## 299P PSYCHOACTIVE CANNABINOIDS AND MEMBRANE SIGNALLING: A PUTATIVE MECHANISM

Gabriel G. Nahas & David J. Harvey[1], New York University School of Medicine, NY 10016, USA and [1]Dept Biochemistry, University of Oxford, UK.

Psychoactive, THC-like cannabinoids partition into the lipid bilayer of the membrane, altering membrane fluidity and activating phospholipases. As a result, increased production of lipid signalling molecules, arachidonyl ethanolamide (AEA), arachidonyl diglycerol (2AG) and arachidonic acid with its cascade of eicosanoids, especially prostaglandins, are generated. Cyclooxygenase inhibitors (indomethacin) block THC-induced prostaglandin biosynthesis. THC and psychoactive cannabinoids also bind persistently to G protein membrane receptors. This binding is associated with allosteric changes in neurotransmitter receptor response, which is increased (dopamine, catechols, GABA), or decreased (acetylcholine, NMDA, opiate ($\mu$ and $\beta$)). These responses are often biphasic. Identified endogenous transmitters to the THC (G protein) receptor are membrane lipids (AEA and 2AG).

It is proposed that AEA and 2AG receptor interaction possesses a physiological function that is to regulate the signalling between boundary lipid and the receptors or enzymes of the membrane. The boundary lipids surrounding the membrane proteins are the vehicles for putative signals between the AEA-G protein receptor and the neurotransmitter receptors. The change of configuration of the G protein receptor catalysed by the lipid mediators would modulate the signalling effect of the membrane on its constituent enzymes and receptors. AEA and 2AG, by-products of tbe membrane phospholipids, would thus be indirect signal modulators of membrane activity. Stimulation of the AEA-G protein receptors might maintain the membrane lipid bilayer in a homeodynamic equilibrium, associated with the transmission of instantaneous signalling to enzymes and neurotransmitter receptors in the lipid bilayer. These signalling mechanisms between membrane phospholigands, the G receptor protein and the lipid bilayer are an ubiquitous property of all membranes and have been identified in brain synapses, gametes, and gonads. The fundamental nature of the AEA and 2AG lipid mediators in controlling membrane function is supported by, in the case of AEA, at least two biosynthetic mechanisms, namely direct enzyme-mediated esterification of phosphatidylethanolamine by free arachidonic acid and, secondly, by a transesterification reaction from phosphatidylcholine. The endogenous natural ligands, AEA and 2AG, which have the same binding sites on the G protein receptor as THC, are signalling molecules generated from the membrane phospholipids and have misleadingly been identified with "cannabinoids".

It is hypothesized that interaction of these lipid mediators with G proteins might initiate signalling through a physical change in protein conformation. Furthermore, the physical signal to the boundary layer lipid would be transduced to the membrane enzyme which initiates the phospholipid-AEA (or 2AG) cycle, thus completing a closed physicochemical loop. Regulation of this process would depend on the interaction of the lipid mediators with their receptors which, in turn, would modulate the signalling function of the lipid bilayer in response to physiological stimuli. In order to perform its putative physiological signalling function, the physicochemical properties of the bilayer must retain their physiological integrity. However, THC alters the physicochemical composition and structure of the membrane and, instead of being rapidly recycled like AEA, must be entirely excreted from the body and will thus remain in the body for a considerable time. THC deregulates the putative membrane signalling in two ways: firstly, by activating the AEA and LAG receptors and, secondly, by altering the physicochemical organization of the boundary lipid bilayer into which it partitions. THC thus deregulates the physiological signalling role of the lipid bilayer, a fundamental feature of all living cells. AEA and 2AG should be regarded as members of a class of fundamental membrane signalling molecules which modulate the activity of G protein receptors, rather than "endogenous cannabinoids".

Marihuana and Medicine. Nahas GG, Harvey DJ, Sutin KM and Agurell S., eds, Humana Press, New York, 1999.

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## 300P THE AGE FACTOR IN THERAPEUTICS

Cameron G Swift, Guy's, King's and St Thomas' School of Medicine, King's College, London

The ageing of populations in both the developed and developing world has highlighted the growth of the pharmaceuticals market for older patients, targeted at disorders of high prevalence and a minority of more or less age-related disorders. In many settings, the escalation of prescribing for older people represents the bulk of market increase in recent years.

Concern over iatrogenic problems has, over the last three decades, motivated investigation of prescribing rationale, medication management and the effect of age on the adaptive mechanisms involved in drug disposition and pharmacodynamics. Those issues have been largely clarified and incorporated into the culture of drug regulation, though there is still a need for further research and for continued interpretation and application within clinical practice.

Together with this, the use of drugs as probes in preclinical and clinical gerontology has enhanced the understanding of age-related processes, particularly at the interface between healthy ageing and disease.

By rectifying the historical exclusion of older patients from preliminary and large scale clinical drug trials, it has been made clear that a positive clinical outcome with modern drug therapy is characteristic of appropriate patient groups of advanced chronological age. There can therefore no longer be any justification for failing to address this dimension of clinical evaluation within drug development programmes and the introduction of clinical guidelines.

There is now a pressing need and opportunity to consolidate this field of enquiry to receive radically the approach taken to therapeutics in late life and to investigate systematically the role of ageing processes in the disorders of older age and their treatment. Such a strategic initiative in age research (as distinct from consideration of ageing as a corollary) will provide a more logical and better informed rationale for the identification, development and application of new drug molecules and therapeutic interventions for the future

## 301P NORMAL AND ABNORMAL AGEING PROCESSES IN MAN

T.B.L. Kirkwood, University of Manchester

The "normal" ageing process in man involves a progressive decline in physiological reserves, increase in frailty and a growing risk of developing a spectrum of age-associated diseases.

Many of these age-associated diseases are, in reality, clinically-diagnosable pathobiological states representing the ends of *continuous* spectra of conditions. For instance, the classic hallmarks of Alzheimer's disease – neuritic plaques and neurofibrillary tangles – are seen in the brains of nearly all individuals over the age of 70, even when there is no outward sign of this disease. Loss of bone mass is a general feature of ageing but does not always result in osteoporosis. Degenerative change in joints is also widespread, although not always associated with osteoarthritis.

To understand the nature of the boundary between normal and abnormal ageing processes, it is important that we understand the nature of the "normal" ageing process. There is now strong evidence that ageing has resulted from a combination of two evolutionary factors in human history. Firstly, the force of natural selection weakens with age resulting in only loose genetic control over the later stages of the life span. Secondly, longevity is secured only at the expense of significant investments in mechanisms for somatic cell maintenance and repair. In our evolutionary past the selection pressure to allocate resources to maintenance was limited by our shorter expectation of life.

These observations are the basis of the disposable soma theory of ageing, which predicts that ageing results from the progressive accumulation of faults in somatic cells and tissues, and that longevity is ultimately controlled through the efficacy of mechanisms for maintenance and stress resistance, such as the antioxidant and DNA repair mechanisms.

The ageing process is thus a normal process that intrinsically generates abnormality. Targetting interventions again specific pathological states associated with abnormal old age therefore has relevance to processes involved in normal ageing as well.

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## 302P NEW MOLECULES FOR OLDER PEOPLE: SELECTIVITY AND SPECIFICITY

R A Yates, Zeneca Pharmaceuticals, Medical Research Dept, Alderley Park, Macclesfield, Cheshire SK10 4TG

Traditionally, those involved in drug research, development and use have shared the objective of "using drugs properly" in the elderly. One conventional approach has been to review clinical safety and efficacy data from a sub set of elderly patients recruited into pivotal pre-registration therapeutic trials and perhaps to further investigate any potentially clinically significant apparently age related difference in subsequent age comparison studies. The limitations of this approach were recognised early, and some more specific studies – such as comparisons of pharmacokinetics in young and old subjects – are almost always incorporated in clinical development programmes for new chemical entities.

It has become increasingly apparent that age itself is not necessarily a primary determinant for clinical pharmacokinetics, pharmacodynamics or therapeutic response to drugs. Exploration of this hypothesis has depended and continues to depend on increasing understanding of the changes in normal physiology and biochemistry that appear to be age-related and the extent to which these influence and are influenced by pharmacologically active agents. Apparently age-related changes have been reported in body composition, organ structure and function in receptor number and sensitivity, hormone levels and response to physiological challenge and in behaviour. There is a large body of literature comparing pharmacokinetics of individual drugs in young and elderly subjects – usually healthy volunteers. Differential pharmacological effects have been described between groups of subjects of different ages, and some treatments for particular conditions will be better tolerated than others by elderly patients.

Expanding understanding of the mechanisms underlying drug action in the elderly, as in other age groups, will require well-controlled studies with very clearly defined, and probably relatively limited, objectives. The continual expansion of understanding of the genetic basis of normal function and of disease will inevitably provide a rich source of hypotheses to be tested in such studies – many of which will be centered on exploration of the activity or otherwise of potential new medicines.

As the population ages and clinical trials in the elderly proliferate there will be further refinement of the modifications to trial procedures appropriate to younger subjects which is sometimes necessary to avoid biased or misleading data from elderly subjects. The expected increased understanding of the diversity of disease mechanisms and increasing specificity in selectivity of new treatment may represent a significant challenge to current drug development processes.

It may be that age of itself will, in time, cease to be considered a relevant co-factor in the assessment of drug action.

Professor Robert Kerwin, Institute of Psychiatry, De Crespigny Park, London. SE5 8AF

The traditional application of genetics to the discipline of pharmacology concerned unusual drug responses that have a hereditary basis. The advent of molecular cloning techniques has had a double effect on pharmacogenetics. Firstly, it has defined a whole series of functional receptor entities that were hitherto unknown. However, they are difficult to study in a clinical pharmacological paradigm because of their low abundance and unknown physiological effects.

Secondly, molecular genetics has revealed that the human genome, including neurotransmitter receptors and enzymes, is more polymorphic and functionally variant than originally thought. This has ushered in a new form of pharmacogenetics that allows functional pharmacodynamic research to be performed by correlating polymorphic variation in genes with variation in drug responses. This allows hypotheses about the locus of action of drugs to be tested.

To illustrate this, our group has adopted this approach to defining the site of action of clozapine. The main new candidates for clozapine's action are the $D_4$ receptor and the 5-$HT_{2a}$ and 5-$HT_{2c}$ receptors. These multiply polymorphic receptors are difficult to study; however, we have correlated response to clozapine with polymorphic variation in these receptors in over 400 patients.

There is no correlation between clozapine response and any of a large range of $D_4$ polymorphism that we have tested, but there are strong associations with 5-$HT_{2a}$ and 5-$HT_{2c}$ variations. These not only predict clozapine response but strongly support the hypothesis that this is an important site of action of the drug: this should stimulate drug discovery into selective 5-$HT_2$ compounds. This approach is gaining momentum rapidly in many areas of medical research and our work suggests the approach may bear fruit in psychopharmacology.

Application of this in ageing disorders is becoming a possibility: for example, allotyping cholinesterase subtypes may improve the use of anti-Alzheimer drugs, and enzyme genetic allotyping can be used to target lower dose regimes.

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Leslie Iversen, Department of Pharmacology, University of Oxford, OX1 3QT

The adult brain has a limited capacity for repair, and in most brain regions no new nerve cells are added after birth. There is a gradual loss of nerve cells and neuropil associated with normal ageing but this is relatively slow and it is not uncommon for centenarians to retain their intellectual capacity. Neurodegenerative disease, however, is accompanied by an accelerated loss of neurones, often targeting particular populations of cells: central and spinal motor neurones in motor neurone disease; basal ganglia dopamine cells in Parkinson's disease (PD); basal ganglia cells in general in Huntington's disease (HD); cerebral cortex in Alzheimer's disease (AD). Imaging results from the OPTIMA project in Oxford from patients with AD suggest that the year by year loss of cortical thickness is accelerated from approximately 1.5% in non-demented subjects to more than 15% in AD patients

Understanding the molecular basis of such diseases and why they target specific populations of neurones remains one of the big challenges for basic neuroscience research. The identification of genetic risk factors for neurodegenerative diseases and the development of genetically engineered animal models represents a major advance, but identifying the gene does not necessarily immediately provide new insight, as the example of huntingtin in HD has shown.

Meanwhile, approaches to the treatment of diseases of the ageing brain remain largely empirical and palliative - attempting to restore some function by remedying the chemical imbalances characteristic of some of these disorders. Refinements of dopaminergic therapy or PD and cholinergic therapy for AD still continue to be made, but few really new approaches to palliative pharmacology can be seen on the horizon. Considerable research effort is being devoted instead to a variety of neuroprotective strategies designed to prevent the progressive loss of neurones in degenerative diseases or to protect against the catastrophic loss of brain tissue following a stroke. Although none of these strategies has so far yielded positive results in the clinic the field is still in its infancy.

Looking further ahead, the replacement of neurones and the repair of damaged fibre tracts may become practical realities within the next few decades. It is already clear that neuronal implants can provide significant therapeutic benefit in PD. If new sources of dopaminergic cells can be identified this could become a routine procedure. The discovery that neural stem cells exist even in the adult brain also offers hope for brain repair, if these cells can be manipulated and used therapeutically.

## 305P TARGETING AGE-RELATED DISORDERS IN THE ARTERIES

David J Webb, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU

Ageing is an important risk factor for cardiovascular disease and is associated with major changes within the cardiovascular system, including a rise in systolic blood pressure, a fall in diastolic pressure, and a consequent increase in pulse pressure. Ageing is also associated with arterial stiffening, an increase in left ventricular mass, endothelial dysfunction and an increased risk of atherosclerotic vascular disease. A better understanding of the interrelation between such changes and the effects of drug treatment is likely to help reduce the burden of cardiovascular disease.

The age-related rise in systolic and mean blood pressure is well described, an associated with an excess mortality that can be reduced by blood pressure lowering. Left ventricular mass, an important independent cardiovascular risk factor, also rises with age, even amongst normotensive individuals. Arterial stiffness plays in important role in both cases.

Arteries are compliant structures and buffer the pressure changes produced by intermittent ventricular ejection of blood. With age, degenerative structural changes and calcification occur within blood vessels (arteriosclerosis) resulting in a stiffer arterial tree. As a consequence, pulse wave velocity (PWV) and the amplitude of the reflected wave both increase, resulting in a change in the shape of the arterial waveform and augmentation of central systolic pressure. Higher systolic pressure is a risk factor for stroke, but also promotes left ventricular hypertrophy, reduces shear stress and further accelerates arteriosclerosis.

Clearly, arterial stiffening has important direct structural and haemodynamic consequences, and recent evidence suggests that it may be an independent cardiovascular risk factor. In addition, ageing and hypertension are associated with endothelial dysfunction, which causes additional functional stiffening of the arteries. Furthermore, endothelial dysfunction leads to an imbalance between the actions of the nitric oxide and endothelin systems that enhances vascular hypertrophy and hyperplasia, increases platelet aggregability, and accelerates atherogenesis.

Recent developments in technology allow the assessment of central arterial pressure and PWV (pulse wave analysis) reliably and non-invasively in the clinic setting. These techniques may also be combined with pharmacological stimuli to provide an assessment of endothelial function. Pulse wave analysis has now been incorporated in a range of major clinical cardiovascular trials that should help to determine the importance of arterial stiffness in the pathogenesis of cardiovascular disease, and the value of arterial stiffness and endothelial function as important therapeutic targets for the future.

Cockroft JR, Wilkinson IB & Webb DJ 1997 Age Ageing 26 (suppl 4):53-60

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## 306P PRIORITIES IN DRUG REGULATION AND DEVELOPMENT

Michael D Rawlins, Wolfson Unit of Clinical Pharmacology, University of Newcastle, Newcastle upon Tyne NE2 4HH

Priorities for the elderly, in drug development and regulation, are legitimate concerns for all of us. They obviously include pharmaceutical industry and drug regulatory authorities but also academia and health professional generally, as well as patients and the public. We all hope, one day, to be the beneficiaries. Moreover, drug development and regulation are but a means to an end: unless effective and cost-effective new treatments and management strategies are adopted into routine use their development and regulation will have been pointless.

Development priorities include new agents to prevent premature death in older people, as well as new treatments to improve their quality of life. The most common causes of premature death are stroke, heart disease and cancer. Priorities for development therefore include the discovery and evaluation of agents for both the primary and secondary prevention of stroke and ischaemic heart disease, as well as strategies for the early detection of the more common malignancies (particularly of the lung, breast, prostate and large bowel). Effective interventions for the treatment of established stroke, together with simpler and safer drugs for the management of acute myocardial infarction, are needed. Furthermore, a high priority should be given to the development of new active cancer agents that would give worthwhile relief, if not cure, of established cancer without imposing a significant burden of iatrogenic disease.

Drugs to improve the quality of life would include novel agents for the treatment of cognitive impairment, Parkinson's disease, osteoarthritis and chronic obstructive airways disease. Currently available drugs for each of these conditions are disappointing. Whilst they all provide some worthwhile - if modest - symptomatic relief, restoration of patients'quality of life is rare, adverse reactions common, and none influence the progression of the underlying disorder.

Knowledge of many of the mechanisms underlying the vulnerability of the elderly to the adverse effects of drugs have been reflected in the regulatory requirements for drugs likely to be used in elderly patients. Dosage requirements for older patients, based on specific clinical data, are now routinely included in the prescribing literature (Summary of Product Characteristics) for new products. Persisting problems have two causes: the heterogeneity of the ageing process itself, and the prevalence of polypharmacy (and hence the inevitability of interactions) amongst elderly people. These issues will need to be better addressed in the future as gerontological and pharmacological knowledge increases.

Finally, these developments from the laboratory and the research clinic will have little public health impact unless they are adopted into routine clinical practice. In some respects this represents the most difficult challenge of all, and one that is to be addressed by the planned National Institute of Clinical Excellence.