CONCEPTUALIZING ADDICTION

Receptor regulation as a unitary mechanism for drug tolerance and physical dependence—not quite as simple as it seemed!

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Abstract
This review examines the development of the unitary hypothesis that both drug tolerance and the drug withdrawal syndrome arise from adaptive regulation of drug receptors in the brain. Although there is still considerable merit in this hypothesis, the author explains that careful evaluation of some of the changes that have been reported suggests that many of these are not good explanations for both aspects of drug dependence. In addition, modern developments in the understanding of receptor mechanisms show that the original hypothesis represents a gross oversimplification of the true situation. Adaptive changes in post-receptor mechanisms are now believed to be at least as important as alterations in the receptors themselves. Equally some receptor proteins, by altering their subunit composition, may be able to adapt to the presence of drugs without producing major changes in the action of the natural transmitter. In conclusion, receptor regulation still seems to play an important role in drug dependence but this role is more complex than was once believed.

Introduction
This is intended as a personal review for non-specialists of a hypothesis that, at one time, seemed capable of explaining some very basic aspects of drug dependence. This hypothesis is that regulation of neurotransmitter receptors in the brain represents a form of “neuroadaptation” to drugs, and that this underlies the phenomena of tolerance and the drug withdrawal syndrome. The concept of neuroadaptation to drugs was probably first articulated by Himmelsbach in the 1940s while working in Lexington, Kentucky where, coincidentally, this review was written. During his studies on opiate addicts Himmelsbach reached the conclusion that tolerance was due to some form of “homeostatic adaptation” in the brain that opposed the effects of the opiate drug. As long as his patients continued to take opiates their brain function remained relatively normal; however, as soon as they were prevented from obtaining their drug, the removal of opiate from the brain left the adaptive mechanism (whatever it was) exposed (or “unbalanced”), and this produced the withdrawal syndrome (Fig.1). This is a simple and brilliant explanation for drug tolerance and physical dependence. It explains many of the clinical and experimental characteristics of drug dependence, and it has dominated mechanistic hypotheses of dependence for more than 50 years. However, Him-
The concept is that the administration of a drug acutely “unbalances” the chemistry of the brain. In order to overcome this effect the brain institutes a homeostatic mechanism, i.e. an “opposing neuroadaptation” that balances the effect of the drug on brain chemistry. While the drug is present in the brain, the system remains in relative balance (i.e. there is evidence of drug tolerance). However, rapid removal of the drug now exposes the adaptation because it is no longer “balanced” by the drug. The resulting functional disturbance is the cause of the drug withdrawal syndrome. In Himmelsbach’s theory, this will continue until the adaptation can be removed and the chemistry of the brain returns to its normal balancing act. Collier’s modification of this hypothesis was to propose that, since drugs act on receptors in the brain, it was logical to suppose that a primary mechanism for neuroadaptation to drugs would be to regulate the numbers of those receptors. This type of adaptation would reduce the effects of the drug, but would also cause alterations when the drug left the brain because the natural transmitters inside the brain also use the same receptors. This modified unitary hypothesis remains implicitly accepted by neuropharmacologists today, but we are beginning to recognize that it represents a gross oversimplification of the complex cellular mechanisms for drug dependence.

A brief history of receptor regulation

Once again this represents a personal view, and many important contributions may have been omitted because of this, or because of insufficient space. Hard on the heels of Himmelsbach’s 1941 hypothesis came a major research finding. This was the “denervation supersensitivity” phenomenon reported by Cannon in 1946.\(^2\) The term describes the increase in responsiveness to the direct application of a neurotransmitter that occurs when a muscle is denervated. This was believed to be an adaptive homeostatic mechanism, but it was not until Emmelin reported in 1961\(^3\) that inhibitory drugs were capable of producing a “pharmacological denervation supersensitivity” that it became clear that it might represent the homeostatic adaptation responsible for dependence in Himmelsbach’s hypothesis. This seems to have been suggested first by Jaffe in 1962 as an explanation for the barbiturate withdrawal syndrome.\(^4\) Three years later, Collier made an extraordinary leap of faith by proposing that the homeostatic adaptations that lead to pharmacological denervation sensitivity and to drug dependence may be based on regulation of the “receptors” that mediate the
Dependence and receptor regulation

effects of the drugs, and those of natural transmitters. Since in 1965 “receptors” were only a vague concept his hypothesis was outrageous and untestable, as well as brilliant. Its publication at that time does credit to the imagination of the Editor of Nature, and to Collier’s powers of persuasion. After this, regulation of receptors rapidly took center stage as a potential explanation for the Himmeslbach hypothesis. However, other cellular adaptations, for example based on enzyme regulation and, much later, regulation of ion channels were also suggested, but captured the popular imagination much less. Then, with the discovery of radioligand binding as a means of measuring receptor numbers in the post mortem brain, Collier’s hypothesis became testable. Evidence came slowly, thus in reviewing the first 10 years of research into receptor regulation, Creese & Sibley reported few findings of direct relevance to drug dependence. There were, however, several reports that numbers of receptor proteins can indeed be regulated by the presence of a drug as a homeostatic mechanism. For example, chronic treatment with dopamine antagonists (the antipsychotic phenothiazines) resulted in an increase in the binding sites representing dopamine receptors in the brain. This type of observation cemented Collier’s “receptor regulation” hypothesis firmly into the psychopharmacological psyche where it has remained, relatively unchallenged, ever since. Put simply, it suggests that the continued presence of a drug acting on specific receptors in the brain causes an adaptive change in the numbers of these receptors, and this causes tolerance to the drug. When the drug is removed, the altered number of receptors now has an impact on the physiological effects of the natural transmitter acting on this receptor, and this functional change causes the drug withdrawal syndrome (Fig. 1).

The beauty of this hypothesis cannot be overemphasized. It combines physiological concepts of adaptation with molecular pharmacology and neurochemistry, and seems capable of explaining drug dependence, one of the most complex of human psychopathologies, at the molecular level. Hypotheses of this aesthetic quality come at a price—they are often accepted much too uncritically and overwhelm the opposition. To an extent this is what has happened with receptor regulation; changes in receptors deemed to be important are often unable to explain tolerance or dependence at even the most basic level. Additionally, hypotheses that attempt to explain tolerance and dependence at the supracellular level, for example by alterations in the “wiring” of the brain, as suggested by Martin in 1966, tend to have been ignored. Nevertheless, receptor regulation in response to drugs of dependence is undoubtedly important in some situations, and the hypothesis provided an enormous stimulus to basic research into mechanisms of drug dependence.

What is receptor regulation?

The regulation of receptor proteins represents physiological homeostasis at the cellular level. Receptor proteins on the surface of nerves are essential for the normal function of the brain because they receive the messages from neurotransmitter molecules that are responsible for all communication between nerves. It is believed that, during development each type of nerve is “programmed” to expect a certain level of input from each transmitter to which it can respond. If this input should stray outside these limits for a significant period of time, then the nerve appears to “sense” that something is wrong, and tries to restore normality by regulating the number of receptor proteins on its surface. Thus, for example, the theory suggests that if nerve A is receiving too few impulses via a specific transmitter because some of the nerves in its vicinity that release this transmitter have died, then nerve A eventually responds by increasing the number of transmitter receptor proteins for this transmitter. Because this “receptor upregulation” increases the chance of transmitter molecules finding an appropriate receptor, this tends to restore the nerve’s response back toward normality. In Cannon’s original terminology, the nerve has become “supersensitive” to the transmitter as a response to relative “denervation”. In this case receptor regulation is therefore an essential homeostatic mechanism, and plays a vital role in maintaining normal brain function in the face of loss of nerves during ageing, or after damage caused by trauma, stroke or disease. Conversely, nerves sometimes also need to protect themselves against overstimulation by neurotransmitters. For example, the major transmitter that excites nerves in our brains is the amino acid, glutamate, and overstimulation of receptors for this transmitter can actually cause nerve cells to...
die via “excitotoxicity”. Simply in order to survive, nerves need mechanisms to reduce their response to excessive levels of this transmitter, and a potential mechanism is for nerves to reduce (or “downregulate”) the number of their receptor proteins for this transmitter. These physiological adaptive mechanisms are thus entirely beneficial under normal circumstances. Unfortunately, changes that may be excellent adaptations physiologically are often completely futile, or even damaging, as responses to drugs.

How do nerves regulate their receptors?
Although we know a great deal about the circumstance in which upregulation or downregulation of receptor proteins by nerves occurs, we are distinctly hazy as to how nerves achieve this. We do know that the receptor proteins are synthesized inside the nerves themselves as a result of signals sent to the genes that encode the proteins. The explanation that suits the “homeostatic” concept best is that the receptor protein itself generates the signal that controls the expression of its own gene. In this “gene regulation” hypothesis, increased activation of a receptor by an agonist drug would send a signal to the gene causing a suppression of the synthesis of the receptor. This would eventually result in a downregulation of these receptor proteins on the surface of the nerve, as the existing receptor proteins gradually “wear out” and are degraded. Conversely, a marked reduction in receptor activation (for example, in the presence of an antagonist drug) would reduce the signal suppressing the gene for the receptor protein. This would then result in an upregulation of the number of receptors as newly synthesized proteins were synthesized and transported to the surface of the nerve.

Once again, the simplicity and beauty of this gene regulation scheme has tended to overshadow the alternatives but, as we become more familiar with the real rather than the hypothetical situation, the alternatives often appear to be just as important. They include increased or reduced rates of degradation of the existing proteins. These mechanisms, like gene regulation, probably require at least several hours or days before they make much difference to nerve cell function. In addition, there may be much shorter-term solutions for the nerve—for example, it may be able to downregulate receptor proteins rapidly simply by removing them from its surface and “sequestering” them in stores somewhere inside the cell. Conversely, nerves may be able to up-regulate receptors by taking pre-formed receptors out of these stores and re-inserting them in the surface membrane. These latter types of receptor regulation appear to be much faster than mechanisms necessary to explain the slow development of drug tolerance and dependence, but this does not mean they are irrelevant. They could, for example, play important roles in limiting the duration of the withdrawal syndrome, and they could be responsible for rapid reinstatement of dependence if relapse occurs after withdrawal. These important possibilities will be discussed later.

So far, receptor regulation has been considered only in terms of alterations in numbers of receptor proteins on the nerve surface. However, alterations in receptor function can occur independently of alterations in numbers of receptors, and this represents another common variation in the response to drugs. Thus, many types of receptors produce their functional effects by causing metabolic changes inside the nerve cell (these are classified as “metabotropic” receptors). For example, some receptors for opiate drugs are linked to inhibition of a key intracellular enzyme, adenylyl cyclase. The nerve can prevent the effects of the opiate drugs on this system by either reducing the number of the receptors (as discussed above), or by reducing the ability of the receptor to link (or “couple”) to adenylyl cyclase, or by increasing the amount of adenylyl cyclase itself. All of these very different changes have been reported under different conditions. They may represent responses to different regimes of exposure to opiates, or simply responses of different types of nerve to the same opiate exposure. In general “metabotropic” receptor systems have a variety of different mechanisms by which they can be regulated, and nerves seem to take advantage of many of these to produce the required homeostatic response. However, as soon as these adaptations involve mechanisms beyond the receptor protein itself, they begin to have additional repercussions for nerve cell function. This is so because other transmitter receptor systems also use the same “coupling” and intracellular enzyme systems to change the function of the nerve cell. This overlap means that an adaptation in response to a drug acting on one specific transmitter system
can have important “heterologous” consequences for the action of other transmitters as well. Conversely, nerves sometimes seem to adapt to drugs by altering the activity of the other transmitter receptor systems, but without directly altering the receptors which are the primary target for the drug. By this stage, the simplicity and elegance of the original concept of receptor regulation have disappeared. Inevitably, these heterologous adaptations are much less easy to predict and to understand than “homologous” adaptations that simply affect the primary drug receptor. As a consequence heterologous adaptations are less studied, but this does not mean that they are any less important in drug dependence.

The metabotropic receptors described above are not the only kind found on nerves in the brain. Just as important are receptors that change the electrical activity of nerves directly by opening or closing tiny electrical pores (“ion channels”) in the surface membrane of the nerve. These “ionotropic” receptors allow the nerve cell many fewer options for adaptation than the metabotropic receptors because their function relies much less on other proteins such as intracellular enzymes. One of the only “heterologous” adaptations in response to drugs acting on these proteins is to change the electrical activity of the nerve cell by regulating other ion channels in the membrane. Alcohol in particular is a drug that has many effects on ionotropic receptors, and heterologous adaptation to alcohol produces generalized changes in nerve cells by this type of mechanism.13 Although ionotropic receptors are very limited in their heterologous adaptive responses they do have an enormous advantage in their potential for homologous adaptation. Thus, not only can they undergo upregulation and downregulation in numbers, but in addition nerves appear to be able to change the molecular structure of these receptors. This is so because the receptors are made up of several different proteins (or “subunits”), and each of these is commonly produced by a separate gene (and so can be regulated separately). There are usually several options available as to how these subunits are combined to make a receptor, and it appears that nerves may be free, to some extent, to choose which molecular sub-type of receptor they express. This is the nerve cell equivalent of “designer drugs”—nerve cells can produce “designer receptors” that may respond differently to drugs of dependence than their original receptors. Whether this really is by design is not yet certain. Nerves do change their receptor sub-unit composition in response to drugs, but whether this is a genuine adaptation or only some irrelevant “epiphenomenon” is in doubt. The implications will be discussed later.

It should already be obvious that “receptor regulation” is a complicated concept, and that there are numerous ways in which it can be achieved. Despite this, most research in drug dependence still remains fixated upon the simple homologous types of adaptation in which receptor numbers are altered as a consequence of gene regulation. As explained above, this is partly because this type of adaptation is much easier to predict and to understand. However, there is another reason. The techniques of neurochemistry have made it relatively easy to investigate changes in receptor numbers that are a consequence of gene regulation. We measure these changes because we can. It is the scientific equivalent of the old adage—“if you want to find your lost key, then look under the light”. What we have found “under the light” is sometimes compelling, but often quite clearly has nothing to do with the keys to understanding drug dependence. The simplest situation, with possibly the most compelling evidence, is described below.

Receptor downregulation in response to agonist drugs

Agonist drugs mimic natural transmitters. Thus they activate receptor proteins in the same way as the natural transmitter, by attaching themselves to the same specific site on the protein as the natural transmitter. When molecules of an agonist drug and the natural transmitter are present together they compete for this “binding site” on the receptor protein. What happens as a result depends on the “strength” of the agonist drug. If it is capable of activating the receptor to at least the same extent as the natural transmitter we call it a “full agonist”, and the receptors can be activated maximally by the combination. However, if the agonist drug is weaker than the natural transmitter then the competition between the two produces a lower response than predicted by the effect of each alone. In this case we call the drug a “partial agonist”. It is the adaptive response to full agonist drugs that is
Table 1. Receptor regulation as a mechanism for tolerance and withdrawal

<table>
<thead>
<tr>
<th>Type of drug</th>
<th>Predicted response</th>
<th>Potential cause of tolerance/withdrawal</th>
<th>Examples drug/receptor</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agonist</td>
<td>Down-regulation</td>
<td>++++</td>
<td>Heroin/opiate R</td>
<td>Post-receptor mechanisms more common.</td>
</tr>
<tr>
<td>Partial agonist</td>
<td>Down- and/or up-regulation</td>
<td>?</td>
<td>Buprenorphine/opiate receptor</td>
<td>Impossible to predict many consequences in brain areas</td>
</tr>
<tr>
<td>Competitive antagonist</td>
<td>Up-regulation</td>
<td>+++</td>
<td>Caffeine/Adenosine R</td>
<td>Other mechanisms more likely for tolerance?</td>
</tr>
<tr>
<td>Non-competitive antagonist</td>
<td>Up-regulation</td>
<td>+++</td>
<td>Alcohol/NMDA R</td>
<td>Alterations in receptor function more important?</td>
</tr>
<tr>
<td>Agonist + desensitization</td>
<td>Up-regulation</td>
<td>−</td>
<td>Nicotine/nicACh R</td>
<td>Futile adaptation? Cause of tolerance/withdrawal unknown</td>
</tr>
<tr>
<td>Co-agonist</td>
<td>Altered R submit composition</td>
<td>+++</td>
<td>Benzodiazepines/GABAA R</td>
<td>Potential mechanism for cross tolerance with alcohol</td>
</tr>
</tbody>
</table>

Note: In general, the receptor regulation hypothesis is a good explanation for several types of drug dependence. It probably fits best for the regulation of receptor proteins in response to chronic exposure to agonist drugs that act on the same site as the natural transmitter. However, even under these ideal conditions, when the receptors concerned are G-protein coupled receptors (as in the opiate R example above) the regulatory response seems most often to be an alteration in post-receptor mechanisms in coupling and second messengers rather than a simple “down-regulation” of receptor numbers. Several other types of receptor regulation in response to drugs seem likely to be relatively “futile” adaptations, and thus incapable of explaining tolerance. For example, a drug that rapidly desensitizes receptors, such as nicotine, causes an up regulation of its receptors, but these are almost immediately rendered non-functional by desensitization in the continued presence of the drug. Finally receptors that function as ion channels are commonly assembled from several different protein subunits. Frequently drugs modulate these receptors by acting on sites distinct from that at which the natural transmitter acts on these receptors. By altering the subunit composition of their receptors nerves may be able to reduce the effect of the drug without altering the effect of the transmitter, thus producing tolerance with no functional implications on removal of the drug. All these considerations suggest that unitary hypotheses for tolerance and dependence based rigidly on receptor regulation are doomed to failure. Himmelsbach’s “homeostatic” hypothesis and Collier’s receptor regulation modification of this can be correct only some of the time. Nevertheless they were a spectacular step forward in our understanding of the mechanisms of drug dependence.

easiest to understand in terms of receptor regulation and the Himmelsbach hypothesis of homeostatic adaptation (see Table 1).

Drugs such as morphine and heroin are full agonists at opiate receptors and so provide an excellent example of this type of regulation. Unfortunately the predicted changes in receptor numbers are only observed in some rather artificial situations in vitro. Nevertheless, this illustrates the fundamental principles, and opiate dependence was the subject both of Himmelsbach’s hypothesis and Collier’s receptor regulation modification of this. When opiate drugs are present in the brain they complement the effects of the natural transmitters (the opioid peptides) on their receptor proteins. This produces the euphoria, relaxation and analgesia associated with the acute effects of these drugs. If morphine or heroin remain in the brain for more than a few hours then receptor regulation theory predicts that the nerves affected by their presence should begin to adapt to this by reducing the numbers of opiate receptors on their surface. This would reduce both the effect of the drugs and of the natural transmitter, and should therefore restore some semblance of normality to these nerve pathways. In this case, relative normality in the presence of the drug is synonymous with drug tolerance, and this hypothetical adaptation is beneficial to the organism. However, if morphine or heroin is now rapidly removed, the nerves that have downregulated their opiate receptor proteins are left with a below-normal input from the natural opioid peptide transmitters.
Until these nerves can remove the adaptive change (i.e. increase their opiate receptors back to a normal level) the organism is predicted to suffer the consequences of an under-active opioid system (dysphoria, anxiety, hyperalgesia, etc.). We know this as the “opiate withdrawal syndrome”. In receptor regulation theory, this syndrome will last as long as the system is out of balance because of the reduced number of opiate receptors.

This is an almost perfect explanation for most of the features of opiate dependence. Unfortunately it is largely theoretical. Now that we know more about the receptor regulations that occur in opiate dependence it is clear that the mechanisms are enormously more complex than a simple change in receptor numbers. As mentioned above, the mechanisms thought to be important in the brain include involving alterations in the opiate receptors themselves, their coupling system (called “G-proteins”), and molecular mechanisms that are increasingly removed from the receptor (see Harrison, Kastin & Zadina for review). Once these many alterations have occurred they begin to affect other transmitter receptor systems, and to have profound effects on nerve function. They probably also become increasingly difficult to reverse, making the withdrawal syndrome progressively more severe, and of longer and longer duration. Thus, this increased complexity does not invalidate the original concept, rather it expands and extends its impact on the organism. Most researchers would probably agree that receptor regulation, as defined in this wider sense, plays an important role in physiological dependence on opiate drugs (as evidenced by tolerance and withdrawal syndrome).

The complex situation described above becomes immeasurably more complicated if we try to predict what will happen in response to a partial agonist drug, such as buprenorphine at opiate receptors. Remember that partial agonists are weaker in terms of receptor activation than the natural transmitter. Thus, in areas of the brain where the natural transmitter is usually released continuously in large amounts, the partial agonist will reduce the “normal” level of activation of receptors, and we would predict an adaptive upregulation of receptors in response to the drug. In contrast, in brain areas where release of the natural transmitter is low or infrequent under physiological conditions, the continued presence of a partial agonist will lead to above normal levels of receptor activation and we would predict a receptor downregulation as the response in this brain area. Complex changes such as this have been reported for chronic exposure to partial agonists such as buprenorphine, but we do not know enough about the normal physiology of the opioid transmitters to know whether these are truly adaptive, and whether they contribute to tolerance or other aspects of dependence (Table 1).

There are other examples of receptor regulation in response to agonist drugs, for example benzodiazepines, but these are complicated by the types of receptor proteins involved and will be discussed later. In general, the receptor regulation in response to chronic exposure to a full agonist fits the Himmelsbach scheme best. However, even this is by no means as simple as we once thought and, as soon as we stray outside the full agonists, we enter another level of complexity. Even adaptations to some full agonists do not fit the expected pattern, particularly when drugs cause rapid “desensitization” of receptors immediately after activating them (see below).

Receptor upregulation in response to an antagonist drug
Antagonist drugs block the effects of natural transmitters. The type of antagonist that is most easy to understand is known as a “competitive antagonist”. These drugs compete with the natural transmitter for the binding site on the receptor protein; however, when the antagonist is bound to the receptor protein it does not activate the receptor. As a result, molecules of the natural transmitter are no longer able to react with as many receptor proteins, and the response of the nerve to the transmitter is reduced. This type of antagonist action is often very strong because the antagonist drugs often bind much more tightly to the receptors than the natural transmitter. Antagonist drugs can therefore cause almost complete block of a natural transmitter at low concentrations in the brain. As mentioned above, one of the first descriptions of receptor regulation in response to drugs applies to the upregulation of dopamine receptors in response to chronic treatment with antipsychotic phenothiazines (see Creese & Sibley). We do not have many instances of competitive antagonist drugs causing dependence, but caffeine is a
reasonable example. Among other actions, caffeine competes with the natural transmitter, adenosine, for its receptors, and this is believed to underlie many of the central stimulant properties of caffeine.

In the receptor regulation theory, the physiological response to a reduced input from a specific transmitter is for the affected nerve to increase the numbers of receptors for that transmitter on its surface. This is an explanation for Cannon’s denervation supersensitivity, i.e. a physiological homeostatic mechanism designed to restore relatively normal function. For example, suppose 20% of the nerves releasing dopamine in a motor pathway die as a result of neurodegeneration during ageing. The nerves receiving input from these nerves might increase their dopamine receptors by 20%, and thus restore normal function. Indeed, this is one of the mechanisms that is believed to delay the onset of Parkinson’s disease in the normal ageing brain; it is an easily understandable homeostatic response. Nerves often use the same strategy to respond to the presence of an antagonist drug. Thus in response to phenothiazines, nerves up-regulate their dopamine receptors; in response to naloxone (an antagonist of opiate receptors) they upregulate their opiate receptors; and in response to caffeine, they upregulate their adenosine receptors. These types of regulation have been thought of mainly as attempts at “neuroadaption” (which they almost certainly are) and thus capable of producing drug tolerance. This assumption about tolerance, however, may not always be true because when the potential consequences of receptor upregulation in response to competitive antagonists are examined more critically, some serious deficiencies appear.

First, remember that competitive antagonist drugs work by binding to the receptor proteins that are the target for the natural transmitter. Increasing the numbers of receptor proteins increases the sites at which the antagonists work, just as much as it increases the number of sites for the natural transmitter. In fact, the level of competition between the drug and the transmitter is exactly the same regardless of the number of receptor proteins. This has very important implications for receptor upregulation as a mechanism for drug tolerance. Imagine that a competitive antagonist is blocking 90% of the effects of a natural transmitter in a nerve pathway. As an adaptive response to this block, the nerves receiving the reduced chemical messages might respond by, for instance, doubling the number of their receptors for this transmitter (an heroic effort for most nerves). At first sight it might be thought that this would overcome the effect of the drug by providing a whole new population of “unblocked” receptors, but this is not the case. Although there are now twice as many receptor proteins as before, the competitive antagonist drug will still be blocking 90% of them. As far as the nerve pathway is concerned, it will have recovered some function (from 10% of normal to 20% of normal), but it is still greatly affected by the presence of the drug. Thus, although receptor upregulation is a common response to antagonist drugs, it is a poor mechanism to explain high levels of tolerance. Adaptations such as this, that are relatively ineffective at restoring normal function, are often called “futile adaptations”.

Thus, in this case, an adaptation that is effective as a physiological response to “denervation” is rather futile in the face of a pharmacological challenge from an antagonist drug (see Table 1).

Although receptor upregulation in response to antagonist drugs may produce little tolerance, this does not mean that it is unimportant in dependence. Suppose, in our example above, we remove the competitive antagonist after receptor upregulation has occurred. In our example, the natural transmitter now has twice as many receptor proteins on which to act, and these are no longer blocked by the antagonist drug. The consequence will be a two-fold increase in the effects of the transmitter on the nerve cells that have adapted in this way. In theory, the consequences of this should be exactly the opposite of the acute effects of the drug. For example, in the case of caffeine, the acute effects of the drug in blocking adenosine receptors is believed to cause stimulation and an increase in the ability to concentrate. On withdrawal from caffeine the “adaptive” increase in adenosine receptors is predicted to cause lethargy and depression. Thus, although receptor upregulation in response to antagonist drugs may be a poor explanation for tolerance, it may sometimes be a good explanation for the withdrawal syndrome (Table 1). There are also implications outside drug dependence. When dopamine receptors are upregulated by chronic treatment with antipsychotic drugs there is relatively little evidence of tolerance to the medication. However, when brain
concentrations of the antipsychotic drop between doses we see the appearance of movement disorders (“tardive dyskinesias”) that are thought to represent overactivity in the dopamine transmitter system. It is by no means certain that this is directly attributable to the upregulation of dopamine receptors but this, and other forms of neuroadaptation to phenothiazines, are implicated. In this context, this disorder might be considered a “phenothiazine withdrawal syndrome”, but phenothiazines have no “dependence liability” and cause little tolerance. Clearly, we are now drifting rapidly away from Himmelsbach’s unitary hypothesis in which the mechanisms for tolerance, dependence and withdrawal are essentially the same. In the case of competitive antagonists we have to postulate other mechanisms for tolerance, such as increased release of transmitters, that are considerably more difficult to measure than receptor upregulation.

Not all antagonist drugs work by competition with the natural transmitter. These “non-competitive” antagonists interact with transmitter receptors at different sites, and are often much weaker in their effects than competitive antagonists. Alcohol is an example of such a drug, and this can be used to illustrate several types of receptor regulation in relation to tolerance and withdrawal (see Table 1). Alcohol is a weak non-selective drug and inhibits, or potentiates, effects of transmitters on several different receptor proteins. One important receptor that is inhibited non-competitively by alcohol is the NMDA receptor (see Littleton & Little). This ionotropic receptor is normally activated by the amino acid transmitter, glutamate, and alcohol is capable of reducing its glutamate-stimulated activity to about 50% of normal. This action may underlie some of the anesthetic and amnestic (memory-reducing) effects of alcohol. In order to overcome this effect of alcohol nerves would theoretically need to double their number of NMDA receptors. However, although several researchers have reported upregulation of NMDA receptor numbers after chronic treatment with alcohol, several other researchers have found no such change (see Rudolph et al.). Even those who have found receptor upregulation usually report only increases of around 20–30% in numbers of NMDA receptors, well below levels that would restore normality. In contrast, almost all researchers agree that during alcohol withdrawal there is increased function of NMDA receptors, and that this contributes to the withdrawal seizures and neurotoxicity that may occur at this time. Once again, “receptor regulation” seems to be important, but the simplicity of the original unitary scheme, that equates the mechanism of tolerance with that of withdrawal, is increasingly obscured. There are other complexities in the relation between alcohol and NMDA receptors that will be dealt with later.

The relation of drug tolerance and dependence to receptor regulation is thus even more tenuous for antagonists than it is for agonist drugs. The next section will add another layer of complexity to agonist drug responses. This section follows the section on antagonists because this type of agonist produces essentially antagonist-like actions when it is present chronically in the brain (which of course is the common situation during the development of dependence). The reason is that these receptors “desensitize” after being activated.

**Receptor regulation in response to agonists that cause desensitization of the receptor**

Many receptor proteins have an automatic “off-switch”. They simply cease to work when molecules of agonist drugs, or of the natural transmitter, are present for more than a few seconds. Why should this be? One answer is that receptors like this are only “supposed” to be activated for a few seconds, and anything longer would be dangerous for the nerve. The nicotinic receptors for the transmitter acetylcholine are a good example of this principle. Acetylcholine is found throughout our bodies and brains and acts as a transmitter between nerves, and between nerves and muscles. It is usually released in very short bursts, and its presence around the nicotinic receptors on which it acts is also limited in time by the presence of an enzyme (acetylcholinesterase) that breaks down acetylcholine rapidly once it has been released. The importance of this mechanism to our survival can be judged by the fact that many of the toxic “nerve gases” work by inhibiting this enzyme. However, as in most good biological systems, there is a “fail-safe” mechanism built in at the level of the nicotinic receptor for acetylcholine. This fail-safe mechanism turns off, or desensitizes, the receptor if acetylcholine or another agonist remains in the vicinity of the receptor for
more than a few seconds. Until acetylcholine (or the agonist drug) is removed the receptor cannot be activated again, and even then it may require some time before it recovers fully. The importance of this additional mechanism to our survival has also been shown recently; in this case by causing mutations in nicotinic receptors, so that they were unable to desensitize. Activation of these mutated receptors by acetylcholine caused degeneration of the nerves that expressed them.\textsuperscript{18} All this demonstrates that this type of “receptor regulation”, i.e. desensitization after activation by an agonist, is important to us physiologically. It is also of great importance in determining the action of one of the most damaging of drugs of dependence, nicotine.

Nicotine, obtained from tobacco, is an agonist at the nicotinic receptors for acetylcholine. The drug is not broken down by acetylcholinesterase, and since people who use tobacco products tend to use them repetitively, nicotine is present in their brain for hours at a time. Fortunately for them their nicotinic receptors, after a brief burst of activation as the first nicotine of the day comes rushing through the brain, shut down almost immediately. In this desensitized state the receptors can no longer be activated by nicotine, or by the natural transmitter acetylcholine. Why people should continue to use nicotine even after the initial response is over is an interesting question. Presumably either the consequences of nicotinic receptor desensitization (relaxation?) are enjoyable, or peaks and troughs in the nicotine concentration in the brain allow partial recovery of the stimulant agonist response for a few minutes. An alternative suggestion is that removal of nicotine from the brain would now cause a “mini-withdrawal”, and that smokers smoke continuously simply to prevent this. Regardless of the reason, this change in the nicotinic receptors has effectively converted the agonist drug into an antagonist, capable of blocking the natural transmitter. The response of nerves to reduced input from the natural transmitter is predicted, of course, to be upregulation of the appropriate receptors, and this is now what happens to nicotinic receptors. Chronic exposure to nicotine results in a significant increase in nicotinic receptors in the brain, a change that has also been found in the brain of smokers post mortem.\textsuperscript{19}

As far as nerve cells are concerned, this receptor upregulation makes complete sense physiologically. Nerves presumably identify that there is a reduction in their input and try to oppose it the only way possible, by receptor upregulation. However, upregulation of receptor proteins in the face of a drug that desensitizes those receptors is probably futile as an adaptive mechanism.\textsuperscript{19} As “new” receptors appear on the surface of the nerve they are almost certainly desensitized within seconds by the nicotine molecules in the brain. If so, they never have a chance to make a difference to nerve cell function, and the acetylcholine system remains effectively disabled. Once again we have a futile adaptation that seems incapable of explaining drug tolerance. However, this does not exclude its theoretical importance in withdrawal. Once nicotine is removed from the brain, the increased numbers of nicotinic receptors can “resensitize” and then are available to be acted on once more by the natural transmitter, acetylcholine. Whether the resultant increased activity in the acetylcholine system contributes to the nicotine withdrawal syndrome is unknown, but this is certainly a possibility (Table 1). A corollary of this is that, once nicotinic receptors have upregulated, and then resensitized after removal of nicotine, then the system might also be more sensitive to the reintroduction of nicotine itself. Behavioral “sensitization”, or “reverse tolerance”, on repeated exposure to drug is a characteristic sign of the effects of stimulants, including nicotine, and it is possible that receptor upregulation may, under some circumstances, contribute to this.

Himmelsbach’s original hypothesis has now all but disappeared. Here we have an agonist drug causing an upregulation of its receptors in defiance of the predicted homeostatic mechanism for an agonist. In addition, this response may be responsible for the opposite of drug tolerance, but yet may still play a role in the withdrawal syndrome. There is worse to come. The interaction between nicotinic receptors and nicotine introduces another important aspect of receptor regulation—nicotinic receptors come in a diversity of subtypes, assembled from different protein “subunits”. Although acetylcholine, the natural transmitter, affects all of the subtypes more or less equally the drug of dependence, nicotine, has much greater effects on some of these receptors than on others. Different nicotinic receptor subtypes are probably also upregulated to different extents in response to nicotine.\textsuperscript{20} This means that the population of
dependencies and receptor regulation

97

receptors affected by the drug is not identical to the population affected by the transmitter. This has implications for the adaptive options open to nerves, and affects both the concept of receptor regulation and the Himmelsbach hypothesis itself.

Implications of receptor subunit composition for concepts of receptor regulation

As mentioned previously, receptor proteins for transmitters come in two major types, metabotropic and ionotropic. In general, metabotropic receptors are made from a single protein that is coupled via a G-protein to other effector proteins that change nerve cell function. In contrast, ionotropic receptors are made from several separate proteins (subunits) and cause direct electrical changes in the nerve cell membrane by opening pores called ion channels. The nicotinic receptor proteins and the NMDA receptor proteins described above are examples of ionotropic receptors, and the drugs that work on these can induce complex adaptive responses in these receptors as a result. However, the example of another type of ionotropic receptor protein, the GABAA receptor, will be used to illustrate the potential importance of the subunit structure in adaptation to drugs. In this way I can introduce the concept of adaptations to two drugs at the same receptor protein, benzodiazepine tranquilizers and alcohol.

The GABAA receptor is normally activated by the transmitter GABA (gamma-aminobutyric acid). Activation opens a channel through the receptor protein which carries chloride ions, and this reduces the electrical excitability of the nerve. GABA acting on these receptors is responsible for the majority of the inhibitory transmitter activity in our brains. The GABAA receptor is made up of five subunit proteins that can be of various types. From our viewpoint the most important subunits are called alpha and beta subunits. The beta subunits bind the natural transmitter GABA, whereas the alpha subunits bind benzodiazepine drugs. When a benzodiazepine drug is bound to its site on the receptor it increases the ability of GABA to cause opening of the chloride channel, and this potentiation of the effect of GABA accounts for most of the tranquilizing and sedative properties of the benzodiazepines. Importantly for our argument, there are at least six different types of alpha subunit, some of which bind benzodiazepines much better than others. However, in general terms the different alpha subunits do not have a major effect on the ability of GABA to activate the receptor. Alcohol also enters the picture because it, too, potentiates the effects of GABA on this receptor. The situation is more complicated for alcohol, and many different subunits have been implicated in alcohol sensitivity. However, it is likely that the alpha subunits are a major influence on the effects of this drug as well as those of benzodiazepines.

The importance of this scheme is that it suggests a mechanism by which the nerve could dramatically alter its response to drugs without markedly affecting its response to the natural transmitter. Let us suppose that benzodiazepines bind best to a specific alpha subunit, call it the alpha1 subunit, and that they bind very poorly to another subunit, call it the alpha6 subunit. Let us also suppose that some nerves can express both of these subunits and can therefore make GABAA receptors that contain one or other of these alpha subunits. In response to the continued presence of a benzodiazepine drug it would make sense for these nerves to switch their production of alpha1 subunits into production of alpha6 subunits. In consequence, the population of GABAA receptors would gradually shift to one in which receptors containing alpha6 subunits predominate. Since these receptors are relatively insensitive to benzodiazepines, this is predicted to reduce the effects of the benzodiazepine drug. It is a good mechanism for tolerance, but it does not necessarily change the effects of the natural transmitter, GABA, because the overall number of GABAA receptors remains unchanged (Table 1). This hypothetical scheme is actually very close to what has been reported for effects of benzodiazepines on nerves—it appears that the total number of GABAA receptors is not altered by drug exposure, but the expression of the gene that encodes the alpha1 subunit is reduced. We do not know for sure that this is an adaptive change, but it certainly could be.

The change in gene regulation leading to altered GABAA receptor composition is not unique to benzodiazepines; something similar happens during chronic exposure to alcohol. This suggests two things. First, it could be that this switch between alpha1 and alpha6 subunits is one of many “standard” physiological re-
sponses, and it is simply a coincidence that it happens to overcome the effects of the drugs. In favor, the genes for these subunits are close together, and may be regulated (inversely) by the same signals; indeed, the reverse switch, to increased alpha 1 subunits, occurs during brain development. Secondly, if the switch really does reduce the effects of the drugs, then it might explain the strong “cross-tolerance” often reported between alcohol and benzodiazepines. If this is true, it has implications for the use of benzodiazepines during detoxification from alcohol. It does not make sense to use drugs to suppress a withdrawal syndrome in which the drug of dependence (alcohol) has altered the receptor target to make these drugs (the benzodiazepines) relatively ineffective. Although the benzodiazepines are undoubtedly useful clinically in suppressing alcohol withdrawal, perhaps we should try to produce drugs that are more (rather than less) effective on the GABAA receptor subtypes produced during chronic exposure to ethanol.

The major implications of this type of receptor regulation in our scheme, however, is that it shows that adaptation to drugs may occur without any impact on the effects on the natural transmitter. Thus, the alterations in GABAA receptor population caused by alcohol and benzodiazepines seem to occur in the absence of any change in receptor number. Theoretically, if the “new” population of receptors is just as sensitive to GABA as was the original population, then the drugs can be withdrawn with impunity—the adaptations in GABAA receptors should have no functional consequences, and should therefore not lead to a withdrawal syndrome. Of course, we know this to be untrue; benzodiazepines, and especially alcohol, can produce extremely severe withdrawal syndromes. Either the withdrawal syndromes from these drugs are due to changes in other systems, or the basic premise, that alterations in GABAA receptor subunits can occur without changing effects of GABA, is wrong. This may be the case because, although GABA does not bind to the alpha subunit of the GABAA receptor, the alpha subunit composition probably does influence its effects on the receptor. Despite our current uncertainty, the theoretical possibility that a homeostatic mechanism may lead to tolerance without also causing a withdrawal syndrome tends to undermine the Himmelsbach hypothesis. According to Himmelsbach, the mechanisms for tolerance and withdrawal should be the same. There are other situations as well where adaptive changes to drugs are capable of explaining tolerance, but which are unlikely to play much of a role in withdrawal. These adaptive mechanisms fail as potential explanations for withdrawal because they are too rapid (see below).

**Implications of rapid forms of receptor regulation**

The Himmelsbach hypothesis of tolerance and withdrawal assumes two characteristics of the underlying homeostatic mechanism. First, that it opposes the acute effect of the drug and so “overbalances” the system when the drug is removed. As seen above, some forms of receptor regulation may be able to simply diminish the effect of the drug without causing this type of overbalancing when the drug is removed. The second important assumption is that the homeostatic adaptation can be removed only slowly when the drug is withdrawn. If the mechanism is removed at the same rate as the drug leaves the brain, then there is no functional disturbance and the mechanism cannot account for the withdrawal syndrome. Several types of receptor regulation are theoretically rapid enough to come close to this ideal, and they will now be discussed briefly. They are important because they may be fundamental to some rapidly developing forms of tolerance, and they may limit the duration of the withdrawal syndrome.

As mentioned above, there are ways in which receptor function can be altered in the absence of any change in the numbers of the receptor protein. Some of these are slow (such as changes in coupling G-proteins) but there are also rapid ways, usually involving a temporary chemical modification of the receptor protein by enzymes inside the nerve cell. The most common modification is called phosphorylation (produced by enzymes called protein phosphokinasers) and it is probably of considerable importance in many ways to drug dependence. First, receptor protein phosphorylation is sufficiently rapid to be the mechanism for acute effects of some drugs—thus it has been suggested that alcohol may affect some receptor proteins by altering their state of phosphorylation. Secondly, immediate homeostatic responses to drugs may involve receptor phosphorylation—for example, the nico-
Dependence and receptor regulation

This rapid homeostatic mechanism may also underlie phenomena such as acute or rapid tolerance, in which a reduced response to a drug is obtainable within minutes or hours on repeated testing. In general, adaptations that are rapidly instituted are also rapidly removed, and this is certainly the case for receptor phosphorylation—enzymes inside the cell (dephosphorylases) can remove phosphate groups within minutes, and thus restore receptor function to normal. Rapid adaptations such as receptor phosphorylation and dephosphorylation can probably reduce the effect of a drug (producing “acute” or “rapid” tolerance) with only very minor consequences when the drug is withdrawn. Clearly this does not fit the Himmelsbach hypothesis—in this case the mechanism responsible for tolerance does not cause a withdrawal syndrome.

There are also regulatory mechanisms that do not change the overall number of receptor proteins in the nerve cell, but that nevertheless reduce the number on the cell surface. We cannot detect these changes by the usual receptor binding studies that we perform on animal or human brain, and so we know very little about whether they are important in vivo. However, experiments in vitro in nerve cell cultures suggest that they may be extremely important. The principle is that the continued presence of an agonist drug leads the nerve cell to “internalize” the receptor proteins that are activated by the drug. Once internalized, these receptors are unavailable to the drug because this binds only to receptors on the surface of the nerve. When discussing receptor downregulation previously, this possibility was ignored—the focus was on gene regulation, in which a reduction in synthesis of receptor proteins gradually led to a reduction in their numbers on the cell surface. This is a slow change (usually taking several days), but the receptor internalization mechanism requires only hours. It probably occurs in response to a variety of drugs of dependence (opiates and benzodiazepines are two examples where this has been shown in vitro) and it may underlie some forms of rapid tolerance. Since the development of physiological dependence, as evidenced by a drug withdrawal syndrome, usually requires considerably longer than this, it is difficult to argue that receptor internalization is instrumental in the development of this type of dependence. However, this does not preclude a role in withdrawal itself, and there are two ways in which receptor internalization could be involved here. First, receptor internalization that has occurred during exposure to the drug may contribute to the functional disturbance during withdrawal. Secondly, if withdrawal is due to an upregulation of some specific receptor, then receptor internalization may be able to restore normality rapidly, and so limit the duration of the withdrawal syndrome. These possibilities will be dealt with next.

Whether prior receptor internalization is ever important in causing withdrawal is uncertain, and depends on how rapidly the process can be reversed. This is a difficult question, and few studies address the role in withdrawal directly. What we know from other experimental situations is that the fate of the internalized receptor proteins varies, depending on how long the situation that caused them to be internalized is maintained. Initially, the receptor proteins are sequestered in internal stores but, from these stores, they can be rapidly “recycled” back to the surface membrane of the nerve if the agonist drug is removed. This is a rapid process, probably rapid enough to limit the duration of a withdrawal syndrome—so, if sufficient receptor proteins can be recycled so as to restore normality, the withdrawal syndrome should be correspondingly brief. However, the longer the receptor proteins remain sequestered inside the nerve cell, the more likely they are to be degraded by the nerve. Once this has occurred, the only way to restore a normal number of receptors to the surface of the nerve is to synthesize more of these proteins. This is a much slower process, and drug withdrawal would therefore lead to a functional imbalance of long duration. Thus, short exposures to an addictive drug should lead to a brief withdrawal syndrome because receptor recycling can occur, whereas long exposures should lead to a much longer withdrawal syndrome because recycling is no longer a possibility.

The converse situation, where the withdrawal syndrome is a consequence of a previous upregulation of receptor numbers, is simpler to understand. If upregulated receptors can be internalized during withdrawal, then this offers a way of rapidly restoring normality and limiting the duration of the withdrawal syndrome. This probably occurs, because there are several examples where upregulation of receptors or ion chan-
nel proteins is reversed more rapidly than would be predicted if they were just degraded at their normal rate. This does not alter the Himmelsbach hypothesis, but it demonstrates that if we are going to apply this to receptor regulation then we need to recognize that several different types of regulation can occur, sometimes simultaneously.

The process of receptor recycling is also potentially important in an area of drug dependence that at least seems to be receiving the attention it deserves: that is, relapse into drug use after detoxification. Our emerging understanding of mechanisms for tolerance, dependence and withdrawal is immensely intellectually satisfying, but it is relatively useless in determining avenues for therapeutic intervention in drug dependence. Thus, although withdrawal and detoxification are important therapeutic problems, they can be managed with a bare minimum of understanding of the mechanisms of dependence. In contrast, we have very little idea of how to deal with the biggest therapeutic problem, i.e. that so many patients relapse after detoxification. In this respect a discussion of the neurochemical basis of the triggers that precipitate relapse is outside the scope of this review; here I am concerned (as was Himmelsbach) with what may happen to homeostatic mechanisms during cycles of dependence, detoxification and relapse. Let us suppose that an individual has been using a drug for many years, and that this has caused an increase in the numbers of a specific receptor on nerves in the brain. According to the Himmelsbach hypothesis, on removal of the drug he (or she) will suffer a withdrawal syndrome caused by the excess numbers of these receptor proteins. It is therefore in the interests of the brain to remove these proteins as rapidly as possible, and theoretically the quickest mechanism is by internalizing and sequestering them inside the affected nerve cells. Once this has occurred the individual is now “drug-free” and no longer “drug dependent” in the sense that the withdrawal syndrome is over. However, suppose he, or she, now relapses into using the drug again. Receptor recycling may now allow the nerves to “reinstate” the homeostatic mechanism responsible for tolerance and dependence much more rapidly than before. This is because the receptor proteins are already formed, they are ready and waiting to be re-used, and do not have to be synthesized anew. In terms of the whole organism this would mean that when physical dependence (as evidenced by tolerance and a withdrawal syndrome) has been induced once, it is very much easier to induce a second (and third and fourth, etc.) time. This is a common belief that pervades much of the descriptive clinical literature, and was remarked on repeatedly by Himmelsbach. If it is truly a consequence of receptor recycling, it is important to know how long after withdrawal this state persists, and how it can be manipulated pharmacologically. Hopefully, a greater understanding of this type of receptor regulation will give us some clues as to effective therapeutic intervention in the process.

These short-term mechanisms for receptor regulation are clearly much less damaging as a response to drugs than the long-term regulatory mechanisms, and prompt the question as to why the brain uses long-term regulation at all. The reason may be that short-term regulation is simply too expensive to the cell in terms of energy expenditure. When a drug is present for a prolonged period it may be more efficient to reduce the synthesis of the receptor proteins for this drug, rather than keeping these in a phosphorylated state, or continually internalizing and recycling them. Like most homeostatic mechanisms, receptor regulation has several layers of complexity and includes short, intermediate and long-term responses to the agents that perturb homeostasis. We are beginning to understand how these may work together to influence drug dependence, but we still have a long way to go.

Conclusions
Receptor regulation is clearly not as simple as was once thought, but this does not invalidate it as an important mechanism in drug dependence. It still offers great hope for therapeutic intervention in drug dependence and, in so far as much of receptor regulation is based on gene expression, it will be of considerable interest to evaluate its role in genetic differences in dependence liability using genetic knock-out techniques. These new molecular techniques, together with our increased understanding of the complexity of the receptor regulatory processes, should help ensure that neurochemical research into drug dependence continues to be exciting. However, this time around we should be more critical of the hypotheses themselves, and of the data generated by these. Himmelsbach’s hypo-
esis of drug dependence and the application of receptor regulation to this, should probably teach us two lessons in research: if a hypothesis is beautiful there is (a) usually some truth in it but (b) not as much as we first thought! As Collier ended his 1965 review on this subject, “Fortunately both suggest further experiments”.

References


