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Use of biomarkers in the discovery of novel anti-schizophrenia drugs

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Schizophrenia is characterized by a diverse symptomatology that often includes positive, cognitive and negative symptoms. Current anti-schizophrenic drugs act at multiple receptors, but little is known about how each of these receptors contributes to their mechanisms of action. Screening of novel anti-schizophrenic drug candidates targeting single receptors will be based on biomarker assays that measure signalling pathways, transcriptional factors, epigenetic mechanisms and synaptic function and translate these effects to behavioural effects in animals and humans. This review discusses current states of the validity of biomarkers in the identification of novel anti-schizophrenic drug candidates.

Introduction

Drug discovery for schizophrenia is hampered by poor success rates. The lack of progress made in bringing better and safer medicines to the schizophrenic patient is because schizophrenia is a complex disease characterized by a diverse symptomatology that is impossible to treat with a targeted approach. The available agents are efficacious for psychosis but do not adequately address other core domains of schizophrenia psychopathology (namely, negative symptoms and cognitive impairment). Hence, novel drugs acting via a new mechanism of action that alleviate the cognitive impairment are badly needed. Furthermore, the phenotypic complexity of this disease limits our ability to form a simple hypothesis and define biological measures [1].

The current medical treatment of schizophrenia consists of drugs acting at multiple receptors, but how the modulation of each of these receptor targets contributes to the anti-psychotic effects is still poorly understood, and the discovery of new anti-psychotic drugs has – to some extent – been serendipitous. Evidence from genetic linkage and association studies has mounted to suggest that some susceptibility genes are etiologic factors for more or less specific illness subtypes, but a causal correlation to a single gene product has not been identified. There is no direct link between the targets through which the current drug act and the susceptibility genes, so it remains unresolved which intracellular proteins mediate the beneficial effect of the treatment.

The next generation anti-schizophrenic drugs are believed to modify targets that interact with the neuronal processes that stabilize cognitive processes in the prefrontal cortex. However, drug discovery for cognitive disorders is particularly troublesome, for several reasons. One major problem is that the only key in validation of putative new targets and identification of new chemical entities with relevant and strong efficacies has been various animal models. Given that schizophrenia is a neurodevelopmental disease involving higher functions that are unique to humans; the modelling of the disorder in less cognitively developed species (such as rodents) represents a considerable challenge. Furthermore, animal models have been backvalidated using known drugs. This approach contains the obvious risk of discovering novel molecules without additional therapeutic benefits. To take novel steps in the research strategy, the industry has to go along new paths and apply new methods to discover innovative medicines for schizophrenia. There is no doubt that discovery and development of novel anti-schizophrenic drugs will take advances in studying the effects on biomarkers and correlate these effects to changes in behaviour. For example, novel classes of anti-schizophrenic drugs are developed based on a battery of *in vivo* models that measure signalling pathways, transcriptional factors, neurochemical changes and behavioural

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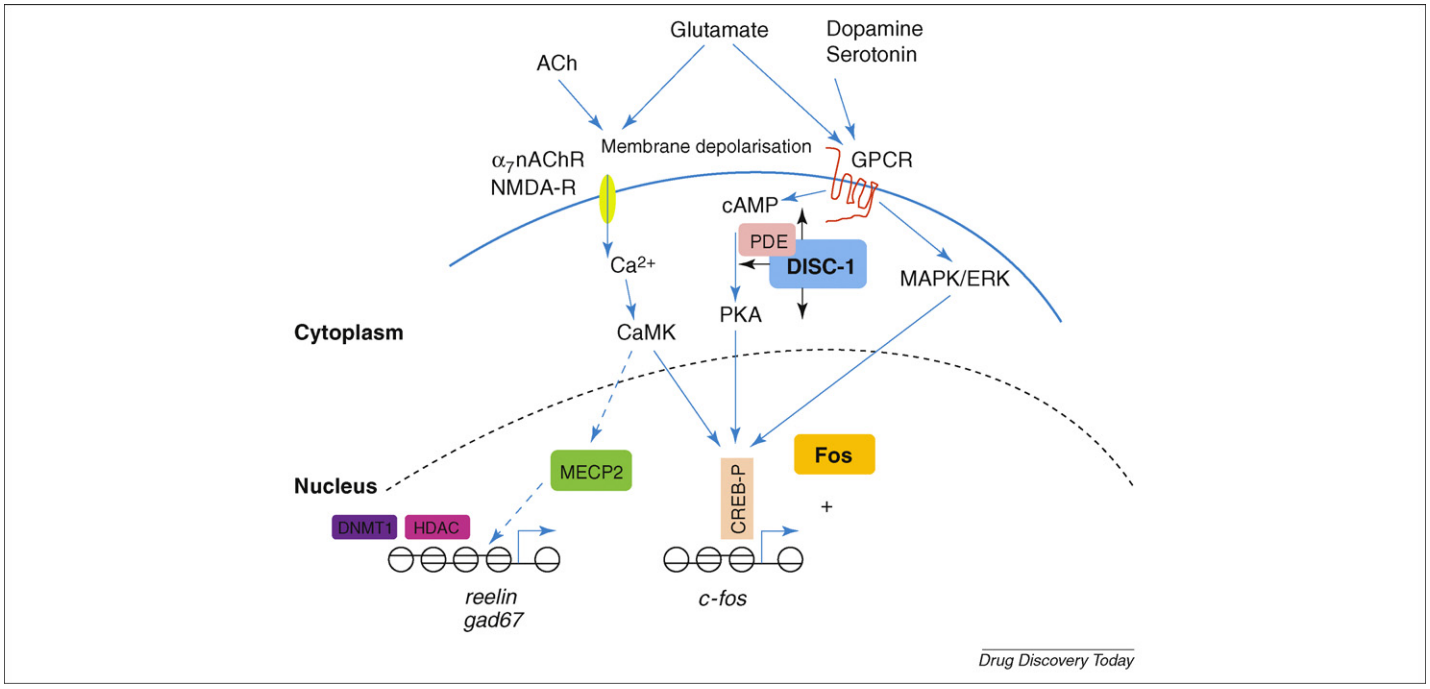

FIGURE 1

This figure illustrates examples of biomarkers that are directly affected by anti-schizophrenia drugs. Schematic illustration of signalling pathways important for mediating the effects of anti-psychotic drugs targeting either ligand-gated or G-protein-coupled receptors (GPCRs). These signalling pathways are regulated by interacting proteins and activate transcription factors that regulate gene expression and, ultimately, also modulate epigenetic events. One example is the cAMP–PKA pathway regulated by several GPCR drug targets that is modulated by DISC1–PDE complexes and subsequently leads to phosphorylation of the transcription factor cAMP response element-binding protein (CREB-P). CREB-P is positively regulating the expression of the *fos* gene, which is an important target for drug action. Other lines of transcriptional regulation are considered to involve activation of ligand-gated Ca^{2+} channels leading to stimulation of the calmodulin kinase (CaMK). This kinase not only phosphorylates CREB but also phosphorylates methyl CpG-binding protein 2 (MECP2), which releases the repressor and stimulates the expression of DNMT1 and HDAC.

effects (Fig. 1), and the selection of new candidates will be based on their ability to modify cellular processes in novel animal models.

Susceptibility genes as biomarkers in drug discovery

Several lines of research have investigated the role of single gene products in the pathogenesis of schizophrenia. Genome scans, linkage disequilibrium and association studies are one important line of productive approaches that have resulted in the identification of several vulnerability genes associated with schizophrenia [2,3]. These include mutations in catechol-O-methyl transferase (COMT), neuregulin 1 and disrupted-in-schizophrenia 1 (DISC1).

These three genes are all of particular interest because their gene products can be associated with cellular processes already known to be relevant for schizophrenia. COMT is an enzyme important in the regulation of extracellular levels of dopamine in the prefrontal cortex [4]. Neuroregulin 1 activates the ErbB4 receptor tyrosine kinase and the non-tyrosine kinases Fyn and PyK2, which phosphorylate the NR2B subunit of the NMDA receptor. Interestingly, neuroregulin 1 knock-out mice show behaviours similar to schizophrenic symptoms that can be reversed with anti-psychotics [5]. The microtubule-associated dynein protein DISC1 is strongly associated with schizophrenia in several patient cohorts [6]. DISC1 is particularly interesting because the expression of non-functional forms results in a decline in neurite function *in vitro* and the impairment of cortical function *in vivo*, and the protein interacts with the phosphodiesterase PDE4B, suggesting a possible role in cAMP-dependent signalling [7–9]. This particular PDE subtype is considered to be an important drug target for schizophrenia [10].

Although these rare genetic variants, such as DISC1, each account for only a small percentage of schizophrenia cases, they are highly penetrant and, therefore, might not only serve in the diagnostics of schizophrenia but also provide clues to the pathophysiology of the disease [11]. These gene products, however, are poor drug targets for small-molecule screening approaches.

Another genetic approach would be to study the effect of common variants that are less penetrant and thereby find novel biomarkers; however, in contrast to several somatic diseases, the identification of such variants have proven difficult in schizophrenia and other psychiatric disorders [12].

Biomarkers reflecting neurodegeneration in schizophrenia

Another line of important results come from postmortem studies revealing a reduction in GABAergic interneurons of the prefrontal cortex [13]. These cells are probably a functional class of inhibitory neurons that also contains parvalbumin [13]. The precise mechanisms behind the reduction of these neurons have not yet been solved, but they are an attractive target for novel treatments because the restoration of their function could be key in treatment. Consequently, the biomarkers that are both expressed in these neurons, and their the function is considered to be involved in working memory and attention, would be particularly attractive

for further studies. Molecules involved in neuronal development and in the regulation of synaptic neurotransmission have been strongly associated with schizophrenia and, accordingly, are relevant biomarkers, but these are still widely distributed in the brain and not selectively expressed in the prefrontal cortex [14].

Acute small-molecule screens using biomarkers

Initial attempts to select novel compounds would involve a simple approach in normal animals. This approach would be to detect whether the drug candidate elicits any immediate changes in biomarker expression, structure, localization or function. To this end, administration of anti-schizophrenic compounds to rodents elicits an increase in a subset of immediate early genes (IEG). It has long been known that all compounds with effect on positive symptoms produce and increase the IEG c-Fos expression in the neurons located in the nucleus accumbens, shell region [15]. The exact mechanisms through which these neurons are activated are unclear; however, increase in dopaminergic and glutamatergic neurotransmission within the nucleus accumbens is considered to be important [16]. Thus, the drug action might not take place directly at the activated neurons in the nucleus accumbens but is probably mediated via other neurons in the brain, such as the entorhinal cortex or the ventral tegmental area [17]. Furthermore, there is no direct evidence that induction of c-Fos is directly linked to the beneficial effects of the drugs *per se*. It is tempting, however, to speculate that because all drugs displaying large differences in their pharmacological profiles produce the same effect on an IEG, these drugs exert a convergent action on this particular IEG and that is crucial for their anti-psychotic effect.

Notably, novel substances that are considered to work via non-monoamine receptors are also active in this assay [18]. This includes, for example, nicotinic cholinergic α_7 agonists [19,20]. Again, it is noted that the activation of Fos by this class of compounds occurs even though the binding sites for the nicotinic cholinergic α_7 receptor have not been reported in the ventral striatum. In contrast to many classical anti-psychotics, nicotinic cholinergic α_7 receptor agonists activate neurons in the prefrontal cortex and in the ventral tegmentum, and it is probable that neurons expressing the receptor in these areas are the primary anatomical substrate for the effects seen in the nucleus accumbens.

Small molecules that are highly selective for metabotropic glutamate receptors (mGluR2 and mGluR3) have robust activity in animal models [21]. Recent findings demonstrate that the anti-psychotic-like effects of mGluR2/3 receptor agonists are absent in mGluR2 knock-out mice [22]. Glutamatergic neurotransmission in the prefrontal cortex (PFC) is inhibited by a mGluR2 agonist [23], but it has not been shown whether this effect leads to change in immediate expression of genes.

Another class of promising small molecules for cognitive enhancement for schizophrenia is Phophodiesterase (PDE) inhibitors [10]. These molecules increase the availability of the second messengers cGMP and cAMP. Whereas most drugs only target one transmitter system, PDEs affect intracellular signalling pathways that are regulated by a variety of transmitters. The cAMP/cGMP-dependent signalling pathways are well known, but the specific pathways upstream and downstream of these messengers that are involved in cognitive enhancement are unknown. Mammalian PDEs are composed of 21 genes and are categorized into 11 families based on sequence homology, enzymatic properties and interaction with drugs [24]. The two most interesting PDE families for schizophrenia are PDE4 and PDE10 but, again, how these specific enzymes affect downstream signalling is unknown [25].

Increase in Fos in the nucleus accumbens is a signal of neuronal activation and not a biomarker for a cellular process relevant in schizophrenia. It is known that increases in intracellular Ca^{2+} and protein kinase A lead to an increase in Fos [26]. Furthermore, inhibition of cAMP response element-binding protein (CREB) mRNA blocks haloperidol-induced Fos expression [27]. Despite the fact that Fos has been a useful marker for neuroanatomical induction of cell activity, it has not brought much understanding about the mechanisms by which the drugs work, and we still need to better understand how IEG couples to more long-term neuronal changes. Other signal molecules such as AKT1 and GSK3β have also been shown to be activated, and these are combined with a lower level of AKT1 protein levels in the brain of schizophrenics [28] and perhaps give a more relevant signal. However, we do not yet know whether other classes of anti-schizophrenic drugs exert an action on these biomarkers in relevant brain structures.

In addition, Erk1/2 and CREB are phosphorylated in the prefrontal cortex by nicotinic cholinergic α_7 agonists [29]. Erk1/2 and CREB are linked to synaptic plasticity and memory formation and might also provide a more specific biomarker than c-Fos [29].

Detection of relevant biomarkers under long-term treatment and in pathological models

The IEG products are perhaps good as indicators for the immediate action of anti-psychotic drugs in normal animals, but they are of little help in determining the long-term consequences of treatment in the psychotic situation. Long-term treatment with anti-psychotics results in changes of ΔFosB/FosB, and this expression is maintained even after the end of the drug treatment [30]. FosB is a member of the AP-1 transcription factor family and is considered to play a part in long-term changes in gene expression. More long-lasting markers are attractive but do not necessarily determine any relevant change in treatment. A more productive approach has been to use agents that are psychomimetics in humans and also induce phenotypes in experimental animals that are presumed to model the same. The disturbances in working memory and attention that are the core cognitive symptoms in schizophrenic patients are difficult, if not impossible, to measure directly in rodent models. One of the preclinical models is based on the knowledge that NMDA receptor antagonists, such as phencyclidine (PCP) and ketamine, exacerbate psychosis in schizophrenic patients and produce positive, negative and cognitive symptoms in normal subjects [31]. Repeated treatment with PCP in normal rats produces a phenotype with similarities to schizophrenia in terms of dysfunctional attentional shifts [32]. More importantly, the same treatment produces a reduction in GABA and parvalbumin mRNA expression [33]. We have recently shown that co-administration of the nicotinic cholinergic α_7 agonist SSR180711 can block the reduction in parvalbumin expression [33]. This emphasizes the nicotinic cholinergic α_7 receptor as a promising novel target for cognitive disturbances in schizophrenia [34].

Epigenetic biomarkers

Schizophrenia shares with other psychiatric disorders not only a genetic predisposition and a contribution from environmental factors but also a long-lasting behavioural abnormality. The illness develops gradually and shows a remitting course, and the response to treatment occurs not immediately but over weeks or months. The requirement for the chronic administration of anti-schizophrenic drugs suggests that long-term changes in cellular function are necessary to produce a beneficial effect. It is, therefore, tempting to suggest that the role of change in regulation of gene expression is an important mechanism by which these drugs affect neuronal function.

Epigenetic mechanisms that exert lasting control over gene expression without altering the DNA sequence have attracted considerable interest because these mechanisms could explain the stable changes in brain function that characterize schizophrenia [35]. There is some evidence that epigenetic mechanisms are involved in the pathogenesis of schizophrenia [36,37]. The majority of the work in this area has focussed on the epigenetic alterations of the reelin and glutamic acid decarboxylase (GAD) promoters because these genes have been shown to be down-regulated considerably in the prefrontal cortex of schizophrenics [13,38].

The reelin gene encodes for a glycoprotein that is expressed in GABAergic interneurons; postmortem tissue from patients with schizophrenia contains lower levels of reelin. So far, however, no drugs have been shown to specifically alter the histone modifications of the reelin promoter. In more general terms, treatment with DNA methylating agents elicited psychotic episodes in patients with schizophrenia, and by contrast, agents that reactivate gene expression – such as inhibitors of DNA (cytosine-5-)-methyltransferase 1 (DNMT1) or histone deacetylases (HDACs) – could improve pharmacological treatment for schizophrenia. Experiments on this mouse model of schizophrenia have shed more light on the epigenetic mechanisms related to the regulation of expression of reelin and GAD67 [36]. Cytosine methylation is believed to provide long-lasting effects on epigenetic memory involved in a wide variety of brain disorders [39]. By contrast, histone modification is considered to be a more dynamic modification that responds to acute drug treatments [40]. It has been shown in mice that valproate (HDAC inhibition) can enhance the DNA demethylation effect of clozapine and sulpiride, providing evidence that targeting both epigenetic markers gives a synergistic effect [36].

The epigenetic biomarkers have still not been explored to a major extent, and their usefulness for drug discovery is not known. However, histone remodelling could contribute to the effect of anti-schizophrenic effects. For example, haloperidol and raclopride induce phosphoacetylation of H3 in the mouse striatum at the c-Fos promoter [40]. It is, therefore, highly relevant to investigate whether drug candidates have effects on these parameters.

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