Anti-psychotic drugs
The anti-psychotic drugs, e.g. haloperidol and chlorpromazine, comprise a large group of drugs that are used to treat the symptoms of schizophrenia [1]. The principal therapeutic effect of the drugs is on the positive (psychotic) symptoms of the disorder, although some drugs, e.g. clozapine, are claimed to have effects on the negative symptoms. After the discovery of dopamine as a neurotransmitter in the brain it was shown that the anti-psychotic drugs were interfering somehow with the actions of dopamine in the brain; on the basis of behavioural studies, this seemed to be via a blockade of dopamine receptors. With the advent of assays for the different dopamine receptor subtypes (stimulation of adenylate cyclase for D1-like receptors and ligand binding assays for D2-like receptors; details of nomenclature of dopamine receptors are in [2,3]) it became clear that there was an excellent correlation between the daily dose of a range of drugs used to treat schizophrenia and the affinities of these drugs for the D2-like receptors. Thus it was concluded that the drugs were acting at D2-like dopamine receptors. After the discovery of the different dopamine receptor subtypes by using molecular biological techniques, these D2-like effects could be at D2, D3 or D4 receptors [2]. A consideration of the affinities of the anti-psychotic drugs for these dopamine receptors shows that some anti-psychotic drugs have a high affinity for the D2 and D3 receptors but a rather low affinity for the D4 receptor [3]. This suggests that occupancy of D2 and D3 receptors might contribute to the anti-psychotic effects but occupancy of D4 receptors is not mandatory for achieving an anti-psychotic effect.

Inverse agonism of anti-psychotic drugs
It has been assumed that the effects of the anti-psychotic drugs at the D2-like receptors are to act as antagonists, blocking the actions of dopamine at the synapse. Prolonged blockade is required because several weeks of treatment are needed to achieve an anti-psychotic effect, but it has been assumed that the drugs are antagonists at dopamine receptors [1]. This idea has recently been challenged through the demonstration that a wide range of anti-psychotic drugs act as inverse agonists at D2 receptors expressed in Chinese hamster ovary (CHO) cells [4]. In these cells dopamine inhibits the stimulation of adenylate cyclase produced by forskolin. The anti-psychotic drugs do not only prevent this dopamine inhibition, but on their own they produce a stimulation of adenylate cyclase. Therefore the D2 dopamine receptors in these cells are constitutively active and the anti-psychotic drugs are preventing this constitutive activation. The stimulation of adenylate cyclase by the drugs is dose-dependent and the potencies of the drugs as inverse agonists correlate well with their affinities for the D2 receptor in ligand binding assays. The maximum stimulation of adenylate cyclase is similar for all the anti-psychotic drugs tested, irrespective of their chemical class and therapeutic efficacy. The aminotetralins UH 232 and AJ 76 are a neutral antagonist and a partial inverse agonist respectively. Thus a spectrum of efficacies from positive (agonists) through neutral (antagonists) to negative (inverse agonist) exist at this receptor.

This inverse agonist property of the anti-psychotic drugs should be reflected at the level of receptor-G-protein coupling and indeed a decrease in basal $^{35}$S-labelled guanosine 5'-[γ-thio]triphosphate ($^{35}$S[GTP[S]]) binding is seen in response to these drugs in membranes of CHO cells expressing the D2 receptor (J. Wilson and P. G. Strange, unpublished work).

Similar, although less extensive, results describing inverse agonism were reported for D2 receptors in pituitary cells [5] and in another study in CHO cells [6]. The anti-psychotic drugs have also been shown to possess inverse agonist activity at D3 receptors [7] and also at D1 and D5 receptors [8], so this is not just a property of the D2 subtype.

For several G-protein-coupled receptors, drugs that were thought to be antagonists have been shown to possess inverse agonist activity [9]. To make these observations it is necessary for the receptor to be active in the absence of...
agonist, i.e. it must be constitutively active. The observations outlined above show that the D2 receptor and several other dopamine receptors can be constitutively active in recombinant expression systems. It is currently unclear whether the D2 receptors are also constitutively active in native tissues but there are suggestions of such agonist-independent activation based on the effects of anti-psychotic drugs at D2-like receptors in the brains (striatum) of experimental animals whose dopamine levels have been severely decreased by lesioning [10,11]. In such animals the drugs elicit responses that are the opposite to those seen with dopamine, suggesting inverse agonism and hence constitutive activation of signalling pathways.

Mechanisms of inverse agonism

If the inverse agonist property of the anti-psychotic drugs is related to their clinical actions it is important to try to understand the mechanism of this inverse agonism in biochemical terms. If this can be understood it might help in the design of new drugs with more selective actions.

A popular mechanism for the actions of G-protein-coupled receptors is the extended ternary complex model [9] (Scheme 1). In this model the receptor can exist in a ground state (R), and a partly activated state (R*) that can couple to the G-protein to form R*G. Agonists are thought to act by stabilizing R* by binding with a higher affinity than to R and by stabilizing R*G by binding with an even greater affinity. In the ternary complex (AR*G), GTP binding in place of GDP is catalysed and the ternary complex dissociates, releasing αGTP and βγ subunits of the G-protein to alter effector activity. In principle, therefore, the ability of an agonist to stabilize the ternary complex will be a major determinant of agonist efficacy [12,13]. For some agonists, however, there is evidence that the ternary complex might not be fully active, i.e. GDP/GTP exchange or ternary complex breakdown might be limiting [12,13].

On the basis of these ideas, there are several possibilities for the mechanisms of inverse agonists. To observe inverse agonism there must be basal activity in the receptor system, i.e. R*G formation, GDP/GTP exchange and ternary complex breakdown must occur in the absence of agonist; this basal activity is then suppressed by the inverse agonist. For opioid receptors it has been suggested that inverse agonism is achieved by stabilization of the uncoupled form of the receptor by the inverse agonist (R/R*) at the expense of R*G [14]. In support of this proposal, inverse agonist binding to these receptors is of higher affinity in the presence of GTP, which uncouples receptor and G-protein. For the β2-adrenergic receptor, however, it has been suggested that inverse agonism is achieved by the higher-affinity binding of the inverse agonists to the R state of the receptor at the expense of the R* state [15]. This proposal is supported by the lower affinity of inverse agonists for mutants of the receptor that are thought to favour the R* conformation [15], although some inverse agonists do not exhibit this differential binding [16]. At cannabinoid receptors [17] and also at muscarinic acetylcholine receptors [18] it has been suggested that inverse agonists act by stabilizing an inactive receptor-G-protein complex. There are therefore several possible mechanisms to account for inverse agonism.

For the D2 dopamine receptor this has not been extensively studied. In one study the binding of the inverse agonist [3H]spiperone was resolved into contributions from two classes of site (of higher and lower affinity) and in the presence of GTP only the higher-affinity sites were seen [19]. This is consistent with the binding of the inverse agonist with higher affinity to the receptor uncoupled from the G-protein. In many studies, however, the binding of [3H]spiperone conforms to a model of a single set of sites and indeed, in the study above, the two states were not seen in all experiments. Also the use of the radioligand [3H]spiperone can be associated with artifacts, owing to its very high affinity for the D2 receptors [20]. Another way to approach the mechanism of inverse agonists at this receptor is to determine the affinities of compounds to the receptor coupled to G-protein and to the free receptor. This requires very careful ligand binding assays for clear conclusions to be drawn [20]. One study has used [3H]quinpir-
ole to label the receptor–G-protein complex and [3H]raclopride to label the free and coupled forms of the receptor [21,22]. No major differences in affinity were seen for several compounds with proven inverse agonist activity. It therefore seems unlikely that a preferential binding of the compounds to the free receptor (R/R*) over the coupled receptor (R*G) can be fully supported here. The alternative mechanism, whereby inverse agonists bind preferentially to R over R*, cannot be evaluated until mutants analogous to those described for the β2-adrenoceptor [15] have been constructed.

We have also been examining mechanisms of inverse agonism for the related serotonin 5HT1A receptor. Clear inverse responses have been obtained for this receptor assayed with [35S]GTP[S] binding, and methiothepin and spiperone exhibit inverse agonist efficacy, suppressing, in a dose-dependent manner, the basal level of [35S]GTP[S] binding (D. McLoughlin and P. G. Strange, unpublished work). For spiperone, ligand binding results show that this compound has a higher affinity for the free form of the receptor (R/R*, assayed with [3H]spiperone binding) relative to the G-protein-coupled form of the receptor (R*G, assayed with 3H-labelled 8-hydroxy-dipropylaminotetralin (8-OH DPAT) binding) [23]. This then provides a potential mechanism for the inverse agonist effects for this compound whereby the drug stabilizes the free form of the receptor and thus decreases the basal activation of the receptor. Experiments in which the inverse agonism of spiperone (to inhibit basal [35S]GTP[S] binding) was tested with different GDP concentrations have shown that the potency of spiperone as an inverse agonist varies as expected from the ligand binding results, i.e. as the GDP is increased, so decreasing the formation of the R*G state, the potency of spiperone is increased. These ligand binding and functional results are consistent with spiperone’s achieving its negative efficacy by stabilizing the forms of the receptor uncoupled from the G-protein and destabilizing R*G.

For methiothepin, ligand binding results show that this compound has similar affinities when assayed versus [3H]spiperone or 3H-labelled 8-OH DPAT, indicating that it has similar affinities for the uncoupled (R/R*) states and coupled (R*G) states of the receptor [23]. These observations rule out the kind of mechanism that is seen for spiperone. In functional studies, the inverse agonist potency of methiothepin to inhibit basal [35S]GTP[S] binding is insensitive to changes in the GDP concentration, in agreement with the lack of sensitivity in ligand binding. Simulations of the extended ternary complex model, however, show that even if methiothepin acted via a preferential binding to R of R*, its potency as an inverse agonist might be affected by GDP. Therefore methiothepin is unlikely to achieve inverse agonism by redistributing the different states of the receptor. One possibility is that methiothepin can bind to the receptor whether coupled to G-protein or not, but in the coupled state it renders it unable to activate the G-protein. This is similar to the mechanisms proposed above for muscarinic and cannabinoid receptors [17,18], in which the inverse agonist stabilizes a non-productive receptor–G-protein complex. Therefore the two inverse agonists, methiothepin and spiperone, seem to differ in their mechanisms of action at the 5HT1A receptor.

**Implications of inverse agonism for clinical effects of anti-psychotic drugs**

The results outlined above establish that the anti-psychotic drugs might be inverse agonists rather than antagonists and that this is a property of the drugs that is independent of the chemical class and therapeutic efficacy. This realization has significant implications for the understanding of their therapeutic and side effects. During acute treatment with the drugs the effects will depend on whether there is constitutive activation of the signalling system linked to the receptor. If there is no constitutive activation, inverse agonists and antagonists will behave identically, suppressing the response to dopamine. If there is constitutive activation, an inverse agonist will suppress this constitutive activation, whereas an antagonist will be without effect on the constitutive activation. Both classes of compound will inhibit the actions of dopamine. There is some evidence for constitutive activation of D2-like dopamine receptors in the brain (striatum) [10,11], but little is known about this and it might be that there are different degrees of constitutive activation in different brain regions. If there is indeed constitutive activation in the signalling system, the receptors might be constitutively desensitized [24]. It is then possible that treatment of the desensitized receptors with an inverse agonist might resensitize the receptors. During chronic treatment with an anti-psychotic...
drug it is likely to be significant that the drugs are inverse agonists. It is a common observation that after chronic treatment of experimental animals and humans with these drugs there is an increase in the number of D2-like receptors in the brain. It has been assumed that this is a consequence of the antagonism of the effects of dopamine, treatment of experimental animals with dopamine and other agonists, leading to a down-regulation of the receptors [25]. It might be, however, that this up-regulation of D2-like receptors is a result of the inverse agonist property of the drug; effects of these drugs on D2 receptor number in recombinant cell lines have been reported [26]. It is unclear whether these effects are important for the anti-psychotic effects of the drugs or whether they contribute to the side effects. It has, however, been proposed that these changes in receptor number are important for the effects of the drugs and account for the delay in the therapeutic effects [1]. Somehow the increase in receptor number alters the sensitivity of dopaminergic synapses and the anti-psychotic effect results. If this is so, the property of inverse agonism will be important for the drugs. These ideas can be tested by finding compounds that are neutral antagonists and testing these for anti-psychotic potential. We have identified the aminotetralin UH 232 as a neutral antagonist [4] but so far this compound has not been tested as an anti-psychotic.

The property of inverse agonism might also be important in understanding another paradoxical observation in respect of anti-psychotic drugs. Studies in vivo of the occupancy of brain dopamine receptors by anti-psychotic drugs have shown that these drugs frequently occupy approx. 70% of the receptors under typical dosages [27] and it is difficult to see how such occupancies can give effects on synaptic action if there is substantial amplification of signal via the receptor. One way out of this paradox is to realize that if the drugs are inverse agonists they will achieve effects at occupancies of much less than 100%.

1 Strange, P. G. (1992) Brain Biochemistry and Brain Disorders, Oxford University Press, Oxford

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