptually driven by the mapping of the human genome in 2001 and an expectation, thus far generally unrealized, that disease-associated genomic targets, their proteomic progeny and related 'pathways' would provide the means to more rapidly and accurately identify new generations of drugs that would be highly specific in their disease-related, beneficial effects and hence more efficacious and freer of side effects than existing drugs [85]

Amgen, Hybritech, Chiron, Cetus, Centor, Genetics Systems, Genetics Institute, Millennium and others, located in San Francisco, San Diego and Boston, that were based on ''pure'' biotechnology platforms. Following suit, many European biotechs were established with VC support and included Celltech, Genset, Xenova, British Biotech, Hybridon, etc., Other ''biotech'' companies, including Agouron, Nova, NeuroSearch, Alkermes and Vertex were VC-funded start-up companies focused on more traditional, chemistry- and pharmaceutics-based drug discovery and delivery approaches. The latter often involved individuals from major pharmaceutical companies who brought critical experience to a process that was academically based, financially driven, but far too infrequently pharmaceutically focused.

The successes of these companies (and a few remain in existence, albeit in different forms, some 30 years later), however transient, drove an intense effort to create and proactively commercialize proprietary, often nascent and immature, technologies from academic and federal research laboratories to form the basis for new companies via the technology transfer process. As a result, for the first time, the established pharmaceutical companies now had competition in acquiring novel, proprietary research findings that, prior to the biotech revolution, were not patented and were readily available in the public domain via publications and meetings. While the scope, validity and negative impact of the patenting strategies for biomedical research that led to the creation of this intellectual property (IP) are now being actively debated [24,25] with Integra's RGD/integrin patents being recently vacated by the US Supreme Court [26], the irrevocable outcomes of the process were major cultural and financial changes in both the way that basic biomedical research was conducted (the apocryphal story of the biology student at Harvard in the 1980s who, moments after registering for first year undergraduate studies, went in search of the patent office) and in the drug discovery process, with an increased focus on the business aspects of the industry [18,23,27,28]. Big pharma now competed with astute VCs and biotech companies for IP, often being forced to use biotech companies as the conduit to acquiring the technology, resulting in numerous collaborations, e.g., Amgen and J&J, Lilly and Synaptic, Hoechst and Massachusetts General Hospital, or acquisitions, e.g., Abbott and Knoll AG/Cambridge Antibody Technologies, Roche and Genentech, Pfizer and Agouron, Pfizer and Idun, etc. In 2004, publicly traded biotechs in the US generated US$ 43 bn in revenue and estimates for 2005 indicated that more than 4400 biotech companies existed worldwide, the majority privately owned [19].

The imperative to build new biotech companies was often driven by financial opportunism rather than any clear, sustained belief that the scientific concepts on which such companies were based would lead to either new paradigms for drug discovery [29,30] or new drugs [31]. In the early days of biotech, investors could count on rapid returns (12–24 months) on relatively modest investments (that in retrospect, given the 8–14 year timeframe for the drug approval process, were more often reminiscent of Ponzi schemes [32] than bonafide endeavors in drug discovery) with big pharma as the customer. An earnest naivety, seductive in its facility to the investing public, also became resident in both the biotech industry and big pharma [30,33], driving the notion that novel, albeit unproven, targets and 'enabling technologies' would revolutionize the drug discovery process in terms of producing safer, more efficacious drugs with reduced timelines, moving these from the research bench to the market place with decreased cost, and hence less risk and increased profitability. In the early 1990s, one highly successful entrepreneur, then a proponent of gene

chip technologies, predicted that the use of the latter in the drug discovery process would reduce the time from compound discovery to market introduction from the typical (and still current) 8–14 year timeframe to no more than 3 years. Despite the disappointment in such claims, drug-related gene microarray profiles in primary neuronal cultures, appropriately used, have proven useful in the retrospective prediction of drug efficacy and potential toxicity and may be anticipated to be an important part of the drug discovery process [34].

An overdependence on enabling technology platforms including high throughput screening (HTS) and various iterations of combinatorial chemistry [14,15,35] in concert with expectations that the technologies, in and of themselves, would be catalysts for cultural and organizational change [36] failed to realize the highly ambitious goals of the 'biotech revolution' as related to drug discovery.

By being able to produce multiples of compounds in the hundreds of thousands to millions with associated database sets, the search for new drugs became almost exclusively a 'numbers game' with limited value being placed on the intellectual insights and debates that a more integrative approach could provide [2,30]. Rather than defining and exploring hypotheses, the science of drug discovery became focused on a routine effort to generate data that was then used to formulate hypotheses [37], an approach that appears to have played a major role in the current dearth of new drugs [38,39].

This led to two casualties. The first, that the biomedical science involved in the drug discovery process became increasingly viewed as a predictable, linear *commodity*, removing the intellectual component, termed the ''turn on the computer, turn off the brain'' mentality [33] that denoted an absence of the need for the skill sets, focus and intuition of ''drug hunters'' like James Black and Paul Janssen [20]. Drug targets, whatever their nature and intrinsic complexity, were viewed as totally interchangeable, static rather than dynamic entities, the latter reflecting aspects of the disease state [21,40,41]. In both, the GPCR/7TM receptor and kinase target areas, compound activity, e.g., the ability of the NCE to recognize the target, is highly dependent on subtle nuances of the target state. For 7TM receptors, key accessory proteins that are frequently absent in transfected cell systems [42,43], receptor dimers [44,45], the β-arrestins and trafficking/internalization of receptors and tissue-specific differences in signaling pathways [43] can markedly alter receptor function and the recognition characteristics of novel ligands requiring that NCEs identified in engineered cell systems be re-assessed for activity in systems where the receptor/target occurs naturally [42]. For protein kinases, the phosphorylation state of the substrate, which may be a function of the disease state, the endogenous ATP concentration and the nature of the natural substrate which is frequently unknown, all alter inhibitor activity as do disease-related constitutive forms of the enzyme [40,41]. Despite the success of Gleevec^{TM}, kinase inhibitor drug discovery – kinomics – remains in its early days [46].

Similarly, compounds identified in the HTS milieu were – and are – frequently classified in binary terms, as either agonists or antagonists, even while pharmacologists were busily using functional molecular systems to classify ligand efficacy in terms of: *partial agonists*, a phenomenon well known from more traditional functional systems, *allosteric modulators* based on enzyme theory and ion channel function [12], *neutral* and *inverse antagonists* and *inverse agonists* [42]. An additional, somewhat unappreciated, nuance was the concept of constitutive 7TM receptor activity, activity in receptor systems that occurred independent of ligand [47] and has been used to redefine target characterization and screening [48].

The second casualty was that pharmacology no longer represented either the basis for the drug discovery process or the partner discipline for medicinal chemistry. The loss of the integrative, hierarchical approach [2] inherent in pharmacology that had provided a more thorough understanding of NCE actions at the molecular, tissue and whole animal levels before their advancement to clinical trials, reduced the ability to interpret data relating to compound efficacy, target selectivity and side effect liability in increasingly more complex systems, the final of which, the intact animal was, and is, considered the closest to the human situation. Instead, this innate complexity was simplified, being replaced by overly reductionistic, exclusionary approaches like in silico molecular modeling [49] and artificially engineered cell lines [42] that while satisfying in a heuristic sense, in the absence of being placed contextually with other data, downplayed or even eliminated considerations of NCE side effects and the ADME properties of compounds.

4. Drugs

NCEs go through many complex iterations on their way to becoming potential drugs [15,50]. Starting as hits, they proceed through a cycle of biological evaluation and chemical optimization to enhance efficacy at the presumed target of interest, to reduce or eliminate side effects and to optimize drug-like properties to become leads. Each NCE entering the process for human clinical evaluation requires several key characteristics to facilitate rapid entry to human trials (Table 2). A drugable NCE has: unique target recognition characteristics (structure–activity properties) to impart affinity and selectivity for the target of interest; the necessary intrinsic efficacy (or lack thereof) to modulate the assumed deficit in cell function associated with the targeted disease state; bioavailablity, usually so that it can be administered by the oral route; metabolic and chemical stablity so that it can be stored after manufacture and easily used by the caregiver or patient, once or twice a

Table 2
Drug characteristics required in an NCE

Synthesis and Chemical Properties	Facile synthesis from readily available starting materials—<8 steps with chiral and chemical purity (>98%) Sustainably good yields Patentable Compound properties Aqueous solubility at physiological pH > 10 μg/ml Solid state properties Physical properties (e.g., pK_a, C log P (<3), stability
Potency	Defined SAR for compound series Active and selective at human drug target in nanomolar range 1–50 nM Greater than 20-fold selective vs. other targets Optimally > 100-fold in 100 + target Cerep/MDS/Upstate profile Equi-active in both expressed and native cell systems Activity against molecular target in preclinical efficacy model
Efficacy	Agonist or antagonist in appropriate cellular systems (native and expressed) Efficacy established in ex vivo system and animal model of disease state PK in pharmacology mode for plasma level associated with efficacy and side effect (PK/PD model)
ADME properties	Metabolically stable in liver S9 preparation in multiple species including human Identification of primary metabolic pathways and routes of elimination Minimal potential for drug–drug interactions Absence of potent inhibition of CYP isozymes in Human recombinant and microsomal systems Minimal induction of CYP3A$ in fresh human Hepatocytes Drug transporter interactions Favorable human P–gp interaction Protein binding (preclinical species and human) Genetic toxicity (e.g., Ames, in vitro micronucleus, chromosomal abberations)—negative Caco-2 permeability Allometric scaling PK dose escalation-rat PK ged/fasted-rat Repeat-dose PK/tolerability in rat
Safety pharmacology	Therapeutic index >30, optimal >100 hERG inactive >30 μM (if active followed by patch clamp and telemeterized rat studies) CV safety (blood pressure, heart rate, dP/dT, etc.) CNS safety (core battery studies–Category A–ICH7A) General behavioural observations Spontaneous motor activity General anesthetic effects Potential synergism/antagonism with general anesthetics Effects on convulsions (proconvulsant activity and synergy with convulsive agents), analgesics Body temperature Effects on GI motility and renal function Repeat-dose PK/tolerability (non-rodent)
Clinical translation	Initial dose prediction from animal efficacy studies Biomarkers for determination of early clinical readout

day; chiral purity with a facile and cost-effective synthetic route; and the necessary novelty to be a patentable entity to recoup the research investment in producing it. Many of these properties were not well appreciated in the early days of HTS when compound libraries from agricultural and photographic company sources were enthusiastically screened with many hits but little success.

Hodgson [51] has cogently noted that ''a chemical cannot be a drug, no matter how active nor how specific its action, unless it is also taken appropriately into the body (absorption), distributed to the right parts of the body, metabolized in a way that does not instantly remove its activity and eliminated in a suitable manner—a drug must get in, move about, hang around and then get out''. Factors involved in defining the ability of an NCE to have appropriate in vivo activity are reflected in Lipinski's 'rule of 5' [52] and its derivations [53] that define the physical properties of an NCE that can favor bioavailability. These include a molecular weight of 500 or less; more than 5 hydrogen bond donors; more than 10 hydrogen bond acceptors; and a C log P value (a standard measure of hydrophilicity) of less than 5.

4.1. Drug selectivity

As a result of the medicinal chemistry activities designed to facilitate their interaction with specific receptors, enzymes or other drug targets, NCEs are selective, to varying degrees, for a specific target. Heuristically, this provides a high level of comfort in advancing compounds to the clinic, especially when a lead has passed through an extensive in vitro target profile as guidelines to identify potential 'off-target' activities that may produce side effects [46,54]. However, even with a target-based approach [55], many effective drugs, e.g., neuroleptics, have multiple target interactions that may account for their efficacy profile with various targets being additive or synergistic with one another [56,57]. Neuroleptics, despite diverse structural motifs, also share a common propensity to interact with the cardiac HERG channel involved in QT prolongation [58]. Many protein kinase inhibitors are

competitive ATP-mimetics making them highly promiscuous in their interactions with members of the kinome family [59]. However, with the emergence of specific, disease-associated mutations in kinase function [40], it now appears possible to identify more selective inhibitors that should be more efficacious as a result of reduced side effect profiles [60].

4.2. Drug effects are both concentration/dose- and structure-dependent

Receptor theory is based on the LMA that states that the effect produced by a compound/drug is proportional to its concentration at the target with the actions of a compound being quantitative rather than being simply qualitative [10]. The concept of a proportional relationship between drug dose and effect dates back to 1776 with studies on the effects of belladonna on mydriatic responses [61]. An NCE can be quantitiatively characterized in terms of its IC_{50}, EC_{50}, Ki or pA_2 value(s) [62] that reflect both the pharmacokinetic (PK) and pharmacodynamic (PD) actions of the compound, the former, the effect of the host environment on the NCE and the latter, the effect of the NCE on the host environment [61]. The dose proportionality inherent in the dose–response curve is however, limited by a number of assumptions, not the least of which is that many PK models are based on a psuedoequilbrium situation between total and free drug concentration in plasma and between free drug in plasma and tissue. Even when a dose–response curve is elevated to the level of a plasma concentration–response curve, the total drug concentration in the biological fluid is not always reflective of the active drug entity leading to the caution that PK models are abstractions rather than ''precise wholly descriptive'' [61].

Modifications in the structure of a discrete pharmacophore can lead to changes in both potency and efficacy, some showing structural linearity in their interactions with the initial target based on minor substitutions on the initial structural template with others resulting in a total change in target specificity in the pharmacophore. The benzodiazepines (BZs) that selectivity interact with the $GABA_A$ receptor provide a rich diversity of compounds with anxiolytic, hypnotic and sedative effects, e.g., diazepam, clonazepam, etc. have a well defined structure–activity relationship while other BZ pharmacophores have a total distinct pharmacology, e.g., tifluadom interactions at the μ opioid receptor based on the fact that the BZ nucleus is among those pharmacophores that are privileged [13].

5. The null hypothesis

In contrast to more modern day technology-based approaches, e.g., gene array chips, that study compound–target interactions almost exclusively at a molecular level using the technology to drive the process [37], more traditional approaches to drug discovery were hypothesis-driven [2]. Compounds that selectively modulated a selected target could then be used as probes to test the hypothesis using whatever technologies were relevant and available. Thus, a simple experiment to ask the question as to whether a novel compound was an analgesic would require being able to show that it had dose-dependent analgesic effects in an appropriate model of pain. A famous examples of this generic approach was Black's syntopic approach to the discovery of the histamine H_2 receptor antagonist, cimetidine [63].

A key feature of a hypothesis-based approach was the *null hypothesis* [12] that entailed performing a series of experiments, the premise of which was that the phenomenon under investigation could be explained in several possible ways. For instance, the hypothesis that an NCE, compound X, acting as a β-adrenoceptor antagonist, would produce a positive outcome in an animal model of hypertension was taken as incorrect. Only when all other alternatives had been considered, used as the basis to generate experimental data and eliminated, was the hypothesis tenable. In the present example, the initial hypothesis would be that there is no a priori assumption that the NCE is different from vehicle. In order to test the null hypothesis, it is therefore necessary to generate a body of data that conclusively tests all possible outcomes. For instance, if compound X is functioning as a competitive β-adrenoceptor antagonist, in addition to generating a dose–response curve for its effects in an appropriate cell/tissue/animal system to derive a quantitative measure of activity, it is also necessary to show that a selective, β-adrenoceptor agonist can dose-dependently overcome the effects of the hypothetical antagonist and that agonists selective for other receptor families, e.g., 5HT, nicotinic cholinergic, do not, thus acting as an initial series of negative controls.

6. The fall of pharmacology

The ability to clone and express proteins of biological interest using the techniques of molecular biology revolutionized biomedical research in the late 1980s and early 1990s to the extent that an additional and necessary criterion for receptor identification and characterization was the ability to clone the receptor. Many new receptors and members of receptor families were rapidly identified leading to an explosion of what were considered to be novel drug targets. In addition, these technologies provided the means to genetically manipulate animals to delete and overexpress receptors and other drug targets [64] adding immeasurably to the biomedical research toolbox.

The perceived importance of these techniques led to their being funded, not as additional tools to enhance the biomedical research endeavor, but to the exclusion of more traditional integrative research approaches to the extent that many pharmacology departments, especially in the

US, did not receive funding unless grant applications contained a molecular biology component. In 1994, only 11% of pharmacology fellowships funded by the National Institute of General Medical Sciences at the NIH involved whole animal research [65]. This inevitably led to a de-emphasis in the teaching of classical pharmacology, an ingenious re-naming of pharmacology departments to incorporate a 'molecular' component somewhere in the department title and, with the aging of the existing population of card-carrying pharmacologists [2], a dearth of expertise. At the time, this was not widely seen as a particular issue although it certainly contributed to the many instances where data showing that single, millimolar concentrations of existing drugs interacting at a novel target were used as evidence that the existing targets, where these drugs were known to act at nanomolar concentrations, were incorrect and that the newly cloned receptors were the 'real' targets for their efficacious actions. In 1992, the Editor of *Nature*, John Maddox, had noted that molecular biology, despite its ''triumphant pervasiveness'' was very much a qualitative science with a real need on the part of its practioners to recognize the importance of the LMA in anticipation of quantitative data interpretation [66]. With the decrease in the teaching of basic pharmacology, the role of the LMA in quantitatively characterizing compounds and systems to generate IC_{50}, EC_{50} and pA_2 values remained in decline. Research papers continued to document the assessment of the effects of only a single compound concentration, usually in the micromolar concentration range, frequently chosen at random, more often than not with a single '*n*' value that did not permit the generation of any estimate of variability, to quantitate compound effects on alterations in gel band intensity, the proteins for which were usually derived from an engineered cell system. Moreover, the use of only a single compound, rather than compounds with varying degrees of activity and a negative control, with the absence of replicated, quantitative data made it nigh impossible to define the SAR for a new compound series, the latter the hallmark of both target and compound characterization, making much of this reported work both ephemeral and unpublishable.

Furthermore, compound selectivity also was often taken on a 'faith' basis. A compound initially reported as being a selective 'X' antagonist became routinely accepted as such without any additional confirmation. A proteomics study of the mitogen-activated protein kinase (MAPK) p38 inhibitor, SB 203580, which has been widely used to define the role of p38 in inflammation and other disease states, showed that this compound was quite promiscuous in its effects inhibiting other kinases with similar or greater potency than p38 kinase [67], raising questions to both its utility as a research probe for p38 effects and the value of the body of data generated using it.

The decline in support of an integrative approach to biomedical research while formally identified in 1994 [65] has only recently led to initiatives to reverse the trend with drug companies, most notably Merck [68] and the NIH [69] funding training in integrative pharmacology.

Major limitations in the physiological relevance of engineered cell systems [42,43] remain. Nonetheless, they continue to be routinely used to characterize new compounds and new receptors leading to an overly reductionistic assessment of ligand–receptor interactions that is infrequently placed in physiological context using systems where the receptors are naturally expressed, e.g., guinea pig ileum, brain slices, vas deferens, etc. [2]. One example of the pitfalls of this approach was the misidentification of the leukotriene LTB_4 receptor [70] as a spurious ATP ($P2Y_7$) receptor [71] due to the fact that the endogenous receptor repertoire present in the cell line used for transfection was not adequately evaluated.

6.1. Animal models

The animal models routinely used to characterize NCEs as part of the hierarchical process [2], while not infallible, are key in providing the necessary evidence of efficacy to advance compounds to the clinical testing stage. The majority of animal disease models do not adequately reflect the human situation with considerable debate on their utility [58,72]. Animal models of chronic pain involving spinal nerve ligation, based on an acute and precise surgical insult, are typically studied for periods of 1–4 months. The development of chronic pain in humans can occur over years or even decades – often with idiopathic causality – potentially resulting in more subtle, more complex and longer lasting synaptic/neuronal changes than those occurring in the shorter-lived animal model. Other animal models of psychiatric diseases like schizophrenia have limited relationship to the human situation. A classical assay to assess NCEs for antipsychotic potential, rat catalepsy, is more a model of dopamine receptor blockade than a model of psychosis with the prepulse inhibition model more accurately reflecting the occurrence of similar phenomena in humans [73]. In depression, iterations on the classical behavioural despair model [74], now nearly 30 years old, remain state-of-the-art despite early inroads in assessing behavioural phenotypes in transgenic mice [75]. Transgenically modified mice, fruit flies and zebra fish have however been used as surrogate models of human disease states with a remarkable degree of success, albeit via a retrospective analysis [64].

Despite their limitations, the inherent complexity of animal models cannot be replicated in other, simpler systems. For example, Spedding et al. [58] have argued that current animal models of psychiatric disorders fail to account for basic aspects of synaptic plasticity that are stress-related that when considered would reflect a more realistic assessment of the relevance of animal models to human disease pathophysiology [76]. Evaluation of the delayed onset of antidepressant action in animals, a well

characterized phenomenon that replicates the human situation, showed that hippocampal neurogenesis was a key component of the mechanism of action of this drug class [77].

Animal models are however routinely used to assess side effect potential [78] – ranging from changes in blood pressure and sedation to overt toxicity – and can also be used to determine the *therapeutic index*—a ratio of the efficacious dose/plasma level of an NCE to the dose/plasma level producing robust side effects and to provide first approximations of compound dosing for clinical trials. For neurodegenerative diseases, NCE effects on behaviours including learning and memory as well as on physiological changes like muscle strength or nerve conduction can be used to assess compound efficacy and related side effects. Such changes can also be correlated with ex vivo biochemical endpoints, e.g., alterations in neurotransmitter or cytokine levels that are useful as an initial 'proof-of-concept' for a mechanism-based hypotheses when assessed in the context of functional measures at the whole animal level. In addition to being used for initial human dosing levels such measurements may also have the potential to be transitioned to biomarker endpoints for clinical trials, providing, with an experimental medicine approach [65], an important translational bridge between preclinical and clinical studies as well as a means to track earlier NCE responses in the human environment [79,80]. The intrinsic value of such data varies depending on the species used due to differences in pharmacokinetics and also in the species-dependent structure or actual presence of the drug target in that species. The sensory neuron-specific *Mrg* GPCRs (SNSRs) involved in pain processing occur in far greater number in mouse (32), than in human (4–6) [81], potentially confounding compound identification and optimization of selectivity, especially when transgenic animals are used.

7. The re-emergence of pharmacology

Over the past 5 years, a variety of articles, many cited above, have questioned the inverse relationship between the resources invested in basic and applied biomedical research and the practical outcomes of this research as measured in terms of new drug introductions—NDAs. The FDA [80] has recently identified the ''strengthening and rebuilding ... [of the] ... relevant disciplines (e.g., physiology, pharmacology, clinical pharmacology)'' as key to understanding NCE effects at the whole animal level and as a process to reverse what has been described as the ''stagnation or decline'' of innovation that the FDA views as contributing to the slow down on new medical therapies. Similarly, the NIH has begun funding courses in integrative and organ systems pharmacology [69] as part of the larger NIH Roadmap initiative [4,82].

In addition to these initiatives, compelling arguments have been made for the use of complex human cell systems for the discovery of new drugs [83,84] and of new uses for approved drugs [57], the latter involving the evaluation of drug combinations using classical pharmacological approaches to assess compound synergy in vitro in conjunction with highly sophisticated, computerized data analysis techniques, e.g., Loewe and Bliss independence modeling.

The systems biology approach has been audaciously viewed as a way to 'rescue drug discovery' [84]. Since compounds with established preclinical efficacy and safety can enter human trials in the absence of a known mechanism of action, it was proposed [84], that drug discovery should move from a 'target-centric' approach, based on the elusive chimera of target validation, to one of compound validation. The mechanism(s) of action of such empirically defined compounds, once in clinical trials, could then theoretically be determined for a fraction of the cost of running a more target-centric-based approach. For those in the industry actually facing such challenges, e.g., establishing the mechanism of action of the antiepileptic, valproic acid as a mood stabilizer, this is far easier said than done. Without a mechanism, it is exceedingly difficult, if not impossible to establish an SAR and identify second generation compounds that have improved efficacy and/or safety.

The re-emergence of pharmacology as an integrative solution to the current state of affairs in drug discovery while positive, still requires additional efforts to avoid such exclusionary paradigms as target-centric [55] versus systems biology [83] as well as the ability to incorporate genome-based approaches [34,85] in an fully integrated manner. This may avoid the treatment of scientific inconsistencies that should provide the stimulus for the inquiring mind (rather than the computer [33]) to better understand why they exist, being viewed as research 'dead ends' unworthy of further research effort [20].

An interesting framework by which to facilitate a pharmacologically based integrative approach may be borrowed from Apple's iPod™. Having identified a need, Apple engineers took existing, off the shelf, hardware and software to create a technologically sophisticated portable digital storage device for music, photographs and books, the utility of which has far exceeded the sum of its parts. In this analogy, as in an optimal drug discovery scenario, appropriate technology was successfully used to reach a previously defined endpoint rather than Apple (e.g., a drug company) searching for a project to justify the existence of their technology. While smoke drums and pithed rats have largely passed into pharmacological history to be replaced by receptor binding, patch clamping, gene arrays, etc., a 'modern-day back-to-the basics approach' [36] using a rubric along the lines of *iPharm* may be what is required to provide a truly integrative framework to understanding compound action at all levels [2] rather than a convoluted 'systems biology' approach that ignores the considerable value of knowing, to the degree possible, the mechanism of

action of a drug. This would help in addressing Milne's concerns [39] that the ''key hurdle ... [in improving productivity] ... lies not with technology but with how we organize to implement new paradigms''.

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