Science and medicine

The twelfth Science and Medicine Conference, the fourth held jointly with the Medical Research Society, took place at the Royal College of Physicians on 23-24 November 1995. This year’s conference included for the first time a session devoted to mechanisms of disease.

G proteins, calcium cycling and cellular physiology

An overview

Professor G Milligan (University of Glasgow) opened the session with an overview of the regulation of the transmembrane signal cascade. Agonists of cell surface receptors linked to G proteins (guanine nucleotide-binding proteins) activate intracellular adenyl cyclases. Changes in levels of any component of this cascade can affect cyclic AMP levels in the cell. For example, cytosolic cAMP levels were elevated, even in the absence of hormonal stimulation, in neuroblastoma gloma hybrid cells modified to have a greater number of β adrenerceptor receptors on the cell surface. When β antagonists were added, the unstimulated cAMP levels in these cells fell, demonstrating that the drug was not acting merely as an antagonist, but as a reverse agonist, causing a decrease in spontaneous receptor activity. Furthermore, certain drugs are only partial receptor agonists and the cell’s response will therefore be determined by receptor concentration. Whereas isoprenaline causes maximal receptor response at very low receptor concentrations (full agonist), salbutamol requires a higher receptor density, and ephedrine higher still, to achieve full clinical effects (partial agonists). This phenomenon can be exploited clinically by choosing partial agonist drugs which will exert their effect only in tissues with high receptor concentrations. Spontaneous mutations of such receptors are already known to cause clinical disease. Examples include mutant luteinising hormone (LH) receptor in the Leydig cells of the testis which causes precocious puberty in males due to excess production of testosterone in the absence of LH.

G proteins are heterotrimetric membrane receptor bound proteins consisting of three subunits, α, β and γ, which catalyse conversion of guanidine diphasate to guanidine triphosphate. They form part of the signalling cascade through which stimulation of the associated cell surface receptors activates the second messenger systems (including adenylyl cyclases). There are multiple classes, including Gα, which stimulate cyclases, Gβ which inhibit cyclases but activate ion channels and Gγ which act via phospholipase C. Spontaneous mutations occur in G proteins, one of which induces constitutive cell proliferation in the pituitary gland causing pituitary adenoma.

Ion channel activation

Professor A C Dolphin (Royal Free Hospital, London) discussed activation of presynaptic GABA, receptors of dorsal root sensory ganglia in the spinal cord. Endogenous or exogenous activation by the GABA, agonist baclofen causes inhibition of synaptic responses by decreasing calcium influx into the cell. Although baclofen decreases calcium influx by only 30%, a large change in effector release results due to the critical dependence of the rest of the signalling cascade on calcium concentration. Inhibition of calcium channels is mediated by G proteins of the Gα class. Professor Dolphin reviewed work suggesting that the G protein β subunit was critical in coupling to the calcium channel. The Xenopus oocyte is a large cell onto which voltage clamps may be applied and DNA injected. Microinjection of antisense oligonucleotides to the β subunit of Gα into these cells depletes their β subunit levels and lowers calcium channel amplitude. This group’s findings may elucidate highly important general physiological principles because opioid, somatostatin and α2 receptors also work via G proteins.

Acetylcholine receptors

Dr M P Caulfield (University College London) has investigated the specificity of G proteins binding to the muscarinic subclass of acetylcholine receptors in neuronal potassium channels. Antisense oligonucleotide technology, which was successfully employed by the previous speaker, failed to determine which G protein class is involved here and this group is now using much larger antisense sequences (comprising hundreds of nucleotides instead of approximately 20) inserted into bacterial plasmids to infect target cells. The talk generated much discussion about the problems of this newly developing technique. Concern was voiced about therapeutic trials for cytomegalovirus infection which are already underway using antisense sequences to block viral replication, as many unanswered questions remain about the mechanisms and specificity of these approaches.

Intracellular calcium cycling

Professor M J Berridge (Babraham Institute, Cambridge) reviewed the functions of the two intracellular channels, ryanodine receptors and inositol

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These alternative release control, triphosphate (InsP₃) receptors, for regulating calcium release from internal stores. Both are under dual control, being activated by calcium itself (calcium-induced calcium release) and also by cyclic ADP-ribose (ryanodine receptors) or InsP₃ (InsP₃ receptors). These alternative agonists increase receptor sensitivity to calcium. When the Xenopus oocyte is filled with fluorescent dye and studied using confocal microscopy, ‘calcium puffs’—flashes of light—are seen as calcium is released from foci of InsP₃ receptors in the endoplasmic reticulum. Most of them die away, but in a few, where sensitivity to calcium-induced calcium release is increased due to higher InsP₃ levels, propagation of the signal throughout the cell can be seen as a spiral wave. This phenomenon has been reported in many cell types, including cardiac myocytes, where sensitisation of ryanodine receptors results in calcium-induced depolarisation and contraction. Abnormal sensitivity to calcium in myocytes could then cause cardiac arrhythmias.

**Calcium and secretory vesicle cycling**

Exocytosis is another calcium dependent phenomenon. Professor R D Burgoyne (University of Liverpool) described the core mechanisms of exocytosis. In retinal bipolar neurones of goldfish, secretory granules are exocytosed within 100 microseconds after calcium influx, whereas in the adrenal chromaffin cell there is a lag of 5–50 milliseconds before granules fuse with the plasma membrane. A series of elegant patch-clamp capacitance experiments using permeabilised chromaffin cells demonstrated the importance of the protein αSNAP in modulating this pathway. It remains unclear how calcium signals regulate the pathway and whether this mechanism applies in all cell types.

**Hypertension—mechanisms of disease**

11β-hydroxysteroid dehydrogenase and hypertension

Dr J Seckl (University of Edinburgh) revealed the relevance to hypertension of 11β-hydroxysteroid dehydrogenase (11β OHSD), whose physiological role is to degrade cortisol. The mineralocorticoid receptor in the distal nephron is bound on both mineralocorticoids and glucocorticoids with equal affinity but is protected from the latter by 11β OHSD. In the rare 11β OHSD deficiency this receptor is stimulated by cortisol, a phenomenon mimicked by ingestion of liquorice which contains the 11β OHSD antagonist glycyrrhizinic acid. Sensitivity of the renal tubule to glucocorticoids is therefore enzyme mediated. The enzyme is also found in the placenta and the epidemiological finding that adult hypertension is correlated with low birth weight led to the hypothesis that attenuated placental 11β OHSD activity might be responsible. Perfusion studies of human placenta support this hypothesis. Thus a story that began with a hypertensive patient with an obscure metabolic defect may turn out to have been a major clue to understanding fetal programming of blood pressure.

**Single gene disorders causing hypertension: GRA and Liddle’s syndrome**

Dr F Karet (Yale University) described two genetic defects causing familial hypertension. In glucocorticoid remediable aldosteronism (GRA) aldosterone secretion is regulated by ACTH. Dr Lifton, at Yale, has recently identified a genetic mutation causing GRA in which a chimeric gene is present with fused nucleotide sequences of 11β hydroxylase and aldosterone synthase genes. In preliminary screening studies, the group has found many normotensive subjects with this disorder, suggesting interaction with other systems controlling blood pressure. Liddle’s syndrome similarly presents with hypertension and hypokalaemia in early adult life. The mechanism underlying this syndrome has now been established as constitutive activation of the amiloride-sensitive distal renal epithelial sodium channel; using genetic linkage analysis single base pair mutations in the gene encoding the beta subunit of this channel have been demonstrated.

**Endothelin as an endogenous vasoconstrictor**

Dr D J Webb (University of Edinburgh) reviewed the role of the endothelins in maintenance of vascular tone. Endothelin-1 is currently the best characterised and the most potent vasoconstrictor known. It acts on endothelin A (ET₁A) receptors causing arterial vasoconstriction and also on endothelin B (ET₁B) receptors mediating release of endothelium-dependent vaso-dilator substances. In forearm plethysmography experiments, Dr Webb demonstrated a slow-onset dose-dependent forearm vasoconstriction following intra-arterial infusion of proendothelin, the precursor to endothelin-1. This was abolished by co-infusion of specific antagonists. Endothelin-1 is now implicated in the pathogenesis of a wide variety of vascular diseases associated with vasoconstriction, including chronic heart failure, hypertension, Raynaud’s disease and renal failure. Furthermore, evidence is accumulating that the endothelins are important in modulating airway constriction and as neurotransmitters.

**Genetic analysis of essential hypertension**

Unlike the advances in these specific syndromes, progress in understanding the genetics of essential hypertension has been much slower. Professor M J Brown (University of Cambridge) reviewed some approaches to this complex polygenic disorder. Genetic linkage studies have demonstrated an increased frequency of genetic polymorphisms in
hypertensive members of single families, and association studies have identified differences in polymorphism frequencies between unrelated hypertensive and non-hypertensive subjects. This field is riddled with difficulties, including variable penetrance of mutant genes, polygenic pathogenesis and late onset disease. However, the computerisation of medical records in general practice has made new insights possible. Many of 6,000 hypertensive patients receiving anti-hypertensive therapy had either none or all their siblings on therapy. In a few cases, 50% of siblings were receiving treatment. These findings suggest that there are many other as yet unidentified single gene disorders causing hypertension.

Cytokine survey

Proinflammatory cytokines

Professor S Dower (Immunex R & D Corporation, Seattle) gave an overview of regulation of proinflammatory cytokines, particularly of the role of cytokine receptors in modulating cytokine action. For example, when tumour necrosis factor alpha (TNFα) binds to its soluble receptor, it becomes unavailable to cellular receptors. Receptors for interleukin-1 (IL-1) are more complex. Two cellular IL-1 receptors have been characterised but only type I is involved in signal transduction. Although the type II receptor has an extracellular domain similar to type I, it is not activated by IL-1. Far from being redundant, however, the type II receptor is an important regulator of IL-1 activity as IL-1 bound to this ‘decoy’ receptor is diverted from the active type I receptor. Other cytokines, such as IL-4, may influence type II receptor numbers and so indirectly influence IL-1 activity. An IL-1 receptor antagonist, an endogenous ligand of the IL-1 receptor without agonist activity but which blocks the action of IL-1, has been identified. Its activity adds a further level of control in the IL-1 system. By evolving homologous structures to some of these molecules, viruses have developed strategies to counter the host’s inflammatory response. The vaccinia virus, for instance, interferes with the host’s cytokine network by encoding a protein that resembles the IL-1 type II receptor.

In response to a question, Professor Dower said there was evidence from animal and clinical studies of sufficient hierarchy in the pro-inflammatory cytokine network to make specific ‘master cytokines’ targets for therapy. However, caution is needed in extrapolating from animal models where anti-cytokine therapy can be precisely timed in relation to an insult, which cannot be done in the clinical situation. Asked whether soluble cytokine receptors could act as a sump or reservoir for cytokines, Professor Dower said there was no experimental evidence of a poorer clinical outcome when these agents had been used.

Cytokine polymorphisms and human disease

Polymorphisms in the interleukin-1 gene family in relation to inflammatory disease was the subject of a talk by Dr A Cox (Royal Hallamshire Hospital, Sheffield). Autoimmune inflammatory diseases tend to cluster in families and have an inflammatory cell infiltrate that must be coordinated by pro-inflammatory cytokines. The genes comprising the interleukin-1 family are located in a small region of chromosome 2 and polymorphisms in this cluster are over-represented in inflammatory diseases. For example, a polymorphism at position -889 in the IL-1α gene is associated with subgroups of juvenile chronic arthritis and related to the ESR, an index of inflammation. However, most polymorphisms are merely markers and it is only in psoriasis that there is a direct association with disease activity. (Excess IL-1β increases the severity of psoriasis, and endotoxin-induced IL-1β production is enhanced by the IL-1β 3953 polymorphism). Dr Cox has adopted the alternative strategy of searching for CA repeats in the IL-1 gene cluster using yeast artificial chromosomes to identify microsatellite markers which will be used for linkage studies in case control studies.

Cytokines and angiogenesis

Dr R Bicknell (University of Oxford) discussed the mechanisms and importance of tumour angiogenesis, the formation of new vessels from existing vasculature. Angiogenesis in tumours is a critical determinant of tumour behaviour, as the vascular density within a tumour influences its metastatic spread. The importance of vasculogenic factors can be investigated by observing the effect of transfecting MCF-7 cells derived from a slow-growing, poorly vascularised breast carcinoma. Cells transfected with vascular endothelial growth factor had a growth advantage in vivo and the tumours formed by these cells had a greater vascular density than controls although they did not metastasise. The mechanism of angiogenesis is not understood but the angiogenic factor, platelet-derived endothelial cell growth factor (PD-ECGF), was recently found to be homologous to thymidine phosphorylase. This is a housekeeper gene which degrades thymidine to thymine and 2-deoxyribose. Thymidine phosphorylase enzymatic activity is necessary for angiogenic activity of PD-ECGF.

Medical Research Society and Association of Young Medical Scientists Plenary Sessions

Gastrin release

Helicobacter pylori infection in the gastric antrum causes duodenal ulceration (DU). Dr I L P Beales (Hammer smith Hospital) reported the effect of IL-8 and H pylori on gastrin release by isolated gastrin-secreting (G) cells. His group had previously demonstrated augmentation
of meal-stimulated gastrin release in DU patients infected with *H pylori*. IL-8, which is expressed in *H pylori* infected antrum, stimulates gastrin release by cultured canine G cells. Extracts of *H pylori* which had no gastrin-secreting activity by themselves, strongly augmented IL-8 mediated secretion. This may provide the explanation for augmented gastrin release in duodenal ulcer patients.

**Amyloid fibril formation**

The lysozyme model of amyloidogenesis was presented by **Dr D R Booth** (Hammersmith Hospital). A single amino acid substitution was found to be responsible for rendering lysozyme amyloidogenic in two separate English kindreds. The enzymatic activity of recombinant mutants was similar to wild-type lysozyme at 37°C, but was lost at 64°C due to protein unfolding activity. The unfolded proteins aggregated and demonstrated typical features of amyloid fibrils when stained with Congo red and when viewed in the electron microscope. Under specific conditions, protein refolding can be obtained to regain enzymatic activity.

**Albumin reabsorption in the renal tubule**

**Dr N Brunskill** (Leicester University) investigated albumin endocytosis by kidney proximal tubular cells. Injury of these cells following reabsorption of protein and leakage of fatty acids into the tubules by damaged glomeruli may cause interstitial scarring. The kinetics of albumin uptake by tubule cells in culture suggest the presence of two receptors with different affinities. Sensitivity to various G protein inhibitors, and the established distribution of G proteins in kidney, implicate a G13α-type protein in this process. This was confirmed by demonstrating increased albumin uptake in tubular cells transfected with G13α.

**Hypertension**

Essential hypertension is characterised by increased peripheral resistance that reflects changes in the microcirculation. **Dr J P Noon** and colleagues (Universities of Edinburgh, Exeter and Glasgow) investigated the microcirculation in the offspring of couples studied in the MRC mild hypertension trial. They found fewer skin capillaries and reduced dermal vasodilatation in the hypertensive offspring of hypertensive parents than in normotensive control subjects. Since hypertension in this group was a manifestation of a familial predisposition, these findings support the idea that microcirculatory changes are important in the inheritance of a familial tendency to hypertension.

The endothelin-2 (ET-2) gene codes for a vasoconstrictor which has been implicated in animal models of hypertension. **Dr P Sharma** (Addenbrooke’s Hospital) screened a population of hypertensive patients and matched controls for the ET-2 gene using a restriction site created by a polymorphism in the untranslated region of the ET-2 gene. Polymorphisms at this site were associated with differences in pretreatment diastolic blood pressure, suggesting that genes at the ET locus influence the severity of hypertension rather than its initiation.

**Dr J Alaghband-Zadeh** and colleagues (Charing Cross and Westminster Medical School and Imperial College) isolated a nitric oxide synthetase inhibitor from the hypothalamus of the spontaneously hypertensive rat. An unusual feature of this inhibitor is that it acts only on endothelial nitric oxide synthetase. The group speculate that the high level of hypothalamic nitric oxide synthetase inhibitor in the spontaneously hypertensive rat may be involved in the pathogenesis of hypertension in this strain.

**An inhibitor of cancer cell proliferation**

**Professor J M Rhodes’** group (University of Liverpool) investigated a mushroom derived lectin that, upon internalisation into a target cell, inhibits cell proliferation. Using confocal microscopy they showed that the mushroom lectin accumulates around the cell nucleus and blocks the transport of heat-shock protein into the nucleus. The lectin therefore inhibits cell proliferation by interfering with protein trafficking through nuclear pores.

**Corneal transplantation**

Corneal transplantation has become a routine clinical procedure but many grafts are still rejected, especially re-transplants. Corneal endothelial cells are the principal target of graft rejection and their viability is critical for graft function. With the aim of reducing rejection, **Dr A J T George** and colleagues (Hammersmith Hospital, Imperial Cancer Research Fund and Moorfields Hospital) have investigated the feasibility of using genetically modified donor corneas in transplantation. Corneal endothelial cells were transfected with a replication-deficient adenovirus containing a β-galactosidase marker gene under the control of a viral promoter. Preliminary experiments in rabbits showed that the endothelial cells were undamaged, the graft functioned and there was at least transient expression of the β-galactosidase.

**α1 antitrypsin**

Mutations in α1 antitrypsin (α1AT), the most abundant circulating protease inhibitor, cause basal emphysema. **Dr D Lomas** (Addenbrooke’s Hospital, Cambridge) hypothesised that accumulation of the mutant enzyme in the liver is due to loop sheet polymerisation. α1AT comprises five β sheets with one mobile loop. The common Z mutation alters the structure of one β sheet which allows the mobile loop of another α1AT molecule to be inserted into the pocket.
so generated, enabling polymerisation to take place. This phenomenon is temperature dependent and rapid control of pyrexia in these patients would therefore be important to prevent this happening. The development of specific small molecule inhibitors of this binding reaction could also have therapeutic potential.

Susceptibility to tuberculosis

Susceptibility to pulmonary tuberculosis is the subject of a collaborative study between Oxford and the MRC laboratories in The Gambia. Host factors influence the outcome of tuberculosis infection, as only 5–15% of those infected develop the disease. Although environmental factors are important, differences in susceptibility amongst strains of laboratory animals suggest that it is also partly genetically determined. Dr C Runwende (John Radcliffe Hospital, Oxford) investigated polymorphisms in the promoter region of the TNFα gene that may contribute to differences in susceptibility. In a case controlled study of HIV-negative tuberculosis patients, they found that heterozygosity for a polymorphism at position -208 in the TNFα promoter was associated with increased susceptibility to pulmonary tuberculosis.

Special lectures

Control of the eukaryotic cell cycle

The Bradshaw Lecture was given by Professor P Nurse FRS (Imperial Cancer Research Fund). Why do cells proceed through the cell cycle in such an ordered fashion and what are the critical check points that prevent a cell from dividing before first having replicated its DNA? Using a series of temperature-sensitive cell-division cycle mutants of the yeast *Saccharomyces cerevisiae*, Professor Nurse’s team found that a kinase was generated during the G2-phase of the cell cycle which triggered mitosis. This same kinase acted as a ‘brake’ preventing cells from entering S-phase. A second kinase with reciprocal action inhibited mitosis but promoted entry into S-phase. Control of the cell cycle is thus coupled to two oscillating regulator complexes that promote legitimate events but prevent illegitimate ones. These observations were not merely confined to yeast but were also described for kinases from frog oocytes and this implies that the mechanism has been conserved throughout evolution. Closely analogous systems operate in mammalian cells. The potential impact of this process on clinical practice includes new targets for cancer therapy, and the exciting prospect of developing novel strategies that exploit the critical control points in the cell cycle. For example, malignant cells could be forced to divide prematurely, before DNA replication has occurred, resulting in a lethal mitosis.

Amyloidosis

In his Goulstonian Lecture Dr P N Hawkins (Royal Postgraduate Medical School) described the use of 123I-serum amyloid P component (SAP) scintigraphy to unravel the natural history of systemic amyloidosis. He showed that appropriate treatment enables amyloid to regress and results in considerable clinical improvement. He reviewed the rapid progress being made in this field, which has become even more significant since the realisation that Aβ amyloid deposits probably cause Alzheimer’s disease.

Conclusion

In the tradition of previous conferences, this was an excellent meeting. The newly introduced * mechanisms of disease* forum was particularly welcome. We came away with a real sense of how the ‘new’ techniques of molecular biology are beginning to impact on ‘old’ clinical problems such as hypertension. It was noticeable that this year, unlike previous years, women were well represented amongst the speakers and the audience.