73P A COMPUTER SIMULATION OF THE SCIATIC NERVE-TIBIALIS ANTERIOR MUSCLE PREPARATION OF THE CAT *IN VIVO* TO TEACH NEUROMUSCULAR PHARMACOLOGY TO UNDERGRADUATE STUDENTS

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Laboratory practicals which use live animals or animal tissue have long been used by pharmacologists. For a number of reasons many courses have reduced the number of practicals in their curricula and some have turned to computer simulations to provide a 'dry-lab' experience which fulfils some but not all of the objectives of the traditional practical class. These are no substitute if animal/tissue handling skills or specific laboratory skills are important learning objectives but can be effective in presenting data in an interactive manner and encouraging students to use it to learn and practice data-handling, data-presentation, data-interpretation and report writing skills.

Here we demonstrate a computer simulation of experiments which may be performed on the cat sciatic nerve-tibialis anterior muscle preparation *in vivo* to illustrate the important differences in the pharmacological action of depolarizing and non-depolarizing blocking agents and teach the essentials of neuromuscular pharmacology. The program was written using Macromedia Director version 6.5 for IBM compatible PCs running Windows (minimum specification: PC 486 running Windows 3.1 or better).

It has several sections accessible form a menu: A *Student Handbook* uses text and graphics to cover: an outline of the process of neuromuscular transmission; the methods (the preparation of the anaesthetised cat, the protocol for nerve stimulation and isometric recording of evoked muscle contractions); a summary of the actions of and the clinical relevance of different types of blocking agents. The ***Experiments*** section presents high-resolution graphic simulations of muscle contractions, in accelerated time, on a scrolling, chart-recorder like display. *Phase I experiments* - compare the action of a non-depolarizing blocker (d-tubocurarine) and a depolarizing blocker (decamethonium). For each there are traces illustrating the effects of: i.v. administration; i.a. administration; blocker + anti-cholinesterase, blocker + a different non-depolarizing or depolarizing blocker; tetanic stimulation; i.a. acetylcholine. *Phase II experiments* - the effects of 4 successive doses of decamethonium followed by the effects of tetanic stimulation and an anticholinesterase

Each experiment has an associated student activity designed to assess understanding of the experimental results. These might be a series of true/false statements or a table to complete. There are also some suggested questions which would form the basis of a report of the experiment.

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74P DIABETIC NEUROPATHIES

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The incidence and severity of diabetes complications are increased by poor control of glycaemia. This implicates raised glucose as a primary component of their aetiology and focuses attention on biochemical aberrations downstream of hyperglycaemia. These include increased oxidative stress, post-translational glycation of proteins and exaggerated flux through aldose reductase and the polyol pathway. Our understanding of the cell biology of diabetic neuropathy, nephropathy and retinopathy indicates that the critical step in their development occurs when these biochemical anomalies cause sustained changes in cell phenotype. In neuropathy this occurs in neurones, Schwann cells and vascular elements of the endoneurium. Thus, we need to identify the transducers that promote these phenotype switches.

This presentation will review the evidence implicating MAP kinases as *de facto* glucose transducers for diabetic neuropathies. *In vitro*, the MAPKs are activated in sensory neurones by raised glucose and this activation is augmented by superimposition of oxidative stress. Activation of ERK or p38 MAPK provides a damage signal, because inhibition of this effect of glucose plus oxidative stress reduces the release of LDH into the culture medium.

We have not yet been able to test the effect of JNK inhibition. All three groups of MAPKs are activated in sensory neurones in diabetic rats and in diabetic patients. Inhibition of p38 activation in diabetic rats reverses neurological function deficits, such as decreased nerve conduction velocity. This latter change is also prevented by treatment of diabetic rats with sonic hedgehog. Thus, we have two dissimilar interventions with a common end-point and it is interesting to speculate on the extent to which the mechanisms differ.

Such effects provide useful tools for dissection of mechanisms and for identification of the transcriptional changes involved.

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There have recently been great strides in the understanding of the basic physiology of pain perception and we now know the identity of some of the plethora of neurotransmitters and modulators that are involved in nociception. This knowledge has yet to deliver its full benefit to those patients who need pain relief and pain is still an unsatisfied medical need. The vast amount of information now available to us from the sequencing of the human genome and that of other animals makes therapeutic advances likely so long as the most appropriate targets are chosen.

Strategies to discover new therapies can arise from refinement of existing drugs, (sometimes by discovery of the mechanism of action of a drug used to treat pain on empirical grounds) or from a consideration of novel gene targets. Refinement of existing drugs probably carries the greatest probability of success but may not constitute a major therapeutic advance. The choice of a completely novel target is more risky but holds out the promise of such a major therapeutic advance. The introduction of the cyclooxygenase-2 inhibitors for the treatment of pain is probably the best current example of the cloning of a new molecular target leading to the discovery and development of drugs with real clinical advantages. Potent non-peptide antagonists at the NK_1 receptor which block the effects of the neuropeptide substance P were also made possible by the cloning and study of receptor function at the molecular level. When these agents were evaluated in animals they showed potential as analgesics but clinical trials failed to confirm this activity although other clinical utilities have since been established (Boyce et al, 2001 for review). Although molecular enabling technologies are now in place we still need better animal tests that are reliably predictive of clinical outcomes. The use of transgenic animals may be one way forward both for predicting target utility and for evaluation of potential drug molecules. This approach is now an important corner stone of modern pain research (Souslova et al, 2000; Akopian et al, 1999).

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76P NEUROPATHIC DISORDERS OF THE ENTERIC NERVOUS SYSTEM

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The enteric nervous system co-ordinates the activity of primary effectors to produce meaningful patterns of behaviour of the gut for the whole organism. In many respects it resembles the central nervous system and indeed the neurons involved are essentially the same. The enteric nervous system is the most peripheral of the neural centres that control gut function and can act independently of the sympathetic and parasympathetic nervous system, and higher neural input into the bowel or may be modulated by it.

The enteric nervous system works with three functional categories of neurons – sensory, inter and motor neurons – and these are connected by synapses into networks that process sensory information and control the behaviour of motor neurons. Multiple connections for logic circuits that decipher codes from sensory neurons and signals from elsewhere in the nervous system interact to produce an integrated motor output.

Abnormalities in the inhibitory or excitatory input may lead to different disease states. A functional obstruction occurs in any condition where inhibitory motor neurons are destroyed or where the muscle coats have lost contractile activity. Without inhibitory controls, the self-excitable smooth muscle contracts continuously and behaves as an obstructing element. Loss or malfunction of inhibitory motor neurons is the pathophysiological basis of this type of condition and it underlies several forms of chronic intestinal pseudo-obstruction, Hirschsprung's disease and achalasia of the oesophagus. In early life this may be due to congenital maldevelopment of the enteric nervous system in which there is disordered colonisation of the primitive gut by neural crest cells – Hirschsprung's disease or congenital aganglionosis, abnormal differentiation of neuroblasts – intestinal ganglioneuromatosis and impaired survival or maintenance of developing nerve cells hypoganglionosis, and perhaps infantile achalasia. The genetic determinants and molecular events which underlie these conditions are beginning to be understood and include the RET/GDNF and ETRB/ET3 signalling systems in Hirschsprung's disease and single point mutations of RET (M918T) in intestinal ganglioneuromatosis. The role of neurotrophic factors in hypoganglionosis and achalasia is yet to be explored but the findings suggestive of these conditions in neurturin and GFRa2 knockout mice suggests that they may play a role in these conditions. In later life, neuropathic degeneration occurs; but apart from autoimmune disease directed against enteric neurons and the toxic effects of drugs, little is known of the underlying disease processes. Neuropathic degeneration in its early stages may be manifest as symptoms which can be confused with the irritable bowel syndrome and other functional gastrointestinal disorders.

There is an increasing body of evidence to show the interaction between mucosal associated lymphoid tissue and the motor apparatus of the gut. The enteric immune system is colonised by populations of immune and inflammatory cells that are constantly changing according to luminal conditions and pathophysiological states. Due to the inability of the physical and chemical barrier of the luminal interface to entirely exclude antigens, the immune system is daily exposed to dietary antigens, bacteria, viruses and toxins causing the mucosal associated lymphoid tissue to be chronically challenged. The motor and secretory responses in the gut of animals sensitised to antigens such as food or bacterial toxins, together with the effects of infection in the human show a direct link between the immune system and the motor apparatus. Immuno-neural integration starts with either detection by epithelial cells of antigen or immunocytes in the lamina propria. The signal is transferred to the enteric nervous system and the appropriate response occurs from the programme library of the enteric nervous system. In general, detection by the enteric immune system and signal transmission to the enteric nervous system initiates a defensive behaviour analogous to the emetic response in the upper gastrointestinal tract resulting in the forceful and rapid propulsion of ingested material over long distances.

Several kinds of immune and inflammatory cells including lymphocytes, macrophages, mast cells and polymorphonuclear cells are putative sources of paracrine signalling to the enteric nervous system. The use of signalling between mast cells and the enteric nervous system is best understood. Antigen evoked degranulation of sensitised mast cells releases a wide variety of paracrine signals that include 5-HT, histamine, platelet activating factor, tachykinins, prostaglandins, cytokines and leucotrienes.

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77P MECHANISMS OF CELL DEATH IN PARKINSON'S DISEASE

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The primary pathology of Parkinson's disease (PD) involves the destruction of nigral dopaminergic cells with the appearance of intracellular proteinous inclusions termed Lewy bodies. The cause of nigral cell death remains unknown but may involve both genetic and enviromental components. The discovery of gene mutations responsible for the occurrence of both autosomal dominant and autosomal recessive forms of early and late onset familial PD has led to interest in the roles of proteins such as parkin, UCH-L1 and Δ-synuclein in nigral cell degeneration. Parkin and UCH-L1 are both important components of the process leading to the ubiquitination of proteins prior to their degradation by the 26S proteasome. While disruption of the normal function of these proteins may explain their apparent cytotoxicity, a general problem in the clearance of abnormal or mutant proteins may also be involved. Indeed, while the majority of cases of PD are not obviously genetic, dysfunction of the ubiquitin-proteasome cycle may also underlie nigral cell death in sporadic PD leading to the formation of Lewy bodies as protective mechanism for the storage of unprocessed and potentially toxic material.

Many mechanisms have been held responsible for nigral cell death in sporadic PD including oxidative stress, mitochondrial dysfunction, excitotoxicity and nitric oxide/peroxynitrite toxicity that form part of the cascade of events underlying neuronal destruction. Cell death appears to be apoptotic in nature and may involve both the neurones and glial cells.

Oxidative stress in the nigra in sporadic PD leads to oxidative damage to lipids, DNA and proteins. The increased protein damage again raises the question of altered protein handling as a common mechanism responsible for cell death in both familial and sporadic PD. Indeed, it could explain why a range of ubiquitinated but unprocessed proteins are found in Lewy bodies in sporadic PD together with products of oxidative/ nitrative attack on proteins.

Examination of proteasomal function in substantia nigra in sporadic PD has shown its enzymatic activity to be reduced. This may result from the loss of structural Δ-subunits and from alterations in the levels of proteins making up the PA28 and PA700 regulatory caps which have also been detected in post-mortem PD tissues. Impairment of proteasomal function in this manner leads to apoptotic cell death of dopaminergic neurones in culture and also following the inhibition of proteasomal activity by intranigral injection of lactacystin in rats.

The finding of altered proteasomal function in sporadic PD coupled the occurrence of oxidative stress and abnormal levels of protein damage may explain Lewy body formation. The concept of a disruption of the ubiquitin-proteasome cycle as underlying nigral cell degeneration brings together in to one hypothesis a potential mechanism responsible for both sporadic and familial PD of early and late onset.

78P AN OVERVIEW OF PRESYNAPTIC RECEPTORS

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Noradrenaline (NA) is the first neurotransmitter for which the existence of presynaptic autoreceptors modulating transmitter release from nerve terminals was documented. The initial experimental evidence was obtained in the peripheral nervous system.

The original hypothesis stated that a negative feedback mechanism, mediated by α-adrenoceptors located on nerve terminals, regulated the calcium-dependent release of the neurotransmitter. Blockade of presynaptic terminal autoreceptors by antagonists enhanced transmitter release during nerve stimulation, while the α-receptor agonists reduced the release of NA. In addition, the interaction between agonists and antagonists that modulate transmitter release was shown to be of a competitive nature. These results were obtained regardless of the α- or β-adrenoceptor type mediating the post-synaptic response of the effector organ. Subsequently, it was demonstrated that the presynaptic terminal autoreceptors were of the α2-subtype and pharmacologically different from the α1-subtype. Today we know that presynaptic terminal autoreceptors are predominantly of the α2A-subtype.

It is of interest that presynaptic autoreceptors exist in the cell body of noradrenergic neurons, where they inhibit the rate of firing of the neuron. These somatodendritic receptors for NA are also of the α2-subtype and were already being studied in the central nervous system in 1970,using clonidine as an agonist. The concept of presynaptic negative feed-control of NA release was also demonstrated for other neurotransmitters in the periphery and in the central nervous system, including acetylcholine, dopamine, GABA, 5HT, histamine and glutamate

In addition to the presynaptic inhibitory autoreceptors, the nerve terminal also possesses presynaptic heteroreceptors which are acted upon by chemical signals present in the synaptic cleft which are different from the neuron's own transmitter. For instance, central serotonergic nerve terminals possess presynaptic inhibitory α2-adrenoceptors, and central dopamine terminals possess presynaptic facilitatory nicotinic cholinergic receptors. Peripheral and central presynaptic autoreceptors and heteroreceptors are potential sites of action of several drugs which can modulate neurotransmitter release and may represent a novel approach to drug discovery. For instance, mirtazepine is an antidepressant drug with central α2-adrenoceptor blocking properties. It enhances NA release by blocking presynaptic terminal α2-adrenoceptors and it also increases 5-HT release by blocking presynaptic α2-hetero-receptors on 5-HT nerve terminals.

79P MAMMALIAN PRESYNAPTIC $GABA_B$ RECEPTORS

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The presence of a GABA receptor on peripheral nerve terminals provided the first evidence for a metabotropic receptor for this inhibitory amino acid. But this site probably has only limited physiological significance in the periphery whereas it appears to play a major role within the brain and spinal cord. Receptor activation has been shown to reduce the evoked release of glutamate, noradrenaline, 5HT, acetylcholine, somatostatin, dopamine, neuropeptides such as substance P, and GABA itself. Thus, hetero- and autoreceptors have been demonstrated and both types appear to be physiologically relevant even though the only clear anatomical evidence for axo-axonic connections is within the spinal cord. Within the hippocampal CA1 region, for example, the GABA released from GABAergic terminals is not only able to act back at autoreceptors on these terminals but is released in amounts sufficient to diffuse to and activate $GABA_B$ heteroreceptors on adjacent excitatory terminals (Isaacson and Hille, 1997, Neuron, 18, 143-152). This reduces the evoked release of glutamate.

Elucidation of the structure of the $GABA_B$ receptor has revealed that whilst it has 7 membrane spanning domains in common with many other G protein-coupled receptors, its only functional state is as a heterodimer. Although the subunits of this receptor dimer, $GABA_1$ and $GABA_2$ and their isoforms are very similar, homodimers are not functional and receptor monomers are not even expressed on the cell surface.

The variation in possible combinations of $GABA_{B1}$ and $GABA_{B2}$ subunits has prompted the suggestion that these may provide the basis for receptor heterogeneity but, as yet, nothing has emerged. Nevertheless, the possibility of receptor subtyping between pre- and post-synaptic sites remains, for example, the pre-synaptic site appears to be insensitive to pertussis toxin whereas this agent readily inactivates the post-synaptic receptor. Also the pre-synaptic receptor is coupled predominantly to membrane Ca^{2+} channels whereas the post-synaptic receptor is coupled to membrane K^+ channels. However, any pharmacological separation between pre- and post-synaptic sites has yet to be established. There are some indications that the subunit structure of the presynaptic receptor may differ from that of the postsynaptic site but this is not a constant feature and we are unable to correlate structure with location. Studies by Raiteri's group (e.g. Trends Pharmacol Sci., 1993, 14, 259-261) have suggested that the nature of the $GABA_B$ receptor may even differ between the type of nerve terminal on which they reside.

The possibility of obtaining pharmacologically distinct $GABA_B$ receptors, particularly between pre- and post-synaptic sites, is attractive. This could provide specificity in the application of the receptor to the potential treatment of neuropathic pain, asthma and drug addiction as well as spasticity, all of which stem from a pre-synaptic locus of action.

80P PRESYNAPTIC OPIOID RECEPTORS

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The opioid-receptor family comprises the "classical" *mu*, *delta* and *kappa* types and the structurally homologous, but pharmacologically distinct, ORL_1 receptor[1].

The *mu* and *kappa* types were defined before the discovery of endogenous peptide agonists; the discovery of the *delta* receptor came from the investigation of the pharmacology of the enkephalins. Unlike morphine, the enkephalins had higher potency at prejunctional receptors in the mouse vas deferens (*delta*), than at those in myenteric neurones of the guinea pig. Subtypes of *mu*, *delta* and *kappa* receptors have been proposed, but the evidence from cloning is for only single gene products. Splice variants are found, but their pharmacological relevance is unknown; otherwise the evidence for subtypes could be explained by post-translational modifications, perhaps involving receptor dimerization.

An important outcome of the cloning exercises was the discovery of a gene encoding a GPCR bearing a high degree of structural homology (~60%) towards the existing opioid receptors. This "Opioid-Receptor-Like" ORL_1 receptor was also functionally homologous, in coupling through $G_{i/o}$ to inhibition of adenylyl cyclase. In general, all four opioid receptor types act through this G-protein, with subsequent important effects on various ion channels. The best characterised are activation of potassium conductances, notably GIRK, and inhibition of high threshold calcium currents. Although an over-simplification, it is convenient to think of the former being responsible for opioid-induced reductions in neuronal activity, and the latter for inhibition of transmitter release.

With the lack of affinity of classical opioids, the attempts to define the pharmacology of ORL_1 have involved the use of the natural agonist nociceptin/orphanin FQ, and of partial agonist peptide analogues; the recently described non-peptide ligands should provide clarification. Some ORL_1 effects in the whole animal are "paradoxical", such as the pro-nociceptive effects that may be attributable to an anti-opioid action. At the cellular level, or in isolated tissues activation of ORL_1 produces effects that are characteristically "opioid", such as presynaptic inhibition of nordarenaline release in the cortex. In the periphery nociceptin produces powerful inhibitions of transmitter release in classic opioid-receptor models of the vas deferens, but also in tissues where the other types are lacking, such as the anococcygeus.

It is likely that the effects of this new member of the opioid-receptor family will all be explicable in terms of inhibition of peri-synaptic activity, much as the "uncharacteristic" stimulatory effects of morphine (e.g. in the VTA-accumbens pathway) are now attributed to disinhibition.

[1] The nomenclature MOP, DOP, KOP and NOP was recently proposed by a re-convened IUPHAR Nomenclature Committee.

81P PRESYNAPTIC CANNABINOID RECEPTORS

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Mammalian tissues contain at least two types of cannabinoid receptor, CB_1 and CB_2. Endogenous ligands for these receptors have also been identified, the most important of these "endocannabinoids" being arachidonoylethanolamide (anandamide), 2-arachidonoyl glycerol and 2-arachidonyl glyceryl ether. CB_1 and CB_2 receptors are coupled through G protein to adenylate cyclase and mitogen-activated protein kinase. CB_1 receptors are also coupled through G protein to certain types of calcium and potassium channel. Whilst CB_2 receptors are expressed mainly by immune cells, CB_1 receptors are found primarily on central and peripheral neurones. Many CB_1 receptors are presynaptic, one consequence of the activation of these receptors being inhibition of evoked transmitter release.

Much of the initial evidence that presynaptic CB_1 receptors modulate transmitter release came from classical pharmacological experiments with isolated strips of myenteric plexus-longitudinal muscle of guinea-pig small intestine, mouse vas deferens and mouse urinary bladder, the measured effect being cannabinoid-induced inhibition of electrically-evoked contractions or contractile transmitter release. These cannabinoid-induced effects were found to be readily opposed by the selective CB_1 receptor antagonist, SR141716A, or by selective CB_1 receptor internalization or downregulation caused by *in vivo* pretreatment with the cannabinoid receptor agonist, Δ^9-tetrahydrocanabinol. It is now clear from experiments in which transmitter release has been monitored directly or indirectly that presynaptic CB_1 receptors modulate transmitter release not only in the peripheral nervous system but also in CB_1-containing areas of brain and spinal cord.

Thus, results from *in vitro* experiments with tissue taken from such areas have indicated that CB_1 receptors can mediate inhibition of the evoked neuronal release of acetylcholine, noradrenaline, dopamine, 5-HT, GABA, cholecystokinin, glutamate, D-aspartate and glycine. There is also convincing evidence that one important role of endocannabinoid molecules is to serve as retrograde synaptic messengers that mediate depolarization-induced suppression of inhibition or excitation by acting on presynaptic CB_1 receptors on glutamatergic or GABAergic neurones following their release from cerebellar Purkinje cells or hippocampal pyramidal cells. Anandamide can also stimulate the release of certain neuropeptides by acting on vanilloid receptors. The potency and efficacy of anandamide at these receptors is rather low. However, there is evidence that this endocannabinoid can be converted by lipoxygenase to metabolites with higher vanilloid receptor efficacy than anandamide.

Evidence is also emerging that non-CB_1, non-CB_2, non vanilloid receptors for cannabinoids are expressed by neurones and that the non-psychotropic plant cannabinoid, (–)-cannabidiol, may be a ligand for one of these receptor types.

82P LARGE ANIMAL MODELS IN STUDIES OF THE PHARMACOLOGY OF ANTI-INFLAMMATORY DRUGS

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Large animal species are used routinely in veterinary pharmacology (a) to elucidate similarities and differences between species in drug pharmacokinetics (PK) and pharmacodynamics (PD); (b) to assist the design of dosage schedules on a species by species basis; and (c) to study disease mechanisms using pharmacological methods. However, the healthy and diseased animals and the disease models used in these studies have potential for wider applications in biomedical research. For example, the use of large animals in studies of inflammatory airway disease, skin disease and joint diseases offers advantages over *in vitro* studies on tissues and cells and *in vivo* studies in laboratory animals. These include ease of animal handling and sequential sampling and the use of cross-over designs to minimise inter-animal variation and increase statistical power. In addition, the traditional approach in drug development of parallel but separate PK and PD studies can readily be replaced by PK/PD analysis in large animal models.

This presentation is concerned with the use of large animal models to investigate the acute inflammatory process and the PK and PD of anti-inflammatory drugs of several classes, but particularly those of the non-steroidal anti-inflammatory drug (NSAID) group. In the horse, cow, sheep, goat, pig and camel tissue cage models of inflammation have been developed to study simultaneously (a) drug pharmacokinetics in plasma and drug distribution into inflamed (exudate) and non-inflamed (transudate) tissue cage fluids (b) magnitude and time course of inhibition of synthesis of eicosanoids and other putative acute inflammatory mediators and (c) magnitude and time course of inhibition of components of the inflammatory response. The investigations have revealed marked species differences in PK and some differences in PD of NSAIDs. Modelling PK and PD data to the sigmoidal Emax equation has enabled calculation of PD parameters (Emax, EC50, N, T1/2keo) for several NSAIDs in a range of species for inhibition *in vivo* of exudate prostaglandin E2 and inhibition *ex vivo* of serum thromboxane B2 in clotting blood. These data tentatively indicate PD parameters for inhibition of cyclooxygenase (COX)-1 and COX-2, respectively, and hence permit determination of COX-1:COX-2 inhibition ratios. For NSAIDs of the 2-arylpropionate sub-group, there is the added consideration that each drug contains a single centre of asymmetry and exists in 2 enantiomeric forms, the PK and the PD of which differ substantially. Potentially, PK/PD analyses may be used to facilitate drug discovery and development; they offer a general framework for interspecies extrapolation and may permit transformation of an effective *in vitro* concentration into a proposed *in vivo* dose for clinical use.

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83P ANTIBIOTIC RESISTANCE: DESIGNING DOSING SCHEDULES ON BASIC PHARMACOLOGICAL PRINCIPLES

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Antibiotic resistance is of considerable concern in animal health because of its direct consequences to the target animal populations and also because of the potential impact that it could have for man. There is compelling evidence that antibiotic-resistant bacterial pathogens selected in animals can be transferred on contaminated foodstuffs to man and may result in human infections recalcitrant to related antibiotics used in man (Molbak *et al.* 1999, Smith *et al.* 1999). Nevertheless antibiotics make a major contribution to efficient animal production, improve the welfare of livestock and may contribute to a reduction in the transfer of zoonotic infections to man.

It is clear, therefore, that every effort must be made to reduce the risk of development and transfer of antibiotic resistance from animals to man. In this context, prudent use guidelines and appropriate decision support systems should be utilised to adequately justify all veterinary use of antibiotics (Choraine, 2000). Furthermore, antibiotics should be administered in strategies which maximise their efficacy and minimise the selection pressure for resistance development.

It has been demonstrated that sub-optimal dosing regimes may confer increased selection pressure for the development of resistance (Blaser *et al.*, 1987, Thomas *et al.*, 1998). In veterinary medicine tissue cage models and *ex vivo* bacterial kill curves have been used to help predict optimal dosing strategies (Shojaee Aliabadi & Lees 2001) and a disease model of bacterial pneumonia used to compare dosing strategies for fluoroquinolones (McKellar *et al.*, 2000). In this danofloxacin was administered by either single intravenous bolus or intravenous infusion at the same total dose and clinical and bacteriological outcome assessed. Bolus administration conferring large maximum plasma concentration was more effective than infusion administration in terms of both clinical and bacteriological cure.

On the basis of this study and extrapolation from rodent studies it is apparent that antibiotics can be categorised according to their action as either concentration dependent, time dependent or co-dependent on concentration and time (Hyatt *et al* 1995). Such generic classification permits appropriate dosage strategies to be predicted for acute systemic bacterial infections. For chronic deep-seated infections optimal dosing strategies have not yet been determined.

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84P RECENT ADVANCES IN THE MECHANISMS OF PAIN

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The pharmacology of pain and analgesia exhibits plasticity in different pain states. Understanding this plasticity may lead to improved therapies for the two major types of pain, neuropathic and inflammatory pain, where nerve and tissue damage leads to alterations at both peripheral and central levels.

At the level of the peripheral nerve, we have a far better knowledge of the nociceptor and receptors for the various chemicals released after tissue damage. Further, drugs acting on novel sodium channels may provide local-anaesthetic-like drugs that target only pain-related activity. The new generation of NSAIDs, COX-2 inhibitors, which lack gastric actions, are examples of how therapy can be improved.

Some of the changes that occur after nerve injury at the level of the peripheral nerve seem to invoke central compensations - calcium channels that are essential for transmitter release are a major link between primary sensory neurones and spinal transmission and gabapentin may act on these changed channels.

In the spinal cord, the release of peptides and glutamate causes activation of numerous receptors. Of major importance is the N-methyl-D-aspartate (NMDA) receptor for glutamate in persistent pain states which, in concert with other spinal systems, generates spinal hypersensitivity. Ketamine blocks the NMDA receptor complex but there is potential for sub-unit selective drugs which lack psychotomimetic effects. The roles of the other receptors for glutamate are less well-understood at present.

Blocking the generation of excitability is one approach but increasing inhibitons may also provide novel analgesics. Opioid actions are via pre- and post-synaptic inhibitory effects on C-fibre terminals, spinal neurones and at supraspinal sites. The recent description of the nociceptin receptor may be another novel target. Combination of NMDA antagonism with an opioid may allow lower doses to be used or restore opioid sensitivity of neuropathic pains.

Potent and selective alpha-2 adrenoceptor agonists are available and the multitude of receptors for 5HT lends hope for future analgesic agents in pains other than headache. A number of other novel targets have recently been revealed such as adenosine receptor agonists. Likewise, antagonists of peptides still remain possible new analgesics.

An intriguing approach would be single molecules with dual pharmacological actions, encompassing targets covered here. At present, this can be achieved by combination analgesia.

85P THE USE OF LARGE ANIMAL MODELS IN PAIN RESEARCH

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Significant advances have been made in the last decade in understanding the pathophysiology of pain in humans and animals, and in parallel, there has been an increasing focus on recognising patterns of pain and managing pain in animals.

It is clear that humans and animals share many similarities in the pathophysiology of pain. Following tissue damage, peripheral and central alterations in nerve fibre and neuronal activity lead to spontaneous pain, hyperalgesia and allodynia. Inflammatory pain is common in animals, and is highly prevalent in large animals, associated with many infectious conditions, trauma and surgery. Neuropathic pain is not commonly described in animals. *In vivo* models of pain, inflammatory and neuropathic, have been characterised in laboratory animals, primarily rodents.

However, increasingly work is being carried out in target species, to understand more clearly species related pharmacology of pain. Use of mechanical, thermal, visceral, chemical and ischaemic models are documented in sheep, horses, and cattle. These have been used in association with pharmacokinetic studies to determine analgesic efficacy in the target species (Welsh *et al.*, 1995). Work in large animal models has also furthered basic understanding of pain mechanisms e.g. the pharmacology of spinal metabotropic glutamate receptors in acute nociceptive pain (Dolan & Nolan, 2000).

Work on naturally occurring pain due to inflammatory disease has been undertaken in large animals. Castration is carried out on most male lambs born in the UK, and studies on the pain associated with this procedure have indicated a distinct behavioural profile associated with pain due to this composite stimulus (ischaemic, neuropathic, inflammatory, depending on the method employed) (Kent *et al.*, 1998; Thornton *et al.*, 1999). Work on clinical disease models has distinct advantages since most disease-associated pain is complex in origin and causality. Use of a surgical laparotomy model, chronic mastitis and chronic 'footrot' in sheep (Ley *et al.*, 1995; Welsh *et al.*, 1994; Dolan *et al.*, 2000) has identified that animals with chronic inflammation have hyperalgesia that is frequently long lasting.

Since animals cannot self-report pain, the use of nociceptive pain threshold measurement has developed as a tool to identify animals with altered nociceptive pathway function, which is suggestive of an initiating stimulus of sufficient intensity to induce clinical pain. Furthermore, the identification of alterations in nociceptive information processing in animals with clinical disease along with altered behavioural profiles has heightened awareness of pain in animals and the need for better management.

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