PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Drugs for a new millennium

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THE ROYAL SOCIETY

Drugs for a new millennium

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A millennium, a century, even a decade is a long time-frame for speculation about anything. Advances in biomedical research in the last few decades have been so extraordinary and escalating at an ever-accelerating pace that any prophecy is a risky proposition. However, it is possible to divine the big, unanswered questions and envisage ways in which they might reasonably be approached in the next few decades, a task which I will try to essay. So many drugs treat so many different medical conditions that a detailed and comprehensive coverage would probably be tiresome. Instead, I will address certain broad themes and diseases that offer both immense challenges and great potential for advances. Rather than review detailed experimental issues, I will confine myself to the 'big picture' issues, providing examples of specific research only in a few instances drawing largely from areas I know best.

Keywords: schizophrenia; neuroleptics; antidepressants; poly(ADP-ribose) polymerase; cocaine; nitric oxide

1. CELL DEATH

A simple-minded approach to future drug development would be to assume that we will find the molecular etiology of all diseases, which in many instances will involve specific abnormalities in one or a group of genes. Treatment would then consist of replacing the missing or abnormal gene. While this approach may ultimately work in a good number of instances, I suspect that we may be able to make major inroads without such information. Moreover, knowing the causes of diseases may not do much for cures. Let me provide a few examples. Immunization is one of the most important advances in all medicine. We now approach immunization by isolating the offending organism, cloning the genes for key proteins and then developing vaccines based on these proteins or related fragments. However, the giant breakthroughs took place hundreds of years ago prior to the isolation of any organism. Vaccination commenced with tissue extracts of individuals infected with cowpox long before cowpox, smallpox or any viruses were understood. The major antibiotics were developed without any understanding of how they might kill bacteria. Similarly, the major drugs in psychiatry emerged from serendipitous events in the absence of molecular insight.

Cell death is fundamental to diseases that afflict the largest number of people and for which treatment is presently gravely inadequate. I am thinking primarily of cancer, as well as stroke and neurodegenerative diseases. In the case of cancer, we wish to kill malignant but not normal cells. In the case of stroke and neurodegenerative diseases as well as myocardial infarcts, we wish to prevent cell death. Accordingly, understanding what is going on in cell death and developing agents that will augment or inhibit the death process may provide therapeutic agents even when we don't know the 'causes' of the individual disease entities.

The decade of the 1990s has witnessed an explosion in cell-death research. One of the first advances was the appreciation that there are at least two distinct ways in which cells die. One of these is called programmed cell death or apoptosis. The other is unprogrammed cell death, generally referred to as necrosis. The best-known examples of apoptotic cell death occur during normal developmental processes. For instance, in the brain, about twice as many neurons are generated in embryonic life than can be accommodated in the adult brain. Neurons that successfully make contact with their target sites survive while the others die by apoptosis. Similar processes take place in other parts of the body. There is much debate as to the type of cell death that occurs in pathologic conditions such as stroke or myocardial infarct. One simple-minded formulation posits that cells will die by an apoptotic route if they have leisure, while necrosis takes place if the tissue insult is overwhelming and there is no time for the delicately coordinated apoptotic path.

In apoptosis a series of events takes place in an orderly sequence involving the activation of various proteases which are called caspases, for cysteine and aspartate proteases. Several distinct caspases act in a cascade vaguely reminiscent of the blood-clotting cascade of complement proteins. If one wishes to interfere with the apoptotic process, then one strategy would be to develop drugs that inhibit various caspases, a current effort underway in the pharmaceutical industry.

Besides the caspases, other proteins either augment or inhibit the death process. The first well-characterized one was an oncogene called bcl-2. Bcl-2 is anti-apoptotic. Other proteins in the bcl-2 and related families are pro-apoptotic. Accordingly, if one wishes to kill cancer cells, a productive approach might be to develop drugs that will block the effects of bcl-2, while if one wishes to preserve certain cells, antagonists of the 'death genes' would be

indicated. Like the caspase area, drug development based on the bcl-2 family is brisk.

2. STROKE AND MYOCARDIAL INFARCT

While stroke and myocardial infarct involve very different organs, they display notable similarities. Most prominently, both are usually caused by clots that occlude blood vessels. Moreover, in both of these conditions, efforts to modulate cell death offer therapeutic promise.

Stroke is one of the three leading causes of death in Western societies, trailing only cancer and myocardial infarct. It is one of the few major diseases for which there remains virtually no treatment. In recent years, the clot-dissolving protein tissue plasminogen activator (TPA) has been approved for the acute treatment of stroke. TPA dissolves clots in cerebral vessels just as in coronary vessels. However, TPA can prevent myocardial damage when administered up to half a day after the onset of coronary symptoms, but is effective in stroke victims only if given within three hours of the first symptoms. While patients with acute chest pain hasten to the hospital, initial symptoms of stroke are typically vague involving dizziness and minor confusion which, in most patients, has happened in the past and has gone away without any treatment. Hence, most stroke patients do not tend to enter the hospital for a day or two. TPA augments bleeding so that damage caused by vascular occlusion is sometimes made worse by TPA-induced haemorrhage. Additionally, many strokes are caused initially by haemorrhage rather than clots in cerebral vessels.

Because of these considerations, stroke presents a major therapeutic lacuna. When I was a medical student, it was thought that blood vessel occlusion in both stroke and myocardial infarct is followed by rapid death of the oxygen-deprived, hypoxic tissue so that treatment is only palliative. Greater optimism has emerged in the last two decades as we have learned that the major tissue damage involves cell death that occurs gradually over a day or two. In stroke, various chemical changes associated with tissue hypoxia trigger a massive release of the amino acid glutamate (figure 1; Lipton & Rosenberg 1994). Besides its role as a constituent of protein, and a key player in intermediary metabolism, glutamate is a neurotransmitter. Glutamate is the major excitatory neurotransmitter and probably the most abundant of all transmitters. In animals, strokes lead to a 50- to 100-fold increase in the release of glutamate. It is felt that this glutamate almost literally excites partially hypoxic cells to death. Evidence for this notion came with the development of drugs that block receptors for glutamate and which, in animals, reduce neural damage in stroke by 50–60%. Some of these drugs are in clinical trial so that in the early decades of the next millennium we may anticipate major benefit. Glutamate synapses are regulated by a complex array of proteins and small molecules, several of which are appropriate targets for drug development. Hence, even if initial glutamate antagonists are not effective drugs, I am confident that this synaptic constellation will lead to valuable treatments.

Since glutamate mediates excitation under physiologic as well as pathologic circumstances, glutamate antagonists may also be useful hypnotic, anticonvulsants and anti-anxiety drugs, while substances mimicking glutamate may be effective cognition enhancers and general stimulants.

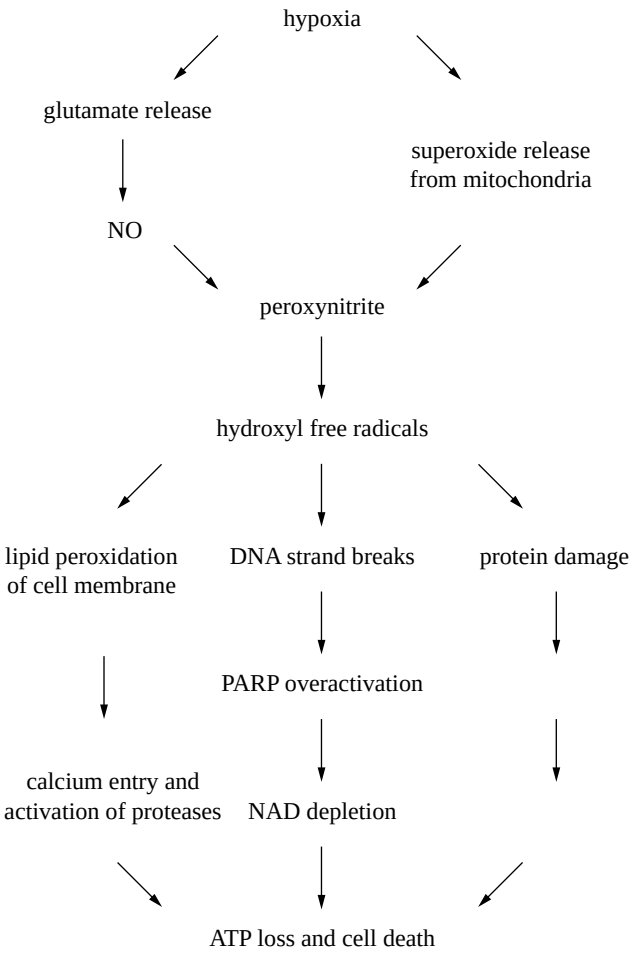

Figure 1. Mechanisms of neuronal damage in stroke.

Exactly how does the 'overexcitation' by glutamate kill cells? A variety of research indicates that a novel gaseous neurotransmitter, nitric oxide, is responsible for doing much of the dirty work of glutamate in stroke and other forms of neurotoxicity (figure 1; Lincoln *et al*. 1997). The nitric oxide neurons have receptors for glutamate, and glutamate stimulates the formation of nitric oxide, which can be released to adjacent cells. Drugs that block the formation of nitric oxide prevent stroke damage. Moreover, mice with targeted deletion of the gene for nitric oxide formation are resistant to stroke damage. Hence, blocking nitric oxide formation may be as good or even better than blocking glutamate effects, especially as nitric oxide is closer to the death process than glutamate.

But substituting nitric oxide for glutamate as a proximal culprit in neuronal death merely begs the question as to 'what kills?' Nitric oxide itself has properties that fit with an executioner's role. Nitric oxide is a free radical, hence it is highly reactive chemically and capable of attacking proteins and other macromolecules. However, nitric oxide is not a particularly lethal free radical. Its ability to kill derives from its interactions with other molecules, especially superoxide. Superoxide is an altered form of oxygen that leaks out of partially hypoxic mitochondria. Superoxide combines quite readily with nitric oxide to form a far more toxic molecule called

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

peroxynitrite. Peroxynitrite in turn degenerates into the hydroxyl free radical, one of the most toxic of all such chemicals. There is an abundance of solid experimental evidence indicating that peroxynitrite and hydroxyl free radicals are the key agents that do the killing.

Even in the absence of nitric oxide, hypoxic mitochondria will generate lethal free radicals. Superoxide can evolve into a hydroxyl free radical without the intervention of nitric oxide. Hence, the machinery for cell death via free radicals is present in non-neural tissues such as heart, which are never exposed to the neurotransmitter actions of nitric oxide. Heart researchers have appreciated for some time that a major portion of the damage following coronary artery occlusion does not simply represent hypoxic tissue dying on the spot. Instead, following the occlusion, blood from collateral vessels pours into the damaged area so that the partially hypoxic tissue is overwhelmed with a superabundance of oxygenation. This leads to high densities of oxygen free radicals, which kill gradually over a period of many hours in a situation analogous to stroke. Superoxide has been extensively implicated in myocardial infarct as well as in stroke. Evidence for this includes the finding that infusion of the enzyme superoxide dismutase, which destroys superoxide, reduces myocardial infarct damage in both animals and humans.

Thus, both in myocardial infarct and in stroke, free-radical damage is crucial. How do the free radicals kill cells? Here things become murkier. When a cell dies, everything falls apart. The cell membrane disintegrates as oxidation damages the lipids that are key components of the membranes. Cell membrane barriers that normally keep ions such as calcium outside of cells become weakened so that calcium rushes into the cell and can cause damage by activating protein-degrading enzymes–proteases, which damage crucial proteins in cells. Free radicals also damage DNA.

Is there any way of distinguishing between the relative importance of these cellular executioners? Much research has focused on calcium-activated proteases such as calpain. Drugs that inhibit these enzymes do offer protection against cell damage in a variety of experimental models. Notable success has also come from exploring the consequences of DNA damage. Coping with DNA damage presents some challenging choices to cells. Limited amounts of DNA damage occur constantly so that cells have evolved extensive enzymatic systems to repair the damage. However, excess damage may not be worth repairing. By analogy, if an automobile has sustained excess damage, it is better to discard the car rather than fix it with the attendant risk of unrepaired defects. Similarly, efforts to repair large amounts of DNA damage in cells may be imperfect, leading to cancerous changes.

Nature seems to have evolved a mechanism that initiates the repair of modest amounts of DNA damage, while the same system destroys cells with excess damage. The mechanism involves an enzyme in the nucleus called poly (ADP-ribose) polymerase (PARP) (figure 1; Pieper *et al.* 1999). PARP has negligible activity under basal circumstances but is activated by DNA damage. PARP uses the oxidative co-factor NAD to donate groups called ADP-ribose to a variety of nuclear proteins, especially PARP itself. These activities expose the damaged DNA molecules to other more conventional repair enzymes. When excess damage to DNA takes place, PARP is massively activated. Its heightened activity consumes the great bulk of NAD in cells. In order to replace the missing NAD, ATP (the universal energy donor) is consumed and the cell dies of energy depletion. While PARP overactivation may be useful in eliminating cells with excess DNA damage, its overactivation in stroke and myocardial infarction might contribute to cell death by energy depletion. Evidence in favour of this notion comes from findings that PARP inhibitors can reduce stroke and myocardial infarct damage in experimental animals substantially. Moreover, mice with targeted deletion of the gene for PARP display 60–80% reductions in stroke damage. PARP inhibitor drugs may be important therapeutic agents and are being explored by the pharmaceutical industry.

3. SCHIZOPHRENIA

Emotional distress is so prevalent that, depending on one's definition of psychiatric illness, more than half of the population has been afflicted at one time or another in their lives. The psychiatric diagnostic and statistical manual contains a broad array of diagnoses. However, one can subsume most of these under three major headings, schizophrenia, manic-depressive illness and anxiety-related disorders. Neurotic and anxiety-associated conditions are most common, especially if one is fairly broad in diagnostic definition. Depression is also prevalent, with serious forms afflicting 10% or more of the population. Rigorous definitions of schizophrenia lead to an incidence of about 1–2% of the population. Thus, schizophrenia is one of the less common psychiatric disorders, with an incidence similar to diabetes. However, it is the most devastating psychiatric disease. Schizophrenia typically appears in late adolescence or early adult life. Victims are so disabled that genuinely productive employment is often not possible for most of their lives. The toll in terms of anguish to the patients and their families is incalculable.

The symptoms of schizophrenia are so foreign to most of us that it is difficult to appreciate their horror. Patients feel that they have lost control of their minds, with thoughts being inserted into their brains. Their delusions are usually terrifying. The common auditory hallucinations are horrifically accusatory. Many psychiatrists characterize the entire adult lives of schizophrenics as a living death. The behaviour of schizophrenics is so disruptive that lives of their parents and siblings are often devastated. Thus, whether calculated in terms of dollars lost to a country's economy or human suffering, schizophrenia may well rank as the number one mental illness.

What is to be done? A plethora of genetic studies over the past 50 years has established a powerful genetic predisposition to the disease. Some of the best evidence comes from twin studies. Monozygotic or identical twins have identical genes, whereas dizygotic or fraternal twins are no more similar genetically than siblings. Twin studies provide a useful approach to separating environmental from genetic factors. If one of a pair of monozygotic twins is schizophrenic, the likelihood of the other twin also

developing schizophrenia is 60–80%. For dizygotic twins the comparable 'concordance' rate is only about 15–20%. Studies of monozygotic twins separated at birth have also been informative. If one co-twin becomes schizophrenic, the odds are greater than 50% that the other will also succumb to the disease.

Many research groups have been searching for the presumed abnormal gene(s). Strategies employed are similar to those that have successfully identified aberrant genes in diseases such as cystic fibrosis and muscular dystrophy. Briefly, one obtains blood cells from most members of large families that have a high incidence of the disease. Then, one seeks commonalities in the genes of individuals inflicted with the illness. There have been hints of specific abnormalities on particular chromosomes of schizophrenics but nothing definitive. In coming decades I would expect researchers to identify one or more genes whose aberrant structure leads to a predisposition to develop schizophrenia. As is thought to be the case for cancer, developing schizophrenia may require alterations in more than one gene. Also as in cancer, schizophrenia may be a collection of different diseases each with its own abnormal genes. Such a situation would make the search for the 'cause' an excruciating challenge.

Even if one finds the abnormal gene, this does not necessarily translate into curative therapy. The abnormal gene might code for an enzyme or a receptor so that one could develop drugs to activate or inhibit the target protein. However, it is equally possible that the disease gene codes for a protein whose function is not readily appreciated. An analogous case involves Huntington's disease. After a 15-year long collaborative investigation by a number of excellent groups, the pathologic gene in Huntington's disease was identified as one coding for a single, large protein called huntingtin. Unfortunately, links between huntingtin, disease symptoms and therapy have been elusive.

The disease is characterized by deterioration of selected brain regions, most prominently the caudate nucleus, which regulates the coordination of movement. Hence, patients with Huntington's disease display grossly abnormal movements, which led to the earlier name for the condition, 'Huntington's chorea'. One would expect huntingtin to be highly concentrated in the caudate nucleus. But huntingtin occurs in similar concentrations throughout the brain, in glial cells as well as in neurons, and in tissues all over the body. This is perplexing, considering that the symptoms are brain specific. The abnormality in the huntingtin protein in patients involves large, repeated sequences of the amino acid glutamine. Researchers speculate that destruction of brain tissue in patients might arise from a 'gain of function' in which the glutamine repeats bind to other proteins which are brain specific. No direct evidence that such huntingtin-associated proteins cause disease symptoms has yet been obtained. The example of Huntington's disease teaches us that finding the abnormal gene is often only the first step in a search for pathophysiology and novel therapy.

The history of drug development for schizophrenia reflects the value of serendipity and may point towards future advances.

Table 1. *History of chlorpromazine development*

1883	phenothiazine synthesized as a dye
1946	promethazine introduced as a phenothiazine antihistamine
1950	promethazine employed as a preanesthetic calming drug by Laborit
	synthesis of chlorpromazine, a very sedating phenothiazine antihistamine
1951	Laborit replaces promethazine with chlorpromazine for pre-surgical anesthesia
1952	Delay and Deniker observe antipsychotic actions of chlorpromazine

(a) *Neuroleptic drug development*

The introduction of the phenothiazine neuroleptic drug chlorpromazine in the treatment of schizophrenia is regarded by many as the most important event in 20th-century psychiatry (table 1; Swazey 1974). Prior to chlorpromazine most schizophrenics could look forward to a lifetime in a state mental hospital. Though chlorpromazine and its successor neuroleptic drugs do not 'cure' the disease, they favourably influence the fundamental symptoms so much that most patients can function reasonably well. Together with the advent of the community mental health movement, the neuroleptics have almost literally emptied out the wards of large state mental hospitals and restored to loved ones patients who in earlier generations would have been lost forever.

The term phenothiazine refers to a three-ringed chemical structure with a sulphur (thia) and a nitrogen (zine) in the centre ring flanked by two phenyl (pheno) groups. Phenothiazine was first introduced as a dye, a product of the 19th-century revolution in the dye industry brought about by advances in organic chemistry. The early 20th century witnessed the beginnings of the modern pharmaceutical industry as organic chemists transformed their expertise in modifying chemical ring structures from dyes to drugs. Among the first drugs developed in this effort were agents designed to block actions of the neurotransmitters acetylcholine and epinephrine (the true neurotransmitters of sympathetic neurons, norepinephrine, was not known until the late 1940s). The prototypic anticholinergic drug, atropine, is the active product of the plant extract belladonna, used for hundreds of years in treating various gastrointestinal and other complaints. The discovery by Sir Henry Dale in 1911 of histamine and his prescient conviction that it played a role in allergies led to an interest in drugs that might block the effects of histamine. The first structures evaluated as antihistamines were somewhat related to atropine. One of them, promethazine, incorporated the phenothiazine ring.

In the late 1940s, Henri Laborit, a French neurosurgeon, speculated that the autonomic, involuntary nervous system employs histamine and is disordered during surgical anaesthesia. Accordingly, he decided to use antihistamines as a key constituent in a pre-anesthetic cocktail of drugs. He was struck by the effectiveness of promethazine in calming patients before surgery—not surprising because of the sedating properties of most antihistamines, especially promethazine. He asked the

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Table 2. *How neuroleptics act—sequence of insights*

neuroleptics at therapeutic doses elicit Parkinsonian effects
Parkinson's disease involves dopamine deficiency
neuroleptics block dopamine (D2) receptors
neuroleptics relieve positive not negative symptoms
clozapine relieves negative and positive symptoms
clozapine successors block serotonin 5-HT2 subtype receptors
future: What transmitter systems best explain drug effects? Are they aberrant in schizophrenia?

Rhône-Poulenc Drug Company to provide him with another antihistamine that might be even more sedating. Chlorpromazine had been synthesized as an antihistamine but was discarded because it was much too sedating for daily use. Laborit was so struck by the 'beautific quietude' the drug elicited in his patients that he urged his psychiatrist colleagues Jean Delay and Pierre Deniker to try it in psychiatric patients. Delay and Deniker administered chlorpromazine to manic depressives and found its calming effect remarkable. They published their first paper in December 1952 and, within a year, the drug was being extensively employed. Psychiatrists tried it in numerous conditions and soon appreciated that it exerted a unique antischizophrenic effect (Ayd & Blackwell 1981).

Initially, clinicians thought that chlorpromazine and related drugs were acting primarily as sedatives. However, when minimally sedating neuroleptics were developed, they were equally therapeutic, while non-neuroleptic sedatives were not effective in schizophrenia. Moreover, analysis of symptom responses indicated selective improvement in uniquely schizophrenic symptoms such as delusions and hallucinations. Accordingly, understanding the molecular mechanism of drug activity might shed light on the disease's pathophysiology.

Clues to the mechanism of action of chlorpromazine came in the first clinical studies of Delay and Deniker (table 2). They had no idea as to what would be an appropriate dose and merely titrated patients till they saw benefit. At therapeutically effective doses, which could vary fivefold in different patients, they invariably noticed neurologic side effects mimicking the symptoms of Parkinson's disease. They dubbed the drugs 'neuroleptics' from the Greek meaning 'to clasp the neuron', suggesting that something involving specific neuronal groups in the brain was crucial for therapeutic effects.

Subsequently, work in numerous laboratories established that neuroleptics act by blocking dopamine receptors, especially a subtype designated D-2 (Carlsson 1988; Snyder 1974). The most prominent group of dopamine neurons in the brain terminates in the caudate nucleus and degenerates in Parkinson's disease so that dopamine deficiency is widely regarded as the proximal cause of symptoms. By blocking dopamine receptors, neuroleptics create a functional deficiency of dopamine explaining the Parkinsonian side-effects. By blocking dopamine receptors in areas of the brain controlling emotional behaviour, such as the limbic system, the drugs presumably exert their antischizophrenic actions.

Knowledge that the drugs act by blocking dopamine receptors led to a 'dopamine hypothesis' of schizophrenia, suggesting that excess dopamine activity in the brain is involved in the genesis of symptoms. This notion was supported by observations that amphetamines, which act by releasing dopamine, markedly worsen schizophrenic symptoms. Numerous studies of post-mortem schizophrenic brains and imaging studies of dopamine receptors in patients have failed to find evidence for specific biochemical abnormalities of dopamine systems in schizophrenia. Nonetheless, even if disruptions in dopamine neurotransmission are not etiologic, it seems likely that dopamine plays some role, at least indirectly, in modulation of symptoms. Certainly, designing drugs to interfere with dopamine neurotransmission provides the surest way of obtaining agents that will be therapeutic in schizophrenia.

Despite their importance in psychiatry, the neuroleptics are by no means cure-alls. Even patients who respond extremely well to neuroleptics remain disturbed. Specifically, though their florid hallucinations and delusions—the 'positive' symptoms of schizophrenia—are alleviated, patients remain emotionally detached from the environment. This 'wallflower' syndrome and related symptoms are commonly designated the 'negative symptoms of schizophrenia' and often are the most disabling ones. The first glimmer of effective treatment of such negative symptoms came with the drug clozapine.

Clozapine was designed as a 'me too' neuroleptic (Healy 1996). Because of major side effects it was withdrawn from the market in most countries after having been available for a relatively short period of time. Then a peculiar thing happened. Psychiatrists all over Europe (the drug had never been introduced in the USA) began complaining that they were losing their most valued agent. Patients who failed to respond to conventional neuroleptics would do well with clozapine. More strikingly, clozapine was relieving the negative symptoms, transforming patients who had been emotionally dead for years into relatively warm, interactive human beings. After controlled trials confirmed these unique properties, the drug was reintroduced to the market with appropriate precautions for monitoring the possibility of agranulocytosis (loss of white blood cells) a potentially life-threatening side-effect.

A search began to ascertain molecular actions that might account for the extraordinary effect of the drug on negative symptoms. By simply looking for drugs that would mimic the biochemical and behavioural profile of clozapine in animals, researchers developed a number of clozapine-like agents. In the USA, three are presently marketed: risperidone, olanzapine and quetiapine. These drugs exert beneficial effects on negative as well as positive symptoms and do not cause agranulocytosis, though most psychiatrists feel that they are not as effective as clozapine in dealing with the negative symptoms.

Determining the molecular mechanism whereby clozapine affects negative symptoms should afford major insight into their genesis. Thus far, there have been many theories but no direct proof. One of the best guesses is that clozapine relieves negative symptoms by blocking subtypes of receptors for the neurotransmitter serotonin. Hence, drugs that block this form of serotonin receptor as well as dopamine D-2 receptors in the right proportions might be ideal.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Table 3. *Psychotomimetic drugs as clues to mental illness*

LSD, mescaline and other psychedelic drugs cause psychosis. But their perceptual and cognitive changes do not resemble schizophrenia
amphetamine and cocaine psychoses mimic subtypes of schizophrenia, but there is no classic thought disorder or negative symptoms
phencyclidine (PCP) psychosis faithfully masquerades as schizophrenia, though some say it resembles mania. PCP is discovered to block NMDA subtypes of glutamate receptors. Glycine and cycloserine, which stimulate NMDA receptors, are antipsychotic

(b) *Drug psychoses*

Drug-induced psychoses provide another approach to understanding schizophrenia without finding the causal genetic abnormality (Hollister 1968). Numerous drugs are psychotomimetic (table 3). For drugs that produce psychoses which closely mimic abnormalities in schizophrenic mentation, one might hope that drug-induced molecular aberrations will closely resemble those of schizophrenic brain. As already mentioned, amphetamines can exacerbate schizophrenic symptoms. Non-schizophrenics ingesting very large doses of amphetamines invariably become psychotic, with disturbances closely resembling an acute paranoid form of schizophrenia. Some researchers have used this information to butress the dopamine theory of schizophrenia. Others argue that the psychosis caused by amphetamine is not close enough to the symptomotology of schizophrenia to be regarded as a valid model of the illness. Psychedelic drugs, such as LSD and mescaline, cause reproducible psychotic behaviour that once was thought to model schizophrenia but has subsequently fallen into disrepute as a valid mimicker of the illness (Grinspoon & Bakalar 1979).

A potentially more fruitful drug psychosis approach to schizophrenia has emerged in recent years from studies of the drug phencyclidine (PCP) (Carlsson & Carlsson 1990). PCP was first developed as an anaesthetic but was found to be psychotomimetic in human subjects. For many years no one knew how it acted. In the 1980s, research into the influences of glutamate, the major excitatory neurotransmitter in the brain, provided clues. Glutamate acts via a number of receptor subtypes of which one of the most studied is the N-methyl-D-aspartate (NMDA) receptor. The NMDA receptor is an ion channel that admits sodium as well as calcium ions following the binding of glutamate. PCP influences the NMDA receptor at its ion channel site to block transmission. This anti-excitatory effect accounts for the anaesthetic qualities of the drug. For reasons that are not altogether clear, blocking NMDA receptors at this site also leads to psychotic manifestations. Psychiatrists debate whether PCP psychosis resembles schizophrenia, manic depressive illness, or neither. The present consensus is that PCP psychosis is the best drug model of schizophrenia yet developed.

Evidence favouring this model has emerged from therapeutic trials in schizophrenia. The model implies that drugs stimulating NMDA receptors should be therapeutic. Since glutamate is an excitatory neurotransmitter, glutamate derivatives that penetrate into the brain and directly activate the receptor would augment activity at all types of glutamate receptor and might cause seizures. Researchers have taken advantage of sites on the NMDA receptor that are unique to this subtype of glutamate receptor. NMDA receptors cannot function unless a unique site on the receptor that interacts with glycine or the D isomer of serine is occupied. A number of groups have administered large doses of glycine or a derivative, cycloserine, to schizophrenics and obtained notably therapeutic responses.

Where might the NMDA research take us? Genes for the various subtypes of NMDA receptors have been cloned. One might ascertain whether schizophrenics display abnormalities in any of these genes. Even if they do not, the possibility of developing therapeutic agents through this receptor are tantalizing. Glycine and cycloserine, the agents already tested, are unsatisfactory drugs. They are electrically charged and do not penetrate the blood–brain barrier very well. Derivatives that have better drug-like qualities may be more effective therapeutic agents and provide tools to further probe the NMDA system in patients. Positron emission tomography scanning and other imaging strategies permit us now to readily quantify and localize neurotransmitter receptors in humans. Such imaging studies comparing schizophrenics and control subjects may be useful.

4. DRUGS OF ABUSE

One major change in societal attitudes towards drugs in the final decade of the 20th century involves judgements about the importance of various classes of drugs. Traditional thinking holds that drugs are important if they relieve symptoms of major illnesses. With the advent of 'managed care' and other pressures on medical costs, a new discipline, 'pharmacoeconomics', has emerged. Pharmacoeconomics attempts to quantify the exact financial impact of each drug. For instance, a drug that relieves congestive heart failure will justify its cost if it reduces the duration of hospitalization.

Viewed from the perspective of pharmacoeconomics, a case can be made, especially in the USA, that the most important pharmaceutical agents are not those that treat disease but those that are employed recreationally and usually illicitly, drugs of abuse. Life for most drug addicts is so dominated by their chosen agents that they do not work and require support from the welfare system. More importantly, addicts often devote their lives to criminal activities that pay for the drug habit. In 1995, it was estimated that 80% of the inmates of federal and state prisons in the USA were there because of crimes related to drug abuse. The cost to the USA from the loss of property associated with this criminal activity is estimated to be US$50 billion per year. For heroin users, crime is focused on securing money to pay for drugs. By contrast, cocaine renders its users paranoid and aggressive, leading to crimes of violence, including senseless assaults and murders. Since most drug-related crime occurs in inner cities, the resulting devastation of civil society has lead to desertion by many citizens of the central cities of the USA. The financial loss from this exodus is so great that its financial impact is probably incalculable.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

(a) *Definitions and varieties*

Drug abuse is a term generally used to designate the self-administration of a drug for recreational rather than therapeutic ends. The term 'abuse' implies that there are adverse effects of such drug use. Agents employed may be legal, such as alcohol and nicotine, or illegal, such as marijuana, heroin and cocaine.

The term 'addiction' implies the repeated use of drugs leading to tolerance, physical dependence and craving. Tolerance simply means that a chronic user will require more of a drug to obtain the desired effect than when the agent was first employed. Because drugs are metabolized more rapidly with chronic use, such 'metabolic tolerance' can occur with non-abusable agents such as antibiotics. With psychoactive drugs, one gets tolerance even when brain and blood levels of the drug are the same in the addicted and naive subject, a phenomenon called 'cellular tolerance'.

Dependence refers to the existence of withdrawal symptoms when drug administration is abruptly terminated. Withdrawal symptoms can be physical, such as the shivering and trembling following withdrawal of opiates, or psychological, such as the depression displayed by individuals stopping the chronic use of cocaine. Thus, there has been differential designation of 'physical dependence' and 'psychological dependence'. Older definitions of addiction required the presence of physical dependence. Since there are not many obvious physical symptoms of withdrawal from cocaine, some writers maintained that cocaine was not technically an addicting drug. Amphetamines, whose actions are quite similar to those of cocaine, were also designated as 'non-addicting' by their manufacturers. However, most authorities now agree that psychological and physical dependence are of comparable importance.

If tolerance and dependence were the totality of addiction, then we would not have a pervasive problem. One can readily withdraw an addict from his or her drug and after a period of weeks, withdrawal symptoms abate. Heroin addicts incarcerated at federal addiction centres such as those in Lexington, KY, USA, are maintained in the drug-free state for one or two years. Unfortunately, when they are returned to their home environment, they often return just as rapidly to the use of the offending drug. Some of this recidivism is due to socioeconomic forces. However, a number of studies have substantiated that individuals return to drug abuse because of compulsive drug-seeking behaviour, which appears to have a biological basis, and which can be demonstrated in various animal species including rodents. Thus, compulsive drug-seeking behaviour is a crucial component in the definition of addiction. It accounts for well-known truisms such as the inability of abstaining alcoholics to take even a single drink for fear of triggering a return to alcoholism.

What are the major classes of addicting drugs? Alcohol and nicotine are probably the most widely abused agents and, in many ways, are more destructive of human life than more tainted substances such as heroin and cocaine. Alcohol is thought to act by facilitating the actions of the major inhibitory neurotransmitter in the brain, γ-aminobutyric acid (GABA) and by diminishing the effects of glutamate, the major excitatory neurotransmitter in the brain. The most extensive research favours a primary action by facilitating the effects of GABA on its receptor sites. In this way, ethanol appears to act in the same or closely similar fashion to other 'downer' drugs such as barbiturates and benzodiazepines (Valium and related substances).

Nicotine is the psychoactive ingredient of cigarettes and definitely accounts for the addicting quality of cigarette smoking. Nicotine mimics the neurotransmitter acetylcholine at one of the subclasses of the nicotinic acetylcholine receptors. Nicotinic stimulation causes enhanced alertness, an apparently beneficial effect, which may account in part for the attraction of cigarettes. When first administered to an individual, nicotine also causes unpleasant dizziness and nausea, but individuals who become regular cigarette smokers quickly become tolerant to these effects. Nicotine probably causes greater compulsive drug-seeking behaviour than any other addictive substance, as it is more difficult to withdraw cigarette smokers than users of any other drug of abuse.

Opiate drugs encompass morphine and its many synthetic derivatives, including heroin. Opiates act by mimicking endogenously occurring morphine-like neurotransmitter peptides termed the encephalins or endorphins.

Many other mind-altering drugs are abused, but most of them are not truly addicting. Examples include marijuana and the psychotomimetic drugs such as LSD and phencyclidine, also known as PCP or angel dust. Though many people use these agents on a regular basis, withdrawal symptoms are minimal and there is little compulsive drug seeking.

(b) *Approaches to treatment: focus on cocaine*

The major classes of abused drugs described above cause different subjective effects and act via different receptor systems. However, those that are addicting all display the same formal properties of tolerance, dependence and compulsive drug seeking. All of them are, by definition, 'rewarding' in that they make people feel 'good' and lead humans and animals to go to considerable lengths to obtain drugs for repeated self-administration. Researchers have looked hard to identify commonalities among these drugs at a molecular level. There is a body of evidence suggesting that a single brain region, the nucleus accumbens in the 'emotional' limbic area of the brain, is a major reward centre for these drugs and uses the neurotransmitter dopamine to secure these effects. In animal studies, lesions of the nucleus accumbens and/or destruction of its dopamine neurons greatly diminish drug-seeking behaviour for numerous reinforcing agents, including nicotine, alcohol, cocaine, and amphetamines and barbiturates.

A molecular basis for addiction has been notably elusive. At the time of writing, there is no accepted mechanism to account for the major features of addiction. With the rapid escalation of new findings in the field, I suspect that the coming one or two decades may well witness major breakthroughs into an understanding of addiction. Such insights may then lead to new therapeutic agents that could influence the addictive process for all drugs of abuse.

In terms of agents to treat drug abuse, the one success story involves methadone for heroin addiction. In the 1960s the pharmacologist Vincent Dole and his wife Marie Nyswander administered large doses of the opiate methadone as replacement therapy for heroin. Methadone is orally active and long acting so that a single oral dose will provide high blood levels and greatly diminish or abolish craving for a period of 24 h. By contrast, heroin must be injected intravenously and is so short acting that 2–4 h following an injection, the addict is already experiencing withdrawal symptoms and seeking frantically for a new dose. Because of this, a heroin user's life is dominated by his or her drug. The methadone maintenance programme developed by Dole and Nyswander, now universally employed in the USA and Europe, has greatly diminished the adverse sequelae of heroin addiction. Once stabilized on methadone, an addict can lead a fulfilling personal and professional life. Being addicted to an opiate does not per se interfere with one's functioning. Indeed, many physician addicts have led effective lives for decades. Though some individuals are concerned that methadone treatment merely replaces addiction to one opiate with addiction to another one, the consensus is that the treatment is of immense value both to the addicts and society. The fact that the patients must remain on methadone for many years is regarded as no worse than the requirement of a diabetic for daily doses of insulin.

The situation with cocaine is quite different. Presently there is no adequate treatment for cocaine addiction. However, recent advances in understanding the drug's molecular actions offer hope. Cocaine inhibits the reuptake inactivation of the neurotransmitter dopamine. Most neurotransmitters, including dopamine, are inactivated after release by being pumped back into the nerve ending that had released them. By blocking this 'dopamine transporter' cocaine facilitates the synaptic actions of dopamine, especially in the limbic system. Studies employing mice with targeted deletion of genes for neurotransmitter transporters indicate that inhibition by cocaine of the serotonin transporter may also contribute to its actions.

Uhl and associates have provided insight into exactly how cocaine influences the dopamine transporter, suggesting potential treatments for cocaine addiction (Kitayama *et al.* 1996). Cocaine does not compete directly at the dopamine recognition site of the transporter (figure 2). Instead, it binds to a separate site which indirectly or 'allosterically' influences the conformation of the dopamine recognition site to inhibit dopamine transport. Thus, in principle, one should be able to develop a drug that would compete with cocaine for its recognition site but not influence that site in the way that cocaine affects it. Instead, such a drug would merely passively occupy the cocaine recognition site and block the access of cocaine. Such a drug would be regarded as a cocaine antagonist. It could be employed in treating cocaine overdoses. In theory, one might be able to use a 'pure' cocaine antagonist to treat addicts such that whenever they administer the drug they would not feel its effects and would soon lose interest. Of course, the addicts might simply not take the antagonist drug so that it would be of use only if a formulation were developed that could be implanted and act for one or two months. Alternatively,

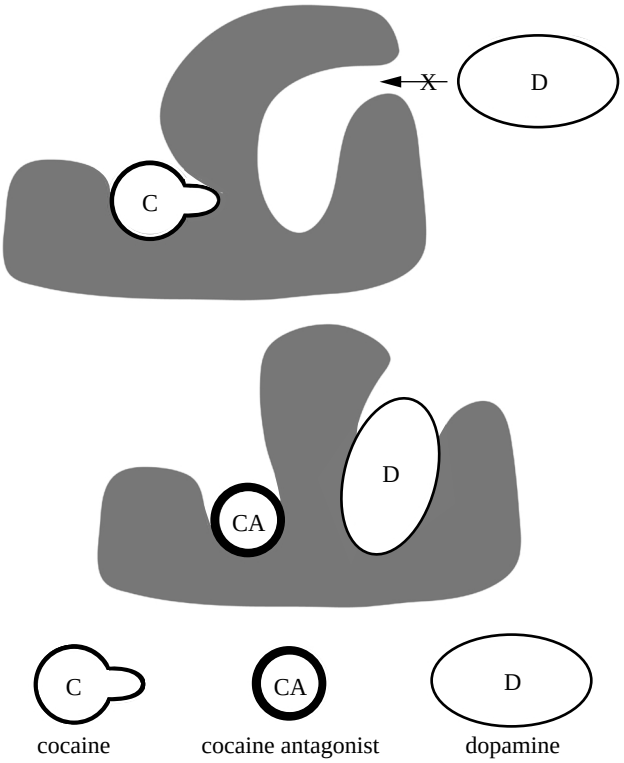

Figure 2. Model of dopamine (D) transporter. The transporter possesses separate but overlapping recognition sites for DA and cocaine. Cocaine inhibits DA transport indirectly as its binding alters the conformation of the DA site to block DA access. A cocaine antagonist binds to the cocaine recognition site but does not elicit any conformational change in the DA site so that cocaine effects are blocked while DA transport remains normal.

one might develop an agent that partially mimics the effects of cocaine to provide a mild level of behavioural stimulation but also acts as an antagonist to prevent the effects of self-administered cocaine. Such mixed agonist–antagonist drugs exist already in the opiate system and are being evaluated as alternatives to methadone treatment. Limited studies in rodents indicate that cocaine antagonists can be developed which, by themselves, have no behavioural effects but which interrupt the influences of cocaine.

Since cocaine is presently regarded as the most dangerous of addictive drugs, approaches to its control by new pharmacologic agents would be a great boon. Rapidly accelerating research into such drug development suggests that within one or two decades, the scourge of cocaine will be abated.

5. CONCLUSIONS AND NEW DIRECTIONS

I have deliberately restricted my coverage to a few topics that illustrate important themes that are likely to lead to major drug advances in the coming century and to diseases that I know best. It seems to me that understanding cell death will provide molecular tools to impact the most common and debilitating diseases, such as cancer, stroke and myocardial infarction. Advances in pinpointing drug-sensitive enzymatic steps in the cell death process have been breathtaking. A decade ago next

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

to nothing was known about this area. Now, an extensive cascade of programmed cell death pathways has been elegantly mapped, and what we know today appears to be the tip of an iceberg. Mental illness poses more complex challenges, which I have addressed through a historical perspective.

For want of space I have not addressed one other broad area that impacts many diseases, angiogenesis. Angiogenesis has been extensively studied in cancer research. Tumours cannot develop unless supported by the growth of new blood vessels. Anti-angiogenic agents do reduce tumour growth in various animal models. Conversely, lack of blood vessel perfusion of tissue is pathogenic in coronary artery disease and in peripheral vascular disease. A number of protein growth factors stimulate blood vessel growth. Examples include basic fibroblast growth factor and vascular endothelial growth factor. In animals, these agents cause sprouting of new blood vessels in the coronary artery system and peripheral vascular beds, and augment blood flow.

Stroke and myocardial infarction are specific disease entities in which pathogenic substances that cause cell death have been identified, such as glutamate, nitric oxide and various oxygen free radicals. Drugs affecting these processes offer dramatic relief in animal models. The next decades will probably witness the development of even better agents and their application in humans. Except for modest therapeutic effects of TPA, there is no treatment at all for vascular stroke, the third leading cause of death in the USA. I expect that alleviation of stroke damage will be one of the most important therapeutic advances in the next few decades.

Neurodegeneration occurs in numerous conditions besides stroke. Such entities include Huntington's and Alzheimer's diseases. Alzheimer's may well yield to molecular interventions in the coming decades, though the path to new drugs is not as clear-cut as for stroke. There is growing acceptance that the deposition of amyloid plaques is key for the pathophysiology of the disease. Amyloid plaques result from the deposition of a 40–42 amino-acid peptide, designated amyloid-beta. The amyloid-beta peptide derives from improper processing of a large precursor protein, designated APP.

Although the majority of cases are sporadic and non-familial, the most important advances have come from the relatively rare genetic forms of Alzheimer's disease. Several forms of familial Alzheimer's disease have been ascribed to specific mutations in APP processing. Even more common forms of familial Alzheimer's disease have been ascribed to mutations in a different family of proteins, the presenilins. Recent evidence indicates that presenilins play a role in processing APP, as pathogenic abnormalities in the presenilins lead to increased formation of the amyloid-beta peptide. A third predisposition to Alzheimer's disease involves increased formation of one of the subtypes of the common blood protein apolipoprotein-E (ApoE). Individuals with high levels of ApoE4 have a high incidence and early onset of Alzheimer's disease, whereas individuals with high levels of ApoE2 are protected. Interestingly, the different subtypes of ApoE also affect APP processing.

Thus, Alzheimer's disease reflects a common phenotype, which can be brought about by different biochemical pathways. One strategy for developing therapeutic agents would be to inhibit the enzymes that give rise to the abnormal plaque-forming processing of APP. Despite vast efforts in numerous laboratories, the exact enzyme proteins have yet to be identified, although the processing steps have been delineated. A step designated alpha-secretase provides physiologic processing of APP. Pathologic processing to the amyloid beta-peptide involves the actions of two enzymes referred to as beta- and gamma-secretase, respectively. Most researchers feel that inhibitors of beta- and/or gamma-secretase will block the formation of the amyloid-beta peptide and retard or prevent the onset of the disease. Though the enzymes have yet to be cloned, several pharmaceutical companies have already identified inhibitors of the beta- and gamma-secretase, which can lower amyloid-beta peptide levels in rodent brain.

In contrast to the well-defined therapeutic strategies in cancer, myocardial infarction, stroke and neurodegenerative diseases, things are far murkier in psychiatry. Numerous groups seem to be coming close to the aberrant genes in schizophrenia and manic depressive illness. However, there have been many false starts. Even if such genes are identified, they may not lead to meaningful therapeutic strategies. Though fundamental understanding of psychiatric diseases lags behind the neurologic disabilities, we already have valuable therapeutic agents. I have not addressed the area of drugs and depression. Effective antidepressants have been available since the mid-1950s and the recent generation of selective serotonin reuptake inhibitors has provided therapeutic benefit with minimal side effects. Pharmacologic strategies continue to point towards novel modes of obtaining more effective and safer drugs so that suffering from these diseases may be considerably alleviated before we have any fundamental understanding of their causation.

In summary, the second half of the 20th century has witnessed a revolution in the development of important therapeutic agents for many different diseases, especially those afflicting the nervous system. The escalating rate of scientific advance portends far more effective drugs, many of which will probably revolutionize treatment and perhaps lead to cures of the major scourges of the human species.

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REFERENCES

Ayd, F. J. & Blackwell, B. (eds) 1981 *Discoveries in biological psychiatry.* Philadelphia, PA: Lippincott.

Carlsson, A. 1988 The current status of the dopamine hypothesis of schizophrenia. *Neuropsychopharmacology* **1**, 179–180.

Carlsson, M. & Carlsson, A. 1990 Interactions between glutaminergic and monaminergic systems within the basal ganglia—implications for schizophrenia and Parkinson's disease. *Trends Neurosci.* **13**, 272–276.

Grinspoon, L. & Bakalar, J. 1979 *Psychedelic drugs reconsidered.* New York: Basic Books.

Healy, D. 1996 *The psychopharmacologists*. London: Chapman & Hall.

Hollister, L. E. 1968 *Chemical psychoses*. Springfield, IL: C. C. Thomas.

Kitayama, S., Morita, K., Dehi, T., Wang, J. B., Davis S. & Uhl, G. R. 1996 Dissection of dopamine and cocaine binding sites on the rat dopamine transporter expressed in COS cells. *Ann. NY Acad. Sci*. **801**, 388–393.

Lincoln, J., Hoyle, C. H. V. & Burnstock, G. 1997 *Nitric oxide in health and disease*. Cambridge University Press.

Lipton, S. A. & Rosenberg, P. A. 1994 Excitatory amino acids as a final common pathway for neurologic disorders. *N. Engl. J. Med*. **330**, 613–622.

Pieper, A. A., Verma, A., Zhang, J. & Snyder, S. H. 1999 Poly (ADP-ribose) polymerase, nitric oxide and cell death. *Trends Pharmacol. Sci*. **20**, 171–179.

Snyder, S. H. 1974 *Madness and the brain*. New York: McGraw Hill.

Swazey, J. P. 1974 *Chlorpromazine in psychiatry*. Cambridge, MA: MIT Press.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES