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Control of Receptor Sensitivity at the mRNA Level

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Abstract

Neurons are able to adjust the sensitivity of receptor-mediated processes according to the level of receptor activation. Extrapolating from our knowledge of other cellular proteins, regulation of receptor mRNA availability would provide a highly economical means of achieving this objective. Epidermal growth factor is able to induce long-lasting increases in its receptor binding by increasing receptor mRNA levels, and similar effects have been shown for other growth factors. Studies on G-protein-coupled receptors, in particular using adrenoceptor clones transfected into cultured cell lines, have shown that changes in receptor number are generally associated with an alteration in receptor mRNA content. At the neuromuscular junction, dramatic increases in nicotinic acetylcholine receptor number are achieved by activating receptor subunit gene transcription. Less information is available concerning the regulation of ligand-gated ion channels in the brain.

Overall, the evidence suggests that receptor mRNA levels are frequently controlled by the degree of receptor stimulation. Receptor mRNA levels are therefore likely to be one of the most important control points for both homologous and heterologous regulation of receptor sensitivity.

Index Entries: Neurotransmitter receptors; gene expression; up-regulation.

Scope

It has been known for many years that the sensitivity of receptor-mediated processes is not fixed rigidly at a predetermined level. In general, cells can adapt to changing degrees of receptor activation by altering the gain of the response to the agonist. It can be assumed that in the majority of cases the change in receptor sensitivity makes the cell better able to meet the demands of the altered neurochemical environment.

From our current knowledge of the mechanisms that cells can utilize to modulate their sensitivity to receptor activation, it is clear that there are many potential points of control, and that in any particular case there are likely to be a number of different levels where the regulatory mechanisms are operating. The cloning of many receptors in recent years has made it possible for the first time to assess whether the regulation of receptor mRNA levels represents an important control point, or whether all the regulation is at the posttranslational, or protein, level. This review attempts to summarize much of the evidence for pretranslational control, and to consider the functional significance of the findings to date. It will not cover intracellular receptors, such as those for the steroids, where there is already abundant evidence for control of sensitivity at the mRNA level. Rather, it will be focused on cell membrane receptors, and in particular those receptors for neurotransmitters or neuromodulators and growth factors. This is where the research in this field is moving fastest at present, and where some general principles are being appreciated for the first time.

Regulation of Receptor Sensitivity

For neurotransmitter receptors, several different forms of altered receptor sensitivity have been defined. Cells can adapt to increase their response to subsequent receptor activation (supersensitivity), and this supersensitivity is frequently linked to an increase in receptor number (upregulation). Similarly, cells can decrease their response to agonists, and here the decreased responsiveness of the signal transduction mechanism is termed desensitization, which may or may not be linked to a fall in the number of receptors (downregulation).

An alteration in the sensitivity of a particular receptor-mediated process is typically observed when there is a significant change in the level of activation by the ligand for that receptor. In this case, where the ligand has affected the sensitivity of its own receptor, the effect (desensitization, downregulation, and so on) is referred to as homologous, to distinguish it from those situations where the sensitivity of a receptor transduction system is affected by activity at a different receptor. These latter effects are termed heterologous, and are far from uncommon in the nervous system.

Other situations where there are long-term changes in receptor sensitivity do not fit into either of these categories. For example, when a receptor appears, along with its effector, during development, the stimuli to induce the receptor system are completely unknown. Such a qualitative increase in receptor sensitivity is clearly going to be based on an increase in mRNA levels, and the induction of gene expression the major point of control. Similarly, when receptor stimulation is linked to cell proliferation or differentiation, the mitotic or phenotypic changes are likely to include effects on other receptors. In these cases, too, the major alterations in the pattern of gene expression within the affected cells make it almost certain that transcriptional controls will be paramount in determining receptor sensitivity. However, this review will concentrate on the evidence that cells with a fairly stable pattern of gene expression, such as those in the fully developed nervous system, can regulate receptor mRNA levels as a means of determining receptor sensitivity.

In many biological systems, there appears to be a fairly simple relationship between the number of receptors occupied by an agonist and the magnitude of the biological response. In these cases, it is clear that an alteration in receptor number will have a proportional effect on the size

of the response. However, there is evidence from both the peripheral and central nervous systems that for high intrinsic activity agonists, such as the endogenous hormones and neurotransmitters, many of the postsynaptic receptors can be considered as "spare," in the sense that they are not needed for the generation of a maximal postsynaptic response. In this situation, increases or decreases in receptor number will not affect the response to a saturating concentration of agonist.

However, even when there are "spare" receptors, alterations in receptor number will affect the speed at which the response is generated and the size of the response to a relatively small release of agonist. From all points of view, then, receptor number has to be seen as an important determinant of cellular reactivity.

Potential Points of Control

A great deal of experimental evidence demonstrates the converse situation: Dramatic changes in the sensitivity of receptor-mediated cellular responses can occur without any alteration in receptor number. In other words, the efficiency of the coupling mechanisms linking receptor activation to cellular effect can show considerable plasticity. However, the number of receptors in the plasma membrane is also a major determinant of the sensitivity of the receptor-mediated process, and is therefore subject to fluctuation according to the level of receptor activity.

The pathway of protein synthesis, from the initial stage of gene transcription, through mRNA splicing and translation to protein processing and insertion into the membrane, provides a number of possible points where receptor number could be regulated. Similarly, the rate of removal of receptor protein from the membrane and the degradation rate are likely to have a profound effect on the number of functional receptors. The amount of receptor protein in the plasma membrane that is available for ligand binding at a given time will therefore depend on the balance between the rate of synthesis/insertion and the rate of internalization/degradation.

The evidence suggests that, in general, there is considerable amplification in the pathway of protein synthesis at the stage of mRNA translation. It has been calculated that, on average, more than 10^4 mol of protein are synthesized per mol of mRNA (Hargrove and Schmidt, 1989). Modulation of the rate of translation would therefore represent an extremely economical way of regulating the rate of protein synthesis. Information from all aspects of cell biology suggests that this is indeed the case, and that changes in the rate of protein synthesis are frequently caused by an alteration in either mRNA availability or translational efficiency (i.e., Lindenbaum et al., 1988; Smith and Liu, 1988; Steinhilber et al., 1988; Theodorakis et al., 1988; Haverstick and Bannon, 1989).

If receptor synthesis is regulated in a similar fashion to that of other cellular proteins, it might therefore be predicted that receptor mRNA levels will be of considerable importance in determining the rate of receptor synthesis. However, even if this is the case, it is far from clear how important the rate of receptor synthesis is, compared to the other factors mentioned above, for regulating the amount of functional receptor protein in the cell membrane.

Consequently, mRNA levels are potentially, but not necessarily, a pivotal point of control for determining receptor levels. The recent evidence that has thrown some light on the role of mRNA levels in regulating receptor sensitivity is summarized below.

Growth Factors

Epidermal Growth Factor Receptor

Epidermal growth factor (egf) is a powerful mitogen in certain cell types, and the regulation of egf receptors has been extensively studied since the egf receptor was identified as a proto-oncogene. The receptor is a large glycoprotein with a single membrane-spanning region and an intrinsic tyrosine kinase activity in the intracellular domain (reviewed by Carpenter and Cohen,

1990). The intracellular consequences of egf activation are proposed to be caused by this tyrosine-specific protein kinase activity.

Following the binding of egf to the receptor, both receptor and ligand are internalized and then degraded (Carpenter and Cohen, 1990). This results in an initial downregulation of cell-surface receptors, with a halftime of around 40 min in human KB carcinoma cells (Beguinot et al., 1985). However, within a few hours, the levels of egf binding are increased relative to untreated cells (Clark et al., 1985), owing to the ability of egf to stimulate the synthesis of its receptor (Clark et al., 1985; Earp et al., 1988; Bjorge et al., 1989). Activation of protein kinase C by phorbol esters induces a similar increase in the rate of receptor synthesis. In both cases, this increase in synthetic rate appears to be caused by enhanced levels of egf receptor mRNA, since there are parallel changes in the rate of synthesis, as assessed by the immunoprecipitation of ^{35}S-labeled receptor protein, and the levels of egf receptor mRNA (Clark et al., 1985; Earp et al., 1988). In general, there is a very good correlation between the rate of egf receptor synthesis and receptor mRNA levels (Astroff et al., 1990), so that in this particular case it can be inferred that mRNA availability is a powerful determinant of the overall synthetic rate.

Since desensitization of the protein kinase C pathway attenuates phorbol ester effects on mRNA levels, but does not interfere with the action of egf (Earp et al., 1988), it is likely that the homologous upregulation induced by egf can proceed independently of protein kinase C.

The increase in egf receptor mRNA levels induced by egf in human KB carcinoma cells was not associated with an increase in transcriptional initiation (Clark et al., 1985), implying that the principle regulation operates at a posttranscriptional level. However, other reports suggest that egf can both increase the rate of transcription of the receptor gene and slow the rate of transcript degradation (Kesavan et al., 1990). All reports agree that cycloheximide treatment potentiates the increase in mRNA levels, implicating a labile protein in a tonic repression of egf receptor synthesis. Recent evidence (Haley and Waterfield, 1991) suggests that there is normally a tendency for transcription to terminate between exons 1 and 2 of the egf receptor gene in human carcinoma cells. Treatment with phorbol esters increases the rate of gene transcription and also removes the block of mRNA elongation.

Thus, within minutes of contacting the cell surface, egf initiates a rapid downregulation of egf receptors, followed by a more protracted upregulation mediated predominantly by an action on mRNA levels. This increase in mRNA levels peaks at around 3 h after stimulation (Earp et al., 1988). Other transmitters, including adrenaline and angiotensin, can produce a heterologous upregulation of egf receptors. This effect also occurs through an increase in synthetic rate mediated by an elevation of mRNA levels, although there is some evidence that the intracellular mechanisms differ from those mediating the homologous egf receptor upregulation (Earp et al., 1988).

Other Growth Factor Receptors

Insulin-like growth factors (IGFs) are peptides that are also mitogenic for certain tissues. The IGF-1 receptor is regulated according to the level of activation by ligands such as IGF-I and IGF-II. However, in contrast to the homologous upregulation observed with the egf receptor, the addition of IGF-II causes a downregulation of IGF-1 receptors (Papa et al., 1991). This decrease in binding is associated with a parallel fall in the levels of IGF-1 receptor mRNA, suggesting that pretranslational controls are very important. A drop in the rate of synthesis occurs coordinately with the decreasing mRNA levels (Rosenthal et al., 1991), providing evidence that in this case too, mRNA levels govern the overall rate of receptor synthesis.

Studies on the insulin receptor raise the possibility that this may be a general rule for the regulation of many growth factor receptors. Treatment of cultured lymphocytes with glucocorticoids promotes insulin receptor gene transcription, leading to a threefold increase in receptor mRNA within 6 h (Rouiller et al., 1988). This causes a threefold rise in the rate of proreceptor synthesis, raising the number of cell-surface insulin receptors by 40%, 18 h after stimulation.

The close relationship between receptor mRNA levels and receptor synthesis is also seen in cultured hepatoma cells, where growth arrest is associated with an increase in insulin receptor number of around 400%. This is paralleled by an increase in insulin receptor mRNA levels, whereas the receptor degradation rate remains unchanged (Hatada et al., 1989).

Less predictably, the homologous downregulation of lymphocyte insulin receptors 18 h following insulin treatment is accompanied by an increase in proreceptor synthetic rate. This may be pointing to a possible upregulation of receptors over a longer period of time. However, the increase in synthetic rate is not caused by an increase in receptor mRNA levels (Rouiller et al., 1988).

Little evidence is available at present concerning the regulation of the receptors for the brain neurotrophic factors. In cultured Schwann cells, activation of adenylate cyclase promotes a decrease in the numbers of nerve growth factor (NGF) receptors, which is accompanied by a decrease in NGF receptor mRNA levels. (Mokuno et al., 1988). In PC12 cells, and also in vivo, NGF treatment increases the levels of NGF receptor mRNA and also the number of NGF binding sites (Doherty et al., 1988; Miller et al., 1991). This effect is because of an enhancement of gene transcription, and provides a potential positive feedback system similar to that seen with the egf receptor.

G-Protein-Coupled Receptors

General Principles

Experiments on receptors linked to G-proteins, in particular the adrenoceptor family, established two distinct and separable phases in the loss of responsiveness to a continuously applied agonist. The first phase—desensitization—occurs comparatively rapidly, and probably reflects both an uncoupling of the receptor from its effector and a transient removal of the receptors from the plasma membrane (reviewed by Lefkowitz et al., 1990). Desensitization undoubtedly plays a major role in modulating receptor sensitivity following sustained agonist stimulation. The rapidity of the effects rules out any contribution of changes at the mRNA level, and receptor phosphorylation is likely to be the main mechanism involved. The second phase—downregulation—represents a decrease in the total number of cellular binding sites, and occurs over a much longer time scale (typically hours rather than minutes), and it is at this stage that suppression of receptor synthesis through a fall in receptor mRNA may play an important role.

Conversely, antagonist treatment in vivo generally causes an increase in receptor number and a supersensitivity to any subsequently applied agonist. There is evidence that this increase in receptor number, or upregulation, does not result from blockade of endogenous agonist, but rather from an intrinsic property of the antagonist itself (Morris and Millan, 1991). To date, there is no evidence that the upregulation and supersensitivity are distinct and separable phenomena. These general principles of regulation appear to hold true for all the different types of G-protein-coupled receptors.

Adrenoceptors

Expression of adrenoceptor clones in cultured cells has made possible a series of studies on the regulation of the receptor mRNAs. It is of interest that an early report (Bouvier et al., 1988) showed a very good correlation, in transfected CHW cells, between the level of expression of β_2 mRNA in different clones and the total β_2 binding capacity. Thus, as detailed above for some growth factor receptors, there is a basis for suspecting that the number of binding sites is often dependent on the cellular level of receptor mRNA.

In a more recent study (Guest et al., 1990), it has been reported that the steady-state levels of β_1 and β_2 adrenoceptor mRNAs are roughly equivalent in 3T3-L1 fibroblasts, and there are also very similar amounts of β_1 and β_2 binding sites. This would imply that the posttranscriptional controls are very similar for these two distinct receptors. Furthermore, when the cells are induced to differentiate, the levels of β_1 mRNA

fall whereas β_2 mRNA levels increase. This is accompanied by a decline in β_1 binding capacity and an elevation of β_2 binding capacity, providing further encouragement for a belief that mRNA levels are of paramount importance for determining receptor number. However, as described below, there are situations where there is a clear dissociation between the levels of a binding site and its corresponding mRNA.

Prolonged exposure of cells to β_2 agonists produces the expected downregulation of β_2 receptor number, and it has now been shown that there is also a fall in the cellular content of β_2 receptor mRNA. In S49 mouse lymphoma cells challenged with isoproterenol, β_2 receptor mRNA declines over 24 h after an initial lag-period (Hadcock et al., 1989a), an effect that is prevented by the presence of a β_2 antagonist. Similarly, isoproterenol or epinephrine applied continuously to hamster DDT_1MF-2 cells produce a slow decline of β_2 mRNA that starts after about 4 h and continues over a 24 h period (Hadcock and Malbon, 1988; Collins et al., 1989). The amount of the decrease can be substantial—down to around 40% of starting levels. The number of binding sites falls to as little as 20% of the initial capacity.

The stimulation of adenylate cyclase, leading to increases in intracellular cAMP and activation of protein kinase A, is likely to be partially responsible for this agonist-induced downregulation of β_2 receptor mRNA. Forskolin is able to decrease β_2 receptor mRNA levels with a similar time course (Hadcock and Malbon, 1988; Hadcock et al., 1989a), with a parallel reduction in the number of binding sites. A similar reduction in β_2 receptor binding and mRNA is seen with prolonged dibutyryl-cyclicAMP treatment (Bouvier et al., 1989; Collins et al., 1989), whereas S49 lymphoma mutants with compromised coupling between β_2 receptors and the relevant G-protein show downregulation of neither receptors nor mRNA (Hadcock et al., 1989a).

However, some other pathway may also be involved, since the decreases in receptor mRNA seen with forskolin are often not as great as those seen with isoproterenol. Furthermore, S49 mutants lacking coupling between the G-protein and adenylate cyclase show downregulation in response to isoproterenol in the presence of only basal levels of cAMP (Hadcock et al., 1989a).

The direct consequence of increased intracellular cAMP is increased β_2 receptor gene transcription (Collins et al., 1989), consistent with the presence of cAMP-response elements in the 5'-flanking region of the β_2 receptor gene. The rather dramatic fall in cellular β_2 receptor mRNA levels observed following treatment with either isoproterenol or forskolin, therefore, implies that another mechanism overrides the increased gene transcription. This in fact seems to be the case, since the mRNA halflife is reported to decrease from 12 h to 5 h in cells treated for 24 h with a β_2 agonist (Hadcock et al., 1989b).

There may be a distinction between the short- and long-term effects of β_2 receptor stimulation on β_2 receptor mRNA levels. Short-term exposure (<4 h) of DDT_1MF-2 cells to adrenaline provokes a considerable increase (over 300%) in β_2 receptor mRNA levels (Collins et al., 1989). Other workers, however, have failed to observe similar increases in β_2 mRNA (Hadcock et al., 1989b; Hough and Chuang, 1990). No significant increase in binding capacity was seen. This rise in β_2 receptor mRNA levels is caused by increased gene transcription (Collins et al., 1989), and since dibutyryl-cAMP produced a similar short-term effect, protein kinase A is probably involved. No destabilization of mRNA is observed at these short time periods.

There are fewer studies on the regulation of β_1 adrenoceptor mRNA, but in C_6 glioma cells, agonist treatment induces a short-term elevation in β_1 receptor mRNA levels that is maximal at 4 h, and is followed by a more protracted fall to below basal levels (Hough and Chuang, 1990). Since no initial elevation in β_2 receptor mRNA was seen in these same cells, there appears to be a differential regulation of β_1 and β_2 receptor mRNAs in the short- but not in the long-term.

In cultured aortic smooth muscle cells, noradrenaline causes a long-lasting downregulation of α_1 adrenoceptor binding. This is accompanied in the short-term by a dramatic fall in α_1 receptor mRNA levels—to 20% of control values at 4 h (Izzo et al., 1990). However, the mRNA levels

have returned to basal levels by 24 h, so it may be that a decreased rate of synthesis is important for receptor number to drop, but a normal rate of synthesis is required to maintain the new equilibrium level.

The short-term fall in α_1 receptor mRNA levels following noradrenaline treatment is greater than that seen when transcription is inhibited totally with actinomycin D, suggesting that reduced gene transcription is not sufficient to explain the effect. It is possible that reduced mRNA stability is able to play a role in the short-term regulation of α_1 receptor mRNA levels.

Conversely, in DDT_1MF-2 cells treated with adrenaline, the downregulation of α_1 binding sites is associated with α_1 receptor mRNA levels which increase over the first 4 h of stimulation, and then decrease to below basal levels over the next 24 h (Morris et al., 1991). No short-term increase in binding is observed. The long-term fall in α_1 receptor mRNA levels is blocked by cotreatment with an α_1 antagonist, and is not owing to decreased mRNA stability. This is therefore in contrast to the homologous downregulation of β_2 receptor mRNA, and suggests that there may be a fall in the rate of gene transcription in this case.

Interestingly, the short-term increase in mRNA content is blocked, not by an α_1 antagonist, but by a β_2 antagonist (Morris et al., 1991). This therefore appears to be a heterologous regulatory mechanism where β_2 receptor stimulation tends to increase α_1 binding by increasing α_1 receptor synthesis. An increase in α_1 receptor mRNA levels, and also α_1 binding sites, is observed with agents that stimulate cAMP formation, implying that adenylate cyclase mediates this action of β_2 receptors. There was no detectable increase in α_1 binding sites following adrenaline treatment, despite the β_2 receptor-mediated increase in α_1 receptor mRNA. The sequestration and degradation of α_1 receptors consequent to direct ligand activation presumably outweigh this action to increase receptor number. This would be similar to the short-term increase in β_2 receptor mRNA following β_2 agonist treatment that is not associated with significantly increased binding capacity (Collins et al., 1989).

It can therefore be proposed that cAMP-dependent short-term increases in receptor mRNA levels are designed to function primarily as a heterologous regulatory mechanism. Where homologous stimulation is also occurring, this effect is overridden, and no upregulation is observed at the protein level.

Transcription of the α_{2A} adrenoceptor gene can also be enhanced by cAMP, and there is a subsequent increase in the number of binding sites (Sakaue and Hoffman, 1991), implying that this is a direct result of the elevated mRNA levels.

Dibutyryl cAMP is able additionally to increase the stability of α_{2A} mRNA slightly, although this seems to be a transient effect that has disappeared after a 24 h period. Experiments studying the effect of growth factors on α_{2A} receptors in HT29 cells have provided other evidence that the rate of gene transcription is the predominant factor regulating α_{2A} receptor number (Devedjian et al., 1991). Here a reduction in the number of functional receptors following growth factor treatment appears to be mediated by a decrease in the rate of gene transcription.

Dopamine Receptors

Whereas all the studies of adrenoceptor gene regulation have used cultured cells, the work on dopamine receptor gene regulation has focused on the situation in vivo, where there are several well-defined models of receptor upregulation. Chronic blockade of dopamine receptors in the rat striatum, or destruction of the dopaminergic innervation, results in an upregulation of dopamine receptors and a behavioral supersensitivity. The upregulation of receptors is so dramatic that it might be expected that experiments to determine the extent of the contribution of mRNA changes would be unequivocal. However, the treatment of rats for about 1 wk with the dopamine antagonist haloperidol has been reported to both decrease (Angulo et al., 1991) and increase (Bernard et al., 1991) dopamine D_2 receptor mRNA. Longer periods of haloperidol treatment either increase D_2 receptor mRNA (Coirini et al., 1990; Angulo et al., 1991; Bernard et al., 1991), or else have no effect (Srivastava et al., 1990; van

Tol et al., 1991). These different results are not easily explained by the different drug doses used. It may be that the processes occurring in vivo are more complex than has been appreciated so far. It is also not clear to what extent the newly characterized D_3 and D_4 dopamine receptors contribute to these increases in striatal dopamine binding that have been assayed using what are now known to be nonselective ligands. In other words, it is at present not possible to relate possible changes in mRNA levels in this model directly to alterations in the binding capacity of the corresponding receptor.

Interestingly, there is more concensus that the receptor upregulation observed in the striatum following dopamine denervation is associated with an increase in D_2 receptor mRNA. (Brene et al., 1990; Coirini et al., 1990; Gerfen et al., 1990; Angulo et al., 1991). The increased receptor mRNA levels appear to be maintained for a number of weeks in parallel with the increased receptor density, implying, perhaps surprisingly, that an increased rate of synthesis is necessary to sustain an increased steady-state level of D_2 receptor binding capacity, in the absence of any ligand activation.

There is some evidence that the increase in D_2 receptor binding in rat pituitary following haloperidol treatment is also associated with an increase in receptor mRNA levels (Lew et al., 1990; Arnauld et al., 1991).

Other G-Protein-Coupled Receptors

In primary cultures of rat cerebellar neurons, functional muscarinic receptors appear in parallel with the m2 and m3 muscarinic receptor mRNAs (Fukamauchi et al., 1991). Treatment with muscarinic agonists induces a gradual decline in muscarinic binding over 24 h. There may be an initial transient increase in m2 receptor mRNA over the first hour of treatment, which is of interest considering the initial increases in adrenoceptor mRNA observed during agonist application *(vide supra)*. Over the next few hours, the levels of both m2 and m3 receptor mRNA fall by up to 50% of control values, although mRNA levels have returned to normal within 24 h (Fukamauchi et al., 1991). It can be assumed that the fall in mRNA levels is an important component of the drop in binding capacity. Muscarinic antagonists not only attenuate these agonist-induced effects, but are also able to increase m2 and m3 mRNA in the absence of any agonist. This is consistent with the ability of antagonists at muscarinic receptors, and other G-protein-linked receptors, to exhibit apparent negative intrinsic activity, and thereby upregulate receptors by an action distinct from the blockade of tonic agonist activity (Soejima and Noma, 1984; Morris and Millan, 1991). This is clearly a fertile field for further investigation. It has recently been reported that rats treated chronically with a muscarinic antagonist showed increased cortical muscarinic binding together with a rise in cortical m1 muscarinic receptor mRNA of 150% (McKinney and Robbins, 1992).

Thyrotropin-releasing hormone (TRH) is able to downregulate TRH receptors in GH_3 cells, and this downregulation is preceded by a fall in TRH receptor mRNA content (Oron et al., 1987). The rapidity of the mRNA decline relative to the mRNA halflife suggests that posttranscriptional controls are important here.

In cultured human thyroid cells, thyrotropin (TSH) is reported to increase the levels of TSH receptor mRNA by over 1000% (Huber et al., 1991). Conversely, in a rat thyroid cell line, TSH has been shown to decrease the numbers of functional TSH receptors and also the abundance of TSH receptor mRNA (Saji et al., 1991). In cells transfected with a functional receptor clone devoid of upstream regulatory elements, this downregulation of receptor mRNA levels is abolished, suggesting the a transcriptional mechanism is paramount.

The luteinizing hormone/chorionic gonadotropin (LH/CG) receptor in cultured Leydig tumor cells is downregulated by LH/CG. This is largely owing to an increase in the rate of receptor degradation, but there is also a significant fall in the content of LH/CG receptor mRNA, mediated via cAMP (Wang et al., 1991). Chorionic gonadotropin administration to rats in vivo also induces a downregulation of LH/CG receptors,

and there is a parallel downregulation of LH/CG receptor mRNA levels (LaPolt et al., 1990; Hoffman et al., 1991).

Ligand-Gated Ion Channels

Neuromuscular Junction Nicotinic Receptors

Since the nicotinic acetylcholine receptor (nAChR) at the neuromuscular junction was the first mammalian transmitter receptor to be cloned, a considerable amount of information has been accumulated concerning the mechanisms of its regulation. The muscle nAChR is a pentameric ion channel that is opened by the binding of ACh. In developing muscle, it is formed of α, β, γ, and δ subunits, in the stoichiometry $\alpha_2\beta\gamma\delta$ (Popot and Changeux, 1984) whereas in adult muscle the γ subunit of the receptor appears to be replaced by a distinct ε subunit (Mishina et al., 1986; Gu and Hall, 1988). The embryonic receptors are distributed throughout the muscle surface, but as the neuromuscular junction forms, the receptors become highly concentrated at the end-plate. They also exhibit other properties distinct from those of the embryonic receptors, including an increased halflife (Schuetze and Role, 1987). Clearly, complex regulatory mechanisms are controlling the sensitivity of nAChRs according to the requirements of the muscle.

Interest has centered on the response of muscle cells to denervation, when large numbers of extrajunctional receptors are synthesized once more (Linden and Fambrough, 1979). Since this effect is prevented by electrical stimulation of the muscle, it is assumed that muscle activity tonically suppresses receptor synthesis outside the neuromuscular junction.

Several systems, including various muscles in the mouse, rat, and chicken in vivo, cultured myotubes, and muscle cell lines, have been used to study the contribution of *de novo* gene transcription to these dramatic changes in receptor sensitivity. The different models have produced considerable agreement on the mechanisms involved. Muscle denervation increases the steady-state level of nAChR α subunit mRNA 50–100-fold in the mouse (Merlie et al., 1984; Goldman et al., 1985), 17-fold in the rat (Goldman et al., 1985), and between 12 and 112-fold in the chicken (Klarsfeld and Changeux, 1985; Moss et al., 1987; Schieh et al., 1988; Tsay and Schmidt, 1989). There is a parallel, although less pronounced, increase in the levels of the mRNAs encoding the β, γ, and δ subunits (Evans et al., 1987; Moss et al., 1987; Schieh et al., 1988). This might be expected on the basis that the synthesis of large numbers of extrajunctional receptors will require increases in the rate of the production of all the component subunits. The different subunit mRNAs are therefore regulated more or less coordinately. The different percentage increases in the levels of the different subunit mRNAs suggests that there are distinct posttranscriptional controls operating.

The elevated mRNA levels are brought about by increased gene transcription (Schieh et al., 1988; Tsay and Shmidt, 1989), although much of this enhanced transcriptional activity is transient. Electrical stimulation of the muscle suppresses the increases in both receptor sensitivity and mRNA levels (Goldman et al., 1988).

The in vitro studies have supported these findings. Blockade of spontaneous electrical activity in myotube cultures by tetrodotoxin increases α subunit mRNA content and receptor binding capacity (Fontaine and Changeux, 1989; Duclert et al., 1990). The increases are abolished by application of a calcium ionophore (Klarsfeld et al., 1989).

The induction of extrajunctional subunit gene expression is clearly the cause of *de novo* receptor synthesis, but the activation of γ subunit gene transcription, which is normally not present in the adult, additionally allows the formation of receptors that can give a greater response to ACh (Jaramillo et al., 1988). Even after denervation, expression of the adult-specific ε subunit remains restricted to the site of the original innervation, (Brenner et al., 1990), indicating that the regulatory mechanisms are substantially different for this gene. To date, this is the only known example where activation of gene expression can produce a receptor with a different subunit composition

to meet changed demands of the tissue, but it would also be an attractive way of altering the sensitivity of multisubunit receptors in the CNS.

The number of receptors over the whole muscle increases dramatically following denervation, and returns to normal if the muscle is stimulated electrically. However, I have been unable to find any indication as to what happens to the number of receptors in the end-plate region. In view of the very high concentration of receptors at the innervated end-plate, it seems likely that in this particular part of the muscle, the concentration of nAChRs actually falls on denervation, and is tonically enhanced by cholinergic activity. This would imply a regulation of subunit gene expression opposite to that operating over the rest of the muscle, which would not be inconsistent with the above evidence on the γ and ε genes. The end-plate region, as the region of synaptic transmission, may prove to be more relevant than extrajunctional muscle when we use neuromuscular nAChRs as a model to predict the response of CNS ligand-gated ion channels to changes in the level of activation. If the above theory is true, then it may be that ligand-gated ion channels in the CNS would normally respond to a rise in the level of activation with a transcriptionally driven increase in receptor number.

CNS Nicotinic Cholinoceptors

The nAChRs in the brain, spinal cord, and autonomic ganglia are probably also pentameric channels that contain both α and β subunits, of which six α and three β have been cloned to date (e.g., Boulter et al., 1986, 1990; Goldman et al., 1987; Nef et al., 1988; Duvoisin et al., 1989). The distinct distributions of the different subunit mRNAs in the brain (Wada et al., 1989; Morris et al., 1990) indicate that there are very likely to be distinct nAChRs in different brain regions with differing subunit compositions.

Chick ciliary ganglion neurons have a high nAChR binding capacity that falls after postganglionic axotomy or preganglionic denervation (Jacob and Berg, 1987). These neurons also contain high levels of α3 subunit mRNA, and these change in parallel with the binding, falling following axotomy of the neuron and after denervation (Boyd et al., 1988), suggesting that mRNA changes are important. Unfortunately, no information is available on what happens to the other subunit transcripts in this model. There is also reported to be a decrease in the content of α3 subunit mRNA in axotomized facial motorneurons, although in this case there is a contrasting increase in the levels of β2 mRNA (Senba et al., 1990). Rather surprisingly, the changes were said to occur in the number of neurons expressing these genes, rather than in the level of cellular expression.

The only well-characterized example of up- or downregulation of CNS ligand-gated ion channels is the increase in central high affinity nicotine binding observed following chronic nicotine treatment (Schwartz and Kellar, 1985). This upregulation following chronic agonist administration appears anomalous considering the evidence from G-protein-coupled receptors. Excessive agonist activation of nAChRs causes a depolarization block to develop, and nicotine has been suggested to function effectively as an antagonist on chronic treatment. However, the above remarks on neuromuscular-junction nAChRs following denervation raise the possibility that the regulatory mechanisms for ligand-gated ion channels may be fundamentally different from those for G-protein-linked receptors, with agonist activation in the former case inducing upregulation through an increase in subunit gene transcription.

The contribution of mRNA changes to this increased binding has been studied so far in a single brain region—the medial habenula nucleus. Here, nicotinic binding increases after a week of nicotine administration, and there is a parallel increase in the levels of all the subunit mRNAs present—α3, α4, β_2, and β_4 (Morris and Barnard, 1991 and in preparation)—with the exception of the β_3 subunit mRNA. However, there is considerable doubt as to whether the β_3 subunit can form part of a functional nAChR in vivo (Deneris et al., 1989). Consequently, it seems likely that, as at the neuromuscular junction, all the constituent subunit mRNAs are regulated in parallel.

$GABA_A$ Receptors

Since the initial cloning of two subunits of the $GABA_A$ receptor (Schofield et al., 1987), many additional subunits have been identified. Functional $GABA_A$ receptors in vivo are multisubunit GABA-gated chloride channels (probably pentameric) that contain α and β subunits, usually along with γ and possibly δ subunits, although there is no evidence so far as to the stoichiometry. Barbiturates and benzodiazepines bind to distinct sites on the $GABA_A$ receptor to potentiate its function.

Despite the multitude of studies on $GABA_A$ receptors over the years, there is to date no consensus as to the ways in which these receptors respond to a sustained alteration in the level of activation. Some authors report that addition of GABA agonists to cerebellar cultures leads to an increase in the number of $GABA_A$ receptors (Belhage et al., 1986), which is dependent on protein synthesis (Belhage et al., 1990). Conversely, other groups have suggested that there is a downregulation of $GABA_A$ receptors on cultured neurons following chronic agonist treatment (Maloteaux et al., 1987; Tehrani and Barnes, 1988; Roca et al., 1990). This downregulation has recently been reported to be accompanied by a large decrease in the levels of mRNA encoding the α_1 and α_2 subunits (Montpied et al., 1991). Another group has observed a fall in $GABA_A$ receptor α_1-subunit mRNA content following treatment of cultured neurons for 4 h with either a $GABA_A$ agonist or a benzodiazepine agonist (Hirouchi et al., 1992), although since binding capacity was not monitored in this study, this fall could have been associated with an increase or a decrease in receptor number.

Chronic benzodiazepine agonist treatment in vivo has been suggested either to leave the levels of benzodiazepine binding sites unchanged (Mohler et al., 1978) or to cause a significant downregulation (Tietz et al., 1986; Miller et al., 1988). The apparent downregulation is reportedly associated with a fall in the levels of α_1 and γ_2 subunit mRNAs in cerebral cortex, but not hippocampus or cerebellum (Heninger et al., 1990; Kang and Miller, 1991). The levels of β_1 mRNA remain unaltered, so in this case, the different subunit genes are seemingly not modulated in parallel. These mRNA changes only appear after 14 d of treatment, whereas binding decreases after 7 d, so the mRNA regulation is unlikely to be the direct cause of the fall in binding capacity.

Another group, studying whole brain rather than individual regions, failed to detect any fall in α_1 or γ_1 subunit mRNAs after chronic benzodiazepine agonist treatment, but did observe a rapid decrease in α_5 subunit mRNA levels, followed by an increase in the levels of mRNA encoding the α_3 and α_6 subunits (O'Donovan et al., 1992a,b). No changes were detected in the content of any of the β subunit mRNAs. These results suggest hat there may be massive regional alterations in the expression of some of the α subunit mRNAs.

Although there seems, therefore, to be general agreement that α_1 subunit mRNA levels fall after receptor activation, more studies are clearly needed to determine the other aspects of the regulation of $GABA_A$ receptor subunit gene expression.

General Comments

From a consideration of all the above evidence, a picture emerges showing that alterations in receptor number are generally associated with changes in receptor mRNA levels. In some cases, the mRNA changes are likely to be the direct cause of altered binding capacity. This is especially true where the mRNA effects precede the changes in receptor binding. In other cases, it is more probable that alterations in gene expression at the mRNA level are just one component of a multilevel control mechanism. The changes in mRNA levels can be induced by alterations either in the rate of gene transcription or in mRNA stability. Where mRNA levels have been related to the rate of receptor synthesis, a good correlation has usually, but not always, been observed.

However we are only at the stage of sketching in a rough outline of the overall effects that are taking place. The intracellular pathways that link

receptor activation to modulation of receptor mRNA levels have been at best only vaguely appreciated. The relationship between receptor turnover and the rate of receptor synthesis needs to be clarified. Above all, only a small minority of the various different neurotransmitter receptors have been studied with respect to their regulation at the protein level, let alone at the mRNA level, so at the present time we are really only guessing at the general principles by which regulatory mechanisms operate. No doubt there are many more facets yet to be appreciated.

A particularly exciting prospect is that of gene switching, where a change in receptor sensitivity is achieved by replacing the expression of one receptor or receptor subunit gene with that of a closely related member of the same protein family. This occurs at the neuromuscular junction during development and following denervation, but has yet to be demonstrated in the CNS.

Furthermore, it is becoming obvious that the posttranscriptional processing of receptor mRNAs can be highly complex. Many receptor mRNAs, including those encoding the dopamine D_2 receptor (Giros et al., 1989), the α_1 subunit of the glycine receptor (Malosio et al., 1991), the γ_2 subunit of the $GABA_A$ receptor (Whiting et al., 1990), and members of the glutamate receptor subunit family (Sommer et al., 1990), are subject to alternative splicing, allowing the generation of closely related protein sequences differing only in a single domain. There is as yet no proof that these distinct mRNAs are actually translated to give distinct proteins, but, assuming that this is the case, the regulation of alternative splicing provides another potential point of control that could be used to modulate receptor function.

Mention was made in the introduction of the advantages for a cell of using the availability of mRNA as a means of regulating receptor sensitivity. We have seen that modulation of receptor mRNA levels definitely occurs, but what, then, are the possible disadvantages for the cell of controlling receptor sensitivity in this way? A small number of mRNA species have been shown to be present in neuronal dendrites, potentially allowing a local modulation of the rate of protein synthesis in the region of synaptic activation. However, no receptor mRNA has yet been observed outside the cell soma, which means that any alteration in receptor mRNA levels will presumably alter receptor number over the whole cell surface. This might well be undesirable when, for example, a central neuron has been activated only through a few synapses in part of its dendritic field.

Consequently, (in the particular case of neurons), it may be possible to predict either that mechanisms exist for targeting newly synthesized receptors out to those distal regions of the neuron where activation has taken place, or, more likely, that receptor mRNA levels are not altered unless there has been a widespread change in the extent of receptor activation over most of the neuronal surface. It is worth remembering in this regard that the expression of receptors in cell lines derived from peripheral tissues may not provide a good model for the more complex regulatory mechanisms likely to operate in neurons.

The period of time involved in receptor stimulation activating transcription factors, and these in turn affecting protein synthesis, means that controls at the transcriptional level are also not going to provide a rapid modulation of the sensitivity of membrane receptors. Less is known about the regulation of mRNA processing, but it seems likely that at this stage too, any control will take some time to exert any appreciable effect. Control at the mRNA level can therefore only be important in long-term changes, brought into operation when a protracted alteration in function is required. For a sensitive, rapid, highly localized control of receptor sensitivity in response to synaptic activation, it would appear that control at the protein level has many advantages over control at the mRNA level.

In any discussion of the regulation of gene expression, particularly in relation to receptors, it should be remembered that it is the amount and nature of the functional protein that is ultimately of importance, and unless this is altered, any genomic regulation will be functionally irrelevant. The above discussion has tended to illustrate what is not known as much as what is already understood. These studies are still at an early stage, although even now, one thing above

all others does seem clear: There are pathways linking receptor activation to an alteration in receptor mRNA availability, and this means that receptor sensitivity beyond doubt frequently is controlled at the mRNA level.

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