HHS Public Access

Author manuscript

Neuropharmacology. Author manuscript; available in PMC 2018 February 20.

Published in final edited form as:

Neuropharmacology. 2017 July 01; 120: 11–19. doi:10.1016/j.neuropharm.2016.03.021.

The Need for New Approaches in CNS Drug Discovery: Why Drugs Have Failed, and What Can Be Done to Improve Outcomes

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Abstract

An important goal of biomedical research is to translate basic research findings into useful medical advances. In the field of neuropharmacology this requires understanding disease mechanisms as well as the effects of drugs and other compounds on neuronal function. Our hope is that this information will result in new or improved treatment for CNS disease. Despite great progress in our understanding of the structure and functions of the CNS, the discovery of new drugs and their clinical development for many CNS disorders has been problematic. As a result, CNS drug discovery and development programs have been subjected to significant cutbacks and eliminations over the last decade. While there has been recent resurgence of interest in CNS targets, these past changes in priority of the pharmaceutical and biotech industries reflect several well-documented realities. CNS drugs in general have higher failure rates than non-CNS drugs, both preclinically and clinically, and in some areas, such as the major neurodegenerative diseases, the clinical failure rate for disease-modifying treatments has been 100%. The development times for CNS drugs are significantly longer for those drugs that are approved, and post-development regulatory review is longer. In this introduction we review some of the reasons for failure, delineating both scientific and technical realities, some unique to the CNS, that have contributed to this. We will focus on major neurodegenerative disorders, which affect millions, attract most of the headlines, and yet have witnessed the fewest successes. We will suggest some changes that, when coupled with the approaches discussed in the rest of this special volume, may improve outcomes in future CNS-targeted drug discovery and development efforts.

Keywords

CNS clinical trials; CNS drug development

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1.1 Introduction

The failure rate for new drugs targeting important central nervous system (CNS) diseases is very high relative to most other areas of drug discovery, a fact reflected in the many pharmaceutical company CNS programs that have been disbanded or significantly reduced (Abbott, 2011; Miller, 2010). This is most apparent in the case of drugs that attempt to alter the course of the disease or condition (disease modifying drugs), and is particularly acute in the area of neurodegenerative diseases (NDDs). In many cases, the drugs that have had demonstrable effects are palliative treatments that have modest effects on disease symptoms and no demonstrable effect on disease progression.

For any disease, it is difficult to discover effective and safe drugs. Discovering and developing a successful drug depends on very detailed knowledge of underlying disease mechanisms and a successful progression from candidate identification to clinical trial design. The pharmaceutical industry (for all the right reasons) is heavily regulated, and it is one of the few industries where, despite the investment of a great deal of capital and time, the majority of efforts result in complete failure. While other industries, such as the aircraft industry, are equally regulated at a certain level, the result of that scrutiny is rarely a completely unusable aircraft, or the irreversible denial of marketing approval for a new airplane. We understand enough about the physics of flight to assure that planes will fly, and an iterative process with regulators makes sure they fly safely. In the discovery and development of new medicines, this is not a certainty: we do not have any *a priori* reason to expect that we can intervene with pharmaceutical agents in any disease, and it is never assured that a drug will be approved for marketing.

Almost miraculously, in many types of disease, such efforts have been very successful, although in very few instances can pharmaceutical treatments be considered cures. Some disease areas, frankly, have proven more tractable than others, and almost all other areas are easier than targeting many types of CNS disease. For example, anti-infective agents target organisms that are foreign invaders in our particular internal ecosystems, presenting almost unlimited opportunity for novel and effective agents to kill pathogens while sparing our own cells. The rapid progress in discovering and developing life-saving medicines in areas such as HIV-AIDS and other viral disease demonstrates that when there is sufficient cooperation between the relevant government agencies, academia and the pharmaceutical/biotech industry, and pressure from patient advocates, progress in such diseases can be very rapid and effective (Fauci, 2003; Hardy, 1994). The same will likely be true with antibiotic resistance and parasitic diseases in the near future, it will just require the political will to do it on a grander scale, and new models for recouping investments for short-term treatments or treatments aimed at third-world patient cohorts. While these types of collaborative efforts have also paid off well in other areas such as cancer and heart disease, thus far they have not led to effective treatments in many of the major CNS disorders. They *have* begun, however, and there is every reason to be optimistic.

In all disease areas, there are several common requirements for designing and implementing a successful effort to discover new treatments. The natural history of the disease or condition *must* be well understood. A potential molecular target has to be identified, and a testable

hypothesis must be generated concerning the role of the new molecular target in either the generation or amelioration of the disease state or condition. A model of the disease must be created that is believed to have predictive validity for use in preclinical tests and that involves induction of the disease, or a mechanistically-related disease, in animals or *in vitro.* A directed program must be initiated to generate molecules to test. If a candidate molecule is identified that fulfills a number of pre-clinical criteria such as dose-dependent efficacy in the model(s), metabolic stability and a sufficient degree of animal safety at multiples of presumed therapeutic doses, a drug candidate may be taken into carefully designed and tightly regulated clinical trials to determine its safety and efficacy. Initially the safety of the drug candidate is tested in healthy human subjects, and eventually in human subjects with the disease. If efficacy is demonstrated that is greater, in the context of the particular disease, than the risks associated with the drug it may be approvable and eventually marketed and made available to patients.

These above steps have been followed in the development of drugs that act on the CNS, but levels of clinical failures are higher than in other therapeutic target areas, most often because of lack of any significant evidence of clinical efficacy. While drugs often fail prior to understanding whether they are efficacious, it is failure for lack of efficacy that is most vexing, expensive and leads to the greatest likelihood of retreat from a disease target. This occurs repeatedly despite seemingly adequate and appropriate preclinical data demonstrating that candidates should work well, and have seemingly adequate clinical safety margins. No one makes the decision to advance a drug into very expensive and time-consuming clinical trials lightly. While there have been some obvious mistakes, usually based on assumptions later proven wrong, the preclinical packages used to propel CNS drugs into the clinic are just as convincing and well-executed as those for any other therapeutic area. Clearly, there is a major disconnect, at least in some CNS sub-disciplines. *Post hoc* analyses may point to specific aspects of a clinical trial that may have contributed to the lack of a positive signal, and such analyses are useful and necessary. Failures due to lack of efficacy, however indicate that there may be serious flaws in the hypothesis. As a result, negative results may be critical to understanding how to make successful drugs. Negative results should therefore be published, but often have not been (Hayes and Hunter, 2012; Jones, 2013).

2.1.1 How bad is the problem in the area of CNS disease?

To any of us who have spent our scientific careers even peripherally involved in the search for treatments for CNS diseases, it comes as no revelation to find out that we work in a tough and unforgiving area. A recent study from the Tufts University Center for the Study of Drug Development pointed to a *part* of the problem set that plagues CNS drug discovery and subsequent development (Kaitlin, 2014). They used data obtained from an analysis of the development (clinical) programs associated with 274 CNS and 1,168 non-CNS investigational compounds, including an analysis of the approval time required for 42 CNS and 345 non-CNS compounds eventually approved by the U.S. Food and Drug Administration (FDA). The study found that success rates for CNS drugs, defined as final marketing approval by the FDA, were less than half of the approval rates for non-CNS drugs for the period 1995–2007 (6.2% vs. 13.3%, respectively). In addition, the time to approval following submission of an application for marketing approval for CNS drugs that *were*

eventually approved was 31% longer than for non-CNS drugs (19.3 months vs. 14.7 months, respectively; the time period sampled was 1999–2013). The study also pointed out that the mean development time was greater, and the number of CNS drugs given priority review by the FDA was significantly lower, relative to non-CNS drugs. These data, while important, fail to convey the complete pattern of CNS-targeted drug failure. Most of the CNS successes that comprise this data set involve additional therapeutic interventions in areas where there has been at least limited success, such as some psychiatric diseases, and are either 'me too' medicines, or somewhat novel medicines for proven targets and tractable conditions (Conn and Roth, 2008; Kaiser and Feng, 2015; McGonigle, 2014; Sukoff Rizzo et al., 2013). There is nothing wrong with discovering incrementally better medicines for old targets; in fact, it is a great thing. It does nothing, however, for the large number of CNS diseases such as the chronic NDDs, for which there is no disease-modifying treatment, despite previous large preclinical and clinical programs (Berk and Sabbagh, 2013; Broadstock et al., 2014; Stanzione and Tropepi, 2011). It also makes the situation look rosier than it is.

2.1.2 Addressing symptoms rather than causes in neurodegenerative diseases

There have been decades of significant advances in our knowledge regarding the basic neurosciences, including neuropharmacology, yet treatment of symptoms rather than cause characterizes most CNS pharmaceutical approaches. For example, most treatments for pain reduce sensation, but do not durably affect the cause of the aberrant sensation. In acute pain, this is acceptable, because the cause is self-limiting due to healing, but in chronic and neuropathic pain when the medication is removed, the pain returns. In psychiatric disorders treatment of symptoms can be very effective, even if accompanied by serious side effects, but again, if medication is terminated, the disease symptoms almost always recur without diminution. Thus, in the absence of other treatments, such drugs have to be taken for life. In both areas, the fact that drugs treat symptoms but do not affect the disease process would be fine if the drugs had few if any side effects impacting quality of life or drug compliance, had no abuse potential, did not often induce tachyphylaxis or other forms of drug resistance, and were not economically challenging to individuals or healthcare systems. Of course, all of those issues do apply to one degree or other (Eisenberg and Suzan, 2014; Gøtzsche et al., 2015; Schug and Goddard, 2014; Uchida and Mamo, 2009).

The worst outcomes (in those areas where programs have been attempted) are seen in the major chronic NDDs, including Parkinson's disease (PD), Alzheimer's disease (AD) and neuromuscular disorders, including amyotrophic lateral sclerosis (ALS; Lou Gehrig's disease). In these disorders the widespread degeneration (death) of neurons (AD) or the more focused death of specific populations of central cells (PD and ALS) leads to increasing dysfunction in individuals, and eventually death. Currently, all of the approved treatments for these diseases are palliative. Symptomatic treatments for these chronic NDDs have been approved: these include dopaminergic modulators for PD (Henchcliffe and Severt, 2011; Radad et al., 2005), cholinergics (Birks et al., 2015; Doody et al., 2012), an excitatory amino acid modulator (Olivares et al., 2012; Plosker, 2015), and recently a combination of the 2 approaches (Greig, 2015) for AD, and a compound that has some effect on ALS symptom progression (Miller et al., 2012; Wagner and Landis, 1997). However, as far as we know, and to the degree it has been tested clinically, these approaches provide symptomatic relief, or as

in the case of Rilutek® (Miller et al., 2012) extend life for several months in ALS (a very rapidly progressing disease), but the patients' function continues to decline with no demonstrable change in slope. Because these are relentless, ongoing degenerative disease processes, the established therapeutic mechanisms depend on augmentation of some aspect of remaining circuitry to *temporarily* restore a degree of function. When remaining circuits are sufficiently diminished, their efficacy disappears.

2.1.3 Approaches for stroke and traumatic brain injury

Another area where little progress has been made despite a decades-long mechanism-based search for effective treatments is the acute NDDs, including stroke (thromboembolic, or ischemic stroke, and hemorrhagic stroke, blockage vs. bleeding, respectively) (Besancon et al., 2008; Cook and Tymianski, 2011; Fisher and Stroke Therapy Academic Industry, 2003; Fisher et al., 2009; Grupke et al., 2015; Kidwell et al., 2001) and traumatic brain injury (TBI) (Agoston and Risling, 2012; Park et al., 2008; Stein et al., 2015). These conditions have been studied and targeted intensively starting in the 1970s, resulting in many large clinical trials in the 1990s and 2000s. This was particularly true for ischemic stroke, where both academia and the pharmaceutical industry felt there was enough information to proceed, and where there was considerable early confidence of success (Hazell, 2007; Kidwell et al., 2001; Muir and Lees, 2003; Young et al., 2007). Stroke is primarily a disease of the aged, and is the second leading cause of death in the world (slightly less in developed countries), with approximately half of all stroke sufferers dying within the first year (Feigin and Lawes, 2003). TBI affects the young at a much higher rate than stroke does, and therefore results in much longer periods of disability and rehabilitation (Roozenbeek et al., 2013; Werner and Engelhard, 2007). While these conditions differ in their proximal causes, cerebral infarct, intracranial vessel rupture or other forms of neuronal injury, their sequelae, the consolidation and worsening of the patient's function, are believed to have similar underlying mechanisms. The development of hypotheses centered on excitotoxic delayed neuronal death (Aarts et al., 2003; Hazell, 2007; Werner and Engelhard, 2007), mediated by the insult-induced abnormal release of neurotransmitters (excitatory amino acids; EAAs), and resulting in toxic intracellular levels of calcium (Ca^{2+}), resulted in an impressive number of complex and large (very expensive) clinical trials which tested a number of hypothetical neuroprotective mechanisms, particularly for cerebral infarct (Cheng et al., 2004; Cook and Tymianski, 2011; Feuerstein and Chavez, 2009; Gladstone et al., 2002; Grupke et al., 2015). To date, not a single *neuroprotective* drug has demonstrated sufficient clinical evidence of efficacy.

There has been one successful class of drug in this area, and it is a disease modifying one. Its success is, however, unrelated to any specific CNS mechanism (it was a cardiovascular drug class), and it does not protect neurons once they are impacted. In the case of cerebral vessel block in ischemic stroke, the block can often be dissolved by so-called clot busting drugs, such as tissue plasminogen activator (tPA; Alteplase; Activase®), provided the drug is delivered within several hours (although that window may be expanding) (del Zoppo et al., 2009; Kidwell et al., 2001; Kwiatkowski et al., 1999). Many patients, however, are ineligible for the use of this treatment because they are unable to receive the drug in time, have concomitant bleeding (which would be exacerbated by treatment), or some irreversible

damage has occurred despite treatment. As a result, only a fraction of patients have function restored. Thus far, attempts to target events producing excitotoxic death, which expands from the core of the stroke area or injury, have failed (Cheng et al., 2004; Fisher et al., 2009; Gladstone et al., 2002; Grupke et al., 2015).

2.1.4 Multiple sclerosis provides an exception

There has been considerable success with one major NDD not in the above grouping, relapsing multiple sclerosis (MS). For this demyelinating disease, several classes of approved medications can actually modify the progression and severity of the disease (Castro-Borrero et al., 2012; Minagar, 2013). The reason for this is instructive. Relapsing MS is an autoimmune disorder (Hagemeier et al., 2012; Inglese, 2006; Keyser, 1988; Lock et al., 2002), and suppression of the immune response and inflammation can be quite effective, although not yet curative. So for one chronic NDD clear identification of a specific and validated disease mechanism quickly resulted in a number of effective treatments (Minagar, 2013).

3.1.1 Why have there been so many failures?

Even at the preclinical stage, it is more difficult to make findings in CNS disease that can be translated into a successful clinical candidate than in most other areas. The brain is a protected compartment (the blood-brain barrier, or BBB) (Begley, 2004; Oby and Janigro, 2006; Pardridge, 2012), and entry of molecules into the CNS is limited and requires special attention by medicinal chemists and whole-animal pharmacologists. Many of the techniques commonly used to increase brain penetration by small molecules, such as increasing lipophilicity, can dramatically reduce solubility, leading to difficulties in drug delivery. Many classes of large molecules, such as peptides and antibodies, will not readily access the CNS without some form of assisted transport (see elsewhere in this issue). Nevertheless, of all the reasons for *clinical* CNS drug failure, this is the least important historically, and rarely resulted in clinical failure. With few exceptions, drugs are not taken into clinical trials if some mechanism for CNS entry cannot be demonstrated, and sufficient brain levels are not reached in animal models. In our further discussion of why CNS drug programs fail, we will assume that the failed drugs passed all preclinical requirements to enter the clinic.

Certain factors can cause clinical drug failures in any disease area, and CNS is no exception. These include an unacceptable pharmacokinetic (PK) profile in humans. While many preclinical models of PK exist with reasonable predictive validity, unexpected metabolic differences can arise and cause the withdrawal of a drug candidate. Usually, this will be detected early in development, unless the final phase of development involves a population of subjects that have not historically been included in safety assessments in healthy volunteers or initial assessments in subjects with the disease. Such populations include women, the aged or chronically ill, and, particularly in the case of women, this factor has resulted in changes in research and clinical trials industry-wide.

Another factor common across disease areas is the problem of both predicted and emergent toxicity. All drugs are assessed for safety, and the yardstick for deciding if a toxicity profile is acceptable will involve a careful and ongoing assessment of the risk/benefit ratio. The

levels of acceptable risk are greater in diseases where the untreated outcome is dire and no effective standard-of-care treatment exists. This is particularly true when a drug regimen is time-limited, and the subject is able to recover and enjoy relief from disease following cessation of treatment. Many oncologic treatments, even the most recent immunotherapies, can be highly toxic, but if successful the disease is in remission and treatment is concluded, at least for a time. Deaths attributable to treatment may even be noted, but a drug may be approved if the population benefit outweighs the individual risk. Because the CNS determines every aspect of our personality and controls all behavior, side-effects, even if not reflecting toxicity in the usual sense, can end the development of a drug if they cause significant neurologic or behavioral dysfunction, or if patients will not take them.

Sometimes incomplete knowledge of the natural history of a disease, coupled with the fear of side effects and even commercial concerns about reimbursement, can contribute to clinical failure that otherwise *may* have resulted in success. In the area of ischemic stroke evidence has accumulated that the area of impacted brain continues to increase long after the 3–4 days post insult that had originally been postulated (Copen et al., 2001; d'Esterre et al., 2012; Dyker and Lees, 1998; Fisher and Bastan, 2012; Lord et al., 2015; Markus et al., 2003; Nour and Liebeskind, 2014; Quast et al., 1993). Nevertheless, some important measures of stroke progression appear stable by 2–3 days, and the side effect profiles of some classes of potential stroke drugs, such as EAA antagonists, were deemed problematic; reimbursement policies at the time also limited most in-hospital stays for stroke patients to 72 hours. Many stroke trials were conducted with drug given for no more than 72 hours, and at lower doses than may have been beneficial. These trials, which were supported by promising preclinical data, failed, and we do not know if longer treatments or higher doses, which would have increased the acute care costs of the study (and treatment, if approved), would have had a different and happier outcome (Cheng et al., 2004; Donnan, 2008; Fisher, 1999; Fisher and Stroke Therapy Academic Industry, 2003; Fisher et al., 2009; Gribkoff et al., 2001; Grupke et al., 2015; Hazell, 2007).

3.1.2 Finding animal models can be a problem

Another common reason for failure is the lack of animal models that have predictive validity (predicting human efficacy) despite having some degree, perhaps even a high degree, of face validity (recreating the disease phenotype or being mechanistically related to the disease). This is probably the most frequently referenced reason for the failure of CNS drugs in the clinic. Whether the failure results from an inadequate disease model or from poor translation of model results into clinical study design, this is a key, perhaps even marquee, problem in CNS translational research.

Looking back at which animals models have proved to be useful provides some surprising wrinkles. Given the poor face validity, and construct validity, of most animal models of neuropsychiatric conditions (i.e., really, what constitutes animal depression, anxiety or psychosis, and how does one measure it?), one would think that predictive validation of models of these diseases/conditions would not be very likely. Nevertheless, some animal models of psychiatric disease, although far from perfect have led to the discovery of a large number of effective medicines (Kaiser and Feng, 2015; Nestler and Hyman, 2010; O'Neil

and Moore, 2003; van der Staay et al., 2009; Weiner et al., 2000; Willner, 1984; Willner, 1986; Willner, 1991). Clearly, these animal models have touched on mechanisms that are also involved in their human counterparts. Conversely, models of chronic pain, ischemic stroke, TBI, and most chronic NDDs, despite actually involving insults and genetic manipulations that produce conditions *mimicking* many aspects of their human disease counterparts, have seldom proven predictive to this point (Bird and Parlee, 2000; Branchi et al., 2003; Cernak, 2005; Gamzu, 1985; le Bars et al., 2001; McGonigle, 2014; Petters and Sommer, 2000; Tordjman et al., 2007; Windisch, 2014). Some examples have become classic cases of how perfectly good research data can become meaningless without information about the differences between humans and animals. The failure of neurokinin (NK1) receptor antagonists in human pain trials is likely one such case (Boyce and Hill, 2004). Chronic and neuropathic pain is most frequently treated with non-steroidal anti-inflammatory drugs (NSAIDS) or, if more serious, opiates. Because both classes of drug can have serious side effects when used chronically, safer and more effective pain drugs are still greatly desired.

Most chronic NDDs have proven problematic, and patients are refractory to disease-modifying treatments to date. Animal models with apparent face validity have, however, been created for all of these diseases. For AD and ALS, these models almost universally involve testing of new drugs in animals that express mutations associated with human disease (Dal Canto and Gurney, 1995; Fisher and Bastan, 2012; Games et al., 1995; Gotz et al., 2004; Spires and Hyman, 2005; Van Dam and De Deyn, 2011). For AD these involve mutations that alter the processing of amyloid or tau proteins, such that animals express AD-like plaques of β-amyloid, tau tangles, or both. These are established CNS biomarkers of AD in humans, and if disease causing they should produce behavioral consequences that mimic human AD (cognitive dysfunction), and *should* predict when drugs will work in humans. In small populations expressing specific mutations, these gene products, altered by the mutation, must have a role in the disease, at least in these individuals. That this has not yet worked, despite decades of drug programs that have targeted toxic β-amyloid accumulation, would therefore seem to be a mystery.

3.1.3 Treating the biomarker may not treat the disease

So why do animal models such as those discussed above not work to predict human efficacy? Well, at least in some cases, they do, depending on whether ‘working’ means to affect disease outcome or affect the biomarker. Mouse models of AD express human mutations believed to be associated with disease. In these AD mice, high levels of amyloid-containing plaques develop, and drugs designed to lower the amount of toxic β-amyloid do indeed do just that. The real problem with the AD mouse models has been demonstrating that the mutation-dependent increase in β-amyloid leads to a behavioral phenotype that resembles the debilitating effect of AD on cognitive function, or that reversing β-amyloid accumulation leads to its amelioration (Windisch, 2014). Recently, the cognitive neurobehaviorist Greg Rose summed it up as a model lacking in all major respects any coherent relationship with the disease it’s supposed to be emulating (Rose et al., 2015). He suggests that, because there is very little correlation between β-amyloid accumulation in plaques and behavioral effects in these models, what is needed is a model based on cell loss

and dysfunction rather than plaque formation. This view is bolstered by recent clinical evidence with a drug based on reducing plaques (Elan/Johnson & Johnson/Pfizer's bapineuzumab). This drug failed in late-stage clinical trials, but in subjects with AD that received the drug the levels of β-amyloid were reduced, plaque deposition was reduced, and phosphorylated tau protein was reduced, all supposed important biomarkers of AD (Panza et al., 2014; Salloway et al., 2014; Vellas et al., 2013). Much the same lack of relationship between efficacy in animal models and human subjects has been seen in ALS (Benatar, 2007; Scott et al., 2008).

It has long been known that amyloid plaques, sometimes at high levels, exist in the brains of many aged individuals that do not display cognitive impairment (Aizenstein et al., 2008; Dickson et al., 1992). Thus, regardless of the cause(s) of AD, an animal model of cognitive impairment due to accelerated aging, or some other model that relies on cell loss in relevant regions, may have better predictive validity. In both ALS and AD the percentage of patients whose disease can clearly be linked to a familial cause is less than 10%, with approximately 90% of patients being 'sporadic', i.e., with no known cause. In ALS animal models, which do result in a disease phenotype that includes neuromuscular dysfunction and death, many compounds have produced notable positive effects, but all have thus far failed clinically. Even if animal models based on these mutations predicted drug effectiveness in the genetically-linked patients, there is hope but no surety that they would work in the vast majority of subjects with these NDDs. Since the problem with CNS drugs is that they often work preclinically, but fail to demonstrate efficacy in the clinic, this is only apparent after the fact, and means that most models leading to CNS clinical failure are too permissive or, as in the case of AD and ALS models, may measure the wrong things (or the right things at the wrong time).

4.1 So how can we improve what we are doing?

4.1.1 Better animal models

In the area of ischemic stroke therapy, the original model paradigm was to use rats that had the drug on board at the time of the infarct. This was rapidly recognized as having little predictive value, and eventually a common paradigm was developed to treat 1–2 hour after an occlusion, usually of the middle cerebral artery (MCAO) in young adult rats. Clinical trials were designed to emulate this, where patients had to be enrolled within a specific time following the onset of the stroke. None of the drugs discovered and taken into the clinic using this or similar paradigms worked in human ischemic stroke (with the *caveat* noted previously that drugs were not dosed for long periods in humans, so it is unclear if they *could* have worked). There is now general agreement that the animal models could have been better designed (Fisher, 1999; Fisher and Stroke Therapy Academic Industry, 2003; Fisher et al., 2009; Philip et al., 2009). Most strokes occur in the aged, yet young adult animals were used. Lissencephalic rodents were used, rather than gyrencephalic species such as cats and monkeys, which more closely resemble the human anatomy. Strokes were identical in induction, both in terms of type, locus and duration within a study, rather than the much less uniform case with human ischemic stroke, and there was little or no attempt to model co-morbidities, such as high blood pressure or diabetes. Obviously, animal models

have to be less time consuming and less expensive than human clinical trials. Nevertheless testing a drug candidate for any disease in a series of models, each less permissive than the last and each more representative of the human disease and the human patients to be treated in terms of age and other potential confounds such as sex and reproductive status, seems to make good sense.

4.1.2 Better hypotheses

Perhaps equally important, but more challenging to the ego, both in academia and in pharmaceutical laboratories and boardrooms, we have to be open to alternative mechanistic hypotheses in intractable diseases, and we have to use negative clinical data to inform future decisions both preclinically and clinically. We need to create preclinical models that incorporate better face validity, and alternative hypotheses that are scientifically sound, into the preclinical path and ultimately into the clinic. Even if the final tests undertaken to propel a compound or approach into the clinic are time consuming and expensive, it will be worth it, both medically *and* economically if this approach better predicts the behavior and efficacy of the drug in clinical trials. While a stroke compound that appeared to be effective when given 2 hours after the onset of MCAO in a young rat may look promising when the brains of these rats are examined histologically several days later, would they still look protected when examined 2 weeks, a month or 6 months later? While it may be impractical to do all of the tests of a stroke drug in aged animals, and particularly in a gyrencephalic species, this might be appropriate at a final go-no go decision point. Companies may balk at the added time and expense, but if they want success in disease areas that are currently un- or under-served in terms of effective medicines, and they want to reduce clinical failure rates, they may need to rethink how preclinical programs advance compounds into the clinic. Timelines should be less important than probabilities of success.

Better models, and better drugs, can only come from a better understanding of disease processes, and new thinking about the composition of the resulting drugs. In drug discovery programs, the association of a molecular target with a disease, or a hypothesis about how manipulation of another target *may* effect processes that lead to disease, usually leads in short order to the creation of high-throughput screening efforts aimed at identifying modulators of the target. This has led to the discovery of numerous highly effective modulators of myriad targets (receptors, ion channels, enzymes, aberrant protein accumulation or distribution). The failure of subsequent clinical trials strongly suggests that, despite all of the outstanding work done to elucidate disease mechanisms, we do not understand as much as we may think we do about many of these conditions. In particular, we do not fully understand the roles of these targets in neuronal systems, or whether modulation of a particular target, and that target alone, will be necessary and sufficient to significantly impact a disease. In many cases, we do not know how an ideal molecule should interact with its target, or whether modulating it can produce the expected results. We tend to simplify.

We (both authors) have long worked to further the understanding of the structure and function of neuronal ion channels, and have contributed to the cloning, expression and modulation of many families of these proteins and their accessory subunits (Brown and Kaczmarek, 2011; Cheney et al., 2001; Dworetzky et al., 1994; Gribkoff et al., 2001;

Gribkoff, 2008; Joiner et al., 1998; Kaczmarek, 2013; Yang et al., 2006; Zhang and Kaczmarek, 2015). Many of these channels are mutated in humans and lead to CNS disease, and we have performed numerous studies, or provided tools, that suggest that their modulators could be useful in diseases as diverse as ischemic stroke and TBI, hearing disorders, epilepsies, and mental retardation, among others (Brown et al., 2010; Brown and Kaczmarek, 2011; Gribkoff et al., 1996; Gribkoff et al., 2001; Gribkoff, 2008; Strumbos et al., 2010; Yang et al., 2006). What we and others have found, however, is that while a channel may have an important role in a particular behavior, and a disease-related mutation may alter some characteristic such as its voltage-sensitivity, simply finding a compound that restores the voltage-sensitivity of the mutant channels may not be enough. For example, in addition to regulating ion flux, many channels have “non-conducting” functions and interact with cytoplasmic signaling molecules that regulate processes as diverse as protein phosphorylation, cell adhesion and protein translation (Kaczmarek, 2006; Pardo and Stuhmer, 2014). Thus, the cause of the disease may lie in perturbations to these other channel functions rather than simply in the change in voltage-dependence. This consideration is likely to apply to many classes of drug targets.

4.1.3 Approaching targets from more than one direction; a lesson from Cystic Fibrosis

Effective therapies may require approaching targets from more than one direction at once, and maybe several targets simultaneously to obtain a useful signal-to-noise ratio in the clinic. An instructive example comes from outside the CNS, and shows how ongoing understanding of the disease target, coupled with changed thinking about the composition of the successful drug approach led to success in treatments for cystic fibrosis (CF). The causal role of mutations of the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride channel that is a member of the ATP binding cassette family of proteins, in CF was established in the late 1980s and early 1990s (Riordan et al., 1989; Riordan, 1993). CF is a disease with widespread effects in the digestive system, reproductive system and, most challenging therapeutically, in the lung, where accumulation of viscous mucous is permissive for infections that cause cumulative lung damage. For some variants CF is fatal in the absence of transplant intervention. It was determined that CFTR is one of the elements responsible for the maintenance of fluid and salt balance across lung epithelia and that disease-causing mutations produce reduction or loss of channel function (Champigny et al., 1995; Riordan et al., 1989; Riordan, 1993). While gene therapy was the unsuccessful initial approach, activators of these channels were discovered very rapidly (Gribkoff et al., 1994; Rowe and Verkman, 2013). It was also noted, however, that, in many cases, the mutated channel was misfolded, and >90% of channel protein never made it to the plasma membrane. In other words, activators could theoretically help to a degree, and probably better in some variants than others, but real therapeutic benefit would have to await a mechanism for translocating misfolded protein to the plasma membrane (a ‘chaperone’) *and* activating the relocated channels (Brown et al., 1996; Rowe and Verkman, 2013). This was the basis for the recent approvals for both an activator and a CFTR chaperone as combination therapy for a very deadly form of CF (Eckford et al., 2012; McIntosh, 2015; Pollack, 2015). While the activator was first approved for a different genetic variant of CF, it really only became a more useful drug as a *combination* affecting two important components of the disease.

4.1.4 Combination therapies may be required

Our penultimate topic of discussion leads from the above example. Most CNS diseases are highly complex. While this is not unique to the CNS, we have been the least successful, in some areas, in dealing with this complexity. It may therefore be unreasonable to think that a single agent will treat many of these conditions. In the case of CF, a disease caused by dysfunction of a single protein, a combination of two agents with different mechanisms led to significant success in the most serious disease phenotype. There is increasing recognition that combination therapies, comprised of the most promising and best-tolerated compounds targeting different putative disease progenitors, or approaching a well-established mechanism by multiple approaches (as in CF), may be the best hope in diseases that have thus far proved completely resistant to therapeutic intervention. This has been successful in anti-infective programs, oncologic drug development and others.

Intractable diseases of the CNS almost certainly have more than one interacting mechanism. Combination therapies for CNS diseases, however, have been slower in acceptance by the FDA and the pharmaceutical industry. The recent approval of a combination of palliatives for AD is an exception, but both agents were first approved individually, and are not disease modifying (Farrimond et al., 2012). Combinations in psychiatric disorders and epilepsy are common, but they combine previously approved drugs in the hope of exerting better levels of efficacy. While in other areas drugs were combined after each had demonstrated efficacy in human disease individually, drug combinations for these neurodegenerative CNS diseases would (at least currently) have to combine compounds that were not efficacious when tested alone. In many respects this is revolutionary. This could be very expensive and time consuming at the level of both preclinical and clinical trials, but major and expensive changes in clinical trial design may be in the future regardless. At a recent conference, representatives of the FDA, including the outgoing Director of the Neurology Division, communicated that such an approach may be more acceptable in the future, as long as the mechanistic justification is strong and the safety profile is sufficient (Perry et al., 2015).

4.1.5 Clinical trials may need to be longer in duration

We discussed earlier the idea that drug trials in stroke may need to be longer in duration than has typically been the case. Recent evidence suggests that success in AD may require intervention at an early time, when there is no disease phenotype present, but when imaging suggests that silent disease processes, which may be irreversible by the time they are obvious behaviorally, have just begun (Chen et al., 2001; Fox et al., 1996; Garber, 2012; Panza et al., 2014; Vellas et al., 2013). This logic may apply to most if not all NDDs. If this is the case, a drug or drug combination may need to be administered for long periods, perhaps many years in otherwise healthy individuals, and there may be no clinical models that involve shortcuts. Of course, this also means that the biomarkers used to identify candidate subjects for clinical trials need a high degree of validation, which is still an important aspect of ongoing research. Approval could take much, much longer, even if ultimately successful in the case of slowly progressive diseases. Companies and relevant government agencies must find a mechanism for undertaking longitudinal clinical studies that are medically meaningful and yet can be economically viable. On all fronts, we simply must take the long view.

5.1 Conclusions

Neuroscience has provided a backbone of testable hypotheses to approach treatment of serious and intractable CNS diseases, such as acute and chronic NDDs, but success has been limited to palliative treatments, or completely ineffective. In addition to the wealth of new approaches in CNS drug discovery and development discussed elsewhere in this special issue of *Neuropharmacology* we suggest some additional factors that could increase success. Careful attention to the natural history of a disease, and the 'neuroecological' milieu of the molecular target of drug action in the disease state, may provide better information for future discovery efforts. Models of CNS diseases and drug therapy regimens that better suit the clinical condition, as well as drug combinations that take the unique step of combining promising but individually ineffective drugs, may improve prospects in the near term, but will require a new level of regulatory, academic and industry cooperation and patience. Clinical trial designs may need to reflect the reality that interventions may have to begin before disease is obvious, perhaps much before, and may require unusual cooperation and patience from all participants. We are optimistic that new treatments are on the horizon, and that renewed efforts in the CNS therapeutic arena can have a much better chance of success.

Acknowledgments

This work has been supported by NIH grant HD067517 and NIH grant DC01919 to LKK.

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