candidate gave him a right answer he would say cheerfully: "That's right. Well done. Now tell me what more you know about this microbe." It was refreshing to see how many borderline candidates cheered up and made a good pass under such intelligent and understanding examination.

Beattie told me of one episode in his early career which reveals both his personal courage and integrity as well as his determination to give a fair deal to a misguided student, a candidate for an honours degree in science. This student had deliberately so planned his answer to an early question in his viva that he set up an argument between his two examiners. As the student had foreseen, this argument became so heated that the candidate's presence became almost superfluous. "All right, you may go!" he was told. The examiners, scowling at one another, agreed: "Second class honours." At this point Beattie, who was present although not examining, asked the examiners to realise what had happened. He argued that this student had a record in his class work and other parts of the examination that surely entitled him to serious

consideration for first class honours. The viva just completed had not really tested that issue. The candidate was recalled, grilled, scolded, and given first class honours. The examiners acknowledged their mistake and thanked Beattie for his intervention. Credit all round.

As an assessor on committees convened to appoint consultants in microbiology Beattie was uncommonly successful. Candidates were often surprised to find that he had not only studied their applications but had actually read their published papers and was ready and anxious to discuss their contents and conclusions. Very few assessors so equip themselves but there is no more certain way of being able to distinguish serious investigative work from mere window dressing. Not surprisingly Beattie's services as an external assessor were in great demand.

In conversation with an Edinburgh friend who knew Colin Beattie only slightly, I found my friend not at all surprised about the good opinion I had formed of Colin. He said: "It's what you would expect. He was at school at Fettes, wasn't he?"

Basic Molecular and Cell Biology

How do receptors at the cell surface send signals to the cell interior?

ROBERT H MICHELL

The cells of the body are bathed in fluids whose major constituents—for example, the ions and proteins of plasma—are held at remarkably constant concentrations over long periods. These extracellular fluids, however, also contain, at very low and constantly changing concentrations, an extraordinary number of chemically diverse molecules that work together to control cell and tissue behaviour and thus integrate body function. Some of these signals, notably neurotransmitters, are released from one cell and recognised by another within a very limited space—for example, acetylcholine acting within the confines of a neuromuscular junction or various transmitters at synapses between neurones. By contrast, classical hormones are disseminated throughout the body (or at least throughout the extraneural tissues) and act on all cells that bear the receptors which recognise them and initiate tissue specific responses.

Between these two geographical extremes lie many other extracellular signals whose actions are localised but directly affect much larger volumes of tissue than classical neurotransmitters at close synapses. Such agents include (a) peptide neurotransmitters that may diffuse away from a nerve ending and act on quite distant cells before destruction; (b) hypothalamic hormones that are carried to the anterior pituitary (their major target organ) by a specialised local circulation before dilution into the entire blood volume; (c) the prostaglandins (prostacyclin from endothelium and thromboxane

A₂ from activated platelets), subendothelial collagen, and adenosine diphosphate (ADP) derived from platelets that regulate platelet behaviour during incipient thrombus formation at the site of blood vessel injury; (d) the platelet derived and other polypeptide growth factors that control the repair of such wounds; and (e) the histamine, leukotrienes, platelet activating factor, and lymphokines that are among the many mediators of inflammatory events. Even the response of an egg to the first contact of a fertilising sperm, before cell fusion, seems biochemically to be little different from the responses of exocrine secretory cells to secretagogues.

So how do all the cells of the body, with their shared origin in a single fertilised egg that has a strictly limited gene pool, manage to mount correct responses to such an astonishing range of external chemical influences? The answer, which has been emerging gradually from research in many laboratories over the past 30 years or so, is remarkably simple and elegant. With the notable exceptions of steroids and thyroid hormones, which enter cells and bind to intracellular receptor proteins that then home on to the chromosomes and control the expression of genes, extracellular stimuli are mostly molecules that are large or hydrophilic, or both. Thus they can interact readily with the cell surface but do not enter cells rapidly enough for the cells to be controlled by intracellular hormone molecules. The complex machinery that discriminates between the many chemical stimuli and then amplifies their signals onwards into the cell interior is built into the cell surface membrane.

Recognition of extracellular stimuli

The highly selective sites at the cell surface that recognise extracellular stimuli and even discriminate between closely related

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molecules (such as the peptide neurohormones oxytocin and vasopressin) were named "receptors" long before we learnt that they are membrane proteins firmly integrated within the structure of the plasma membrane. In addition, early proposals that single agents could influence cells by their interactions with more than one type of receptor—for example, α_1 , α_2 , and β receptors for catecholamines, and muscarinic and nicotinic receptors for acetylcholine—provided the most satisfactory explanations for striking heterogeneities in the quantitative pharmacological characteristics of tissue responses to these agents. Such multiple receptors, with their proved or potential applications as drug targets, have more recently been shown for various other stimuli—for example, histamine, dopamine, serotonin, and vasopressin—and we now know that each type of receptor is a unique protein.

Thus the number of individual receptor proteins encoded within the genome of every nucleated cell must be rather more than the sum of all of the different chemical signals that are recognised at any time during our lives by any of the cells of the body (maybe a few hundred genes). However, at any one time most of this gene family will be turned off in any individual differentiated cell, so that the cell expresses receptors for and responds to only that limited range of stimuli for which it is "a target cell." The control of this selective gene expression is little understood, but it is immensely important. As an example of what might happen if it went wrong, consider the endocrine mayhem that would ensue if the various cell populations of the pituitary that secrete hormones were suddenly randomly to interchange their receptors and thus secrete their hormones in response to the wrong hypothalamic releasing factors. Moreover, each cell's receptor status is constantly susceptible to environmental regulation rather than in a static condition. For example, when lymphocytes that have been stimulated by antigens proliferate and differentiate into mature immune effector cells this depends on the correct sequential expression of a series of receptors for lymphokines; and liver cells isolated from the body show reciprocal changes in responsiveness to α adrenergic and β adrenergic stimuli within a few hours.

The onward transmission of receptor signals to the cell interior

The genetic load on the cell of encoding a huge variety of receptors would be further exacerbated if each type of activated receptor was coupled to an additional and unique machinery that transmitted its (and only its) message onwards into the cell. Fortunately this is not the case. The biochemical machinery of the plasma membrane seems to translate the immensely complex

Some examples of the mechanisms of signal transmission used by receptors

Receptors with intrinsic ion channels

Acetylcholine (nicotinic receptors with Na^+ channel)
GABA and glycine (with Cl^- channels)

Receptors with intrinsic protein tyrosine kinase activity

Platelet derived growth factor Epidermal growth factor
Insulin Insulin like growth factor 1

Receptors that activate adenylate cyclase

Adrenergic (β_1 and β_2) Histamine (H_2)
Vasopressin (V_2) Adrenocorticotrophic hormone
Glucagon (proposed G_2 receptor) Prostacyclin
Thyroid stimulating hormone Parathyroid hormone

Receptors that inhibit adenylate cyclase

Adrenergic (α_2) Prostaglandin E_1 (in some tissues)
Acetylcholine (muscarinic, probably one subtype) Adenosine (one subtype)
Opiate

Receptors that activate PtdInsP_2 hydrolysis

Adrenergic (α_1) Histamine (H_1)
Acetylcholine (muscarinic, probably one subtype) Vasopressin (V_1)
Glucagon (proposed G_1 receptor) Angiotensin
Substance P Thyrotrophin releasing hormone (TRH)
Gonadotrophin releasing hormone (GnRH) Thromboxane A_2

language of extracellular stimuli to which all cells are exposed into a very much simpler language of ionic, electrical, and chemical signals that controls intracellular events. Some of the better understood mechanisms, and the receptors that use them, are summarised in the figure and the box and are briefly described below.

Signalling through the opening of cell surface ion channels

The main permeability barrier within cell surface membranes is provided by the hydrocarbon sidechains of membrane lipids, and the membranes normally have a very low permeability to small ions such as Na^+ , Ca^{++} , and Cl^- . Ion pumps driven by adenosine triphosphate (ATP) in the membranes constantly build ion gradients

A diagrammatic representation of some of the mechanisms of transmembrane signalling used by cell surface receptors. At the left are shown receptors possessing intrinsic protein tyrosine kinase activity, alongside the control of adenylate cyclase by stimulatory and inhibitory receptors (indicated as R) that is mediated by G_s and G_i . At the top is the receptor stimulated hydrolysis of PtdInsP_2 by PIC, leading to intracellular accumulation of 1,2-DAG, $\text{Ins}(1,4,5)\text{P}_3$ and Ca^{++} . At the right are the actions of receptors possessing intrinsic ion channels on membrane potential and hence on cellular electrical activity and Ca^{++} permeability. Many, but not all, of the intracellular effects of changes in the cellular concentrations of cyclic AMP, 1,2-DAG, and Ca^{++} are caused by the phosphorylation and dephosphorylation of particular target proteins by the various kinases and phosphatases that these intracellular messengers regulate, as depicted in the centre.

across this barrier (notably to keep intracellular Na^+ concentrations at least 10-fold lower than the 100 mmol/l of extracellular fluid, and intracellular Ca^{++} concentrations 10 000-fold lower than extracellular Ca^{++} concentrations). These ion gradients and others dependent on them, such as the excess of extracellular over intracellular Cl^- , allow two types of signalling controlled by receptors. Firstly, they give rise to a resting membrane potential (with the intracellular surface of the membrane electrically negative relative to the outside) that is perturbed whenever there are selective and transient increases in membrane permeability to particular ions. Secondly, the Ca^{++} concentration in the cytoplasm is held so low (at about $0.1 \mu\text{mol/l}$) that small ion movements can substantially raise this value: a change in cytoplasmic Ca^{++} can therefore serve as an effective intracellular signal.

Some receptor proteins, almost certainly a small minority of the total, carry within themselves both the recognition sites through which they respond to extracellular stimuli and the mechanisms for onward transmission of the stimulus. Notable among these is a family of receptors, typified by the fast acting nicotinic acetylcholine receptors (NAChR) of skeletal muscles and ganglia, in which binding of an agonist triggers the rapid opening of an ion channel. The NAChR is a five subunit ($\alpha_2 \beta \gamma \delta$) protein of molecular weight about 280 000 that spans the cell surface membrane from outside to inside.¹ Indeed, each of its subunits spans the membrane and contributes amino acid residues to a protected pathway that can rapidly pass cations, particularly Na^+ , through the membrane barrier. When acetylcholine binds to the extracellular portions of the α subunits this ion channel opens for a few milliseconds, allowing Na^+ to flow down its gradient and thus reduce the membrane potential. The best known result of such membrane depolarisation is the initiation of long distance electrical signals in the form of action potentials, as in nerve cells and at the neuromuscular junction. A major effect in some other cells, however, is to cause local changes in membrane permeability to key ions. For example, stimulation of NAChR causes catecholamine secretion from the adrenal medullary cells of some species, because the membrane polarisation triggered by receptors opens plasma membrane Ca^{++} channels that are potential controlled and thus leads to exocytosis of the hormone triggered by calcium.

The inhibitory neurotransmitters γ -aminobutyric acid (GABA) and glycine also have receptors whose structures include an ion channel. However, when these channels open, triggered by neurotransmitters, Cl^- , a negatively charged ion, flows into "stimulated" cells. Since this Cl^- flux tends to raise the membrane potential it increases the resistance of neurones to activation. The structures of these inhibitory receptors are known in much less detail than that of the NAChR, but the recent cloning of the genes for some of their subunits has shown striking sequence homologies with the subunits of the NAChR.^{2,3} Thus these three receptors, which all have intrinsic ion channels, albeit with different ion specificities, seem to be members of a single family of proteins that has evolved from a common ancestor.

Receptors with intrinsic protein tyrosine kinase activity

Receptors that send their messages to the cell interior by controlling enzymes associated with membranes seem to be of two types: those in which the enzyme active site is an intrinsic part of the receptor protein and those that control an enzyme indirectly, through the agency of an intermediate coupling protein. As we shall see, however, these two groups may not be as sharply distinguished as was thought a year or two ago.

The clearest examples of receptor proteins with enzyme activity are the receptors for epidermal growth factor, platelet derived growth factor, insulin, and insulin like growth factor 1.⁴ All of these phosphorylate tyrosine residues both within the receptors and in other proteins, an activity that is stimulated by hormone binding. In the epidermal growth factor receptor the hormone binding site and the kinase domain are at opposite ends of a single polypeptide chain spanning the membrane, whereas in the insulin and insulin like growth factor 1 receptors they are on different polypeptide chains of

a disulphide bridged $\alpha_2\beta_2$ tetrameric protein. Although these receptors share with several of the proteins encoded by oncogenes the ability both to phosphorylate protein tyrosine residues and to control cell growth, extensive studies have so far failed either to show that protein tyrosine kinase is essential to hormone action or to identify key intracellular target proteins phosphorylated during hormone action.

Receptors that control the formation of intracellular second messenger molecules

About 30 years ago the late Earl Sutherland and his colleagues discovered that catecholamines (acting through β receptors), glucagon, and various other hormones transmit their messages to the cell interior by stimulating the synthesis by adenylate cyclase of cyclic adenosine monophosphate (cyclic AMP), an intracellular nucleotide "second messenger" that is present at only micromolar concentrations. In its turn, a rise in the intracellular concentration of cyclic AMP activates a protein kinase stimulated by cyclic AMP that phosphorylates (and thus activates or inactivates) key enzymes of hormone regulated metabolic pathways, including glycogen breakdown and lipolysis. At the time of this discovery Sutherland acknowledged that cyclic AMP was likely to be only the first of many intracellular "second messengers" that mediate hormone actions, but he could hardly have guessed how long it would take to identify others and the extent to which work on adenylate cyclase would come to influence the studies on other signalling systems.

A very substantial proportion of the many receptor species at the cell surface are now known to control cells by influencing adenylate cyclase activity: some stimulate cyclic AMP formation while others inhibit it. Thus all of the extracellular information delivered to these diverse receptors is summarised for the cell interior simply as a rise or fall in the intracellular concentration of cyclic AMP. The first stage of this simplification is a division of receptors into a stimulatory group and an inhibitory group, each of which communicates with adenylate cyclase through a different coupling protein dependent on guanosine triphosphate (GTP): these stimulatory and inhibitory G proteins are known as G_s and G_i respectively. Although it is clear that activation of the trimeric ($\alpha\beta\gamma$) G proteins by stimulated receptors provokes their dissociation into free α subunits and $\beta\gamma$ complexes, with the quantities of the freed α_s and α_i within the membrane regulating adenylate cyclase activity, the molecular details of the stimulatory/inhibitory interplay between G_s , G_i , and adenylate cyclase remain a matter of some argument.⁵

A second large family of receptors feeds its input to the cell through a very different signalling system based on the hydrolysis of a minor membrane phospholipid component (phosphatidylinositol 4,5-bisphosphate, abbreviated to PtdInsP_2) by a specific phospholipase (abbreviated as PIC). As long ago as 1953 Hokin and Hokin discovered that stimulation of receptors activates inositol phospholipid metabolism, and I recognised in 1975 that inositol lipid hydrolysis somehow leads to a rise in cytoplasmic Ca^{++} concentration,⁶ but it was not until the early 1980s that the exact role of PtdInsP_2 hydrolysis in transmembrane signalling was defined.^{7,9}

As with adenylate cyclase, control of PIC activity by receptors is indirect, with a G protein serving as an intermediary. The G protein or, more likely, G proteins that play a part in receptor-PIC coupling are yet to be identified. PIC catalysed hydrolysis of PtdInsP_2 yields two products, each of which has a unique role as an intracellular messenger. Inositol 1,4,5-triphosphate ($\text{Ins}(1,4,5)\text{P}_3$), the water soluble fragment, diffuses into the cell interior, while 1,2-diacylglycerol (1,2-DAG), the lipid soluble fragment, remains associated with the cytoplasmic surface of the cell membrane.

Within cells there is a membrane compartment, probably a portion of the endoplasmic reticulum, into which Ca^{++} is continuously pumped by an ATP driven pump, so holding the cytoplasmic Ca^{++} concentration of "resting" cells at approximately $0.1 \mu\text{mol/l}$. When receptors cause a rise in the intracellular concentration of $\text{Ins}(1,4,5)\text{P}_3$ this compound binds to its own "receptors" on the membrane enclosing this Ca^{++} store and triggers

a rapid release of Ca^{++} into the cytoplasm: the cytoplasmic Ca^{++} concentration often rises briefly to $\geq 0.5 \mu\text{mol/l}$ within seconds after the application of a stimulus. $\text{Ins}(1,4,5)\text{P}_3$ is inactivated both by dephosphorylation and by entry into a complex pathway that interconverts several previously unknown inositol polyphosphate isomers, the biological functions of which are still uncertain.¹⁰ 1,2-DAG, the other messenger molecule formed from $\text{PtdIns } P_2$, activates a protein kinase (protein kinase C; PkC) that can also be activated by phorbol ester tumour promoters. The latter compounds were originally of interest because they enhanced the tumour yield in skin treated with carcinogens, but they were later found partially to mimic some effects of cell stimulation (such as platelet aggregation), so suggesting that they might act by subverting some normal cellular signalling process. Some of the intracellular targets of Ca^{++} and PkC are known: for example, Ca^{++} (in combination with the intracellular Ca^{++} receptor protein, calmodulin) activates glycogen breakdown, smooth muscle contraction, and the pumping of excess Ca^{++} from cells; and PkC regulates a plasma membrane ion channel that exchanges intracellular H^+ ions for extracellular Na^+ and thus controls intracellular pH. However, we understand neither the mechanisms by which these two signals often synergise in the activation of cells nor those by which they control longer term cell decisions such as the choice between proliferation and differentiation.

The future

We have now identified, and partially understand, some of the biochemical mechanisms by which cells respond to extracellular controls, several of which are reviewed in reference 11. In the future we must explore the details of the known signalling processes and identify as yet unknown number of others. A known mechanism that was not mentioned above is the control of intracellular cyclic guanosine monophosphate (cyclic GMP) concentration: in the

retina the photoreceptor protein rhodopsin activates a G protein (G_t or transducin) that controls the enzyme that degrades cyclic GMP. Gene cloning and protein purification studies have recently identified an unexpectedly large number of G proteins, including the proteins that are encoded by the *ras* oncogenes that are frequently mutated in human tumours: these have not yet been assigned roles in known signalling pathways. And other evidence has begun to accumulate for novel biochemical reactions under G protein control: examples include a membrane K^+ channel; phospholipase A_2 (which liberates free arachidonate and thus initiates eicosanoid synthesis); a phospholipase C that liberates 1,2-DAG from phosphatidylcholine rather than $\text{PtdIns } P_2$ (and which may therefore activate PkC without an accompanying rise in intracellular Ca^{++} concentration); and another phospholipase C that is activated by insulin and liberates 1,2-DAG and a glycan derivative of inositol monophosphate as potential intracellular mediators of insulin action. Doubtless other mechanisms will emerge, some of them from quite unexpected quarters, over the next few years.

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How To Do It

Use electronic mail

MICHAEL S BUCKINGHAM

The National Health Service is a large user of the Post Office. New technology in the form of word processors linked by the telephone system may result in letters being replaced by electronic mail. What follows is based on my own experience of the introduction of such a system into a district general hospital.

Introduction

Information may be transmitted from one person to another in a number of ways. Talking face to face is informative, direct, and immediate but may not be convenient. Talking by telephone is also immediate but is slightly less informative and may entail delay while

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the second party is located. Letter writing is much slower but has the advantage of providing "hard copy" which may be filed and used for future reference. This is important for continuing patient management and for legal purposes. Good overall communication between hospital and community depends on a combination of these three systems of exchanging information.

Changes in the office

In the past, many referrals of patients were made by handwritten

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