185P IMIDAZOLINE RECEPTORS IN HYPERTENSION

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Drugs with an action on the central nervous system have been employed for over 40 years in the treatment of hypertension. Reserpine in the 1950s, methyldopa from the 1960s and clonidine in the 1970s were introduced into clinical practice. While their efficacy is not disputed, the frequency of symptom side effects has limited their use in recent years.

The availability of better tolerated drugs active at extracerebral sites has resulted in a marked reduction in the use of centrally acting drugs. The most common side effects were sedation (all central drugs) depression (reserpine and methyldopa) and dry mouth and withdrawal reactions (clonidine). All three classes were believed to act by modifying catecholamine function in the brain and periphery: in the case of methyldopa by formation of a 'false' transmitter, alpha methylnoradrenaline, and in the case of clonidine as an agonist at 'atypical' α_2 receptors in the brain stem. These receptors were considered to be the site and mechanisms not only of the blood pressure fall but also the central side effects, including sedation.

In recent years evidence has accumulated of a new class of receptors which preferentially bind imidazoline drugs and lead to hypotension but not sedation. These imidazoline receptors and putative endogenous ligands have provided the rationale for a new group of drugs which are imidazoline-preferring compounds. Rilmenidine and moxonidine are examples of this group and have been introduced into clinical practice as antihypertensive drugs. These agents have less affinity for α_2 adrenoceptors than clonidine and are relatively selective ligands at the imidazoline I_1 receptor which is linked to inhibition of sympathetic outflow in the rostralventrolateral medulla of the brain stem. These I_1 receptors have also been identified in renal tubules, platelets and adrenal medulla.

Clinical trials with rilmenidine and moxonidine confirm comparable efficacy compared to other established classes of antihypertensive drugs. Side effects, particularly dry mouth and sedation, are less common than with clonidine in controlled trials. Reversal of antihypertensive actions is gradual over 3-4 days, unlike clonidine, and withdrawal reactions have not been reported.

The precise role of imidazoline-preferring agents in hypertension remains to be established. They offer an alternative strategy in patients with side effects on other classes of drugs; they can be useful in a wide range of patients with few absolute or relative contraindications and can be used safely and successfully in combination with most other drugs in patients requiring more than one drug. There is potential for the further improvement of the selectivity of this class of drug and there are possible additional indications, including heart failure patients.

186P THE PHARMACOLOGICAL BASIS OF DIABETES THERAPY: AN OVERVIEW

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Non-insulin dependent diabetes mellitus (NIDDM) has been described by the British Diabetic Association as the hidden epidemic, affecting an increasing proportion (over 5%) of the adult population of the UK and accounting for 10% of the National Health Service's total budget. In addition to dietary control, the principal aims of drug treatment are to facilitate the disposal of blood glucose following ingestion of carbohydrate and to blunt the rise of plasma free fatty acids which ensues from lipolysis. In the insulin-resistant NIDDM patient these beneficial effects can be achieved by restoring insulin sensitivity.

Very many natural products have been examined for their potential hypoglycaemic activity and no less than 343 medicinal plants identified (Rahman & Zaman, 1989). However, with the exception of the sulphonylureas, whose hypoglycaemic properties were discovered somewhat serendipitously in the early 1950s, advances in the pharmacological treatment of diabetes have been notably lacking. The first biguanide (Synthalin B) appeared in 1926 and eventually led to the development of dimethylbiguanide (Metformin) in 1957. The stimulation of insulin release by activation of the K^+-ATP channel-linked sulphonylurea receptor and the potentiation of glucose transport (by the biguanides for example) have remained the principal targets for drug action. Thiazolidinediones such as troglitazone initially proved successful in this last respect, but the early compounds were associated with unacceptable toxicity. In addition, it now appears that a novel imidazoline binding site on pancreatic β cells may be activated to provoke insulin release (Chan *et al.*, 1994). The association of NIDDM with obesity has also directed interest to the anti-diabetic potential of anti-obesity drugs, and a large number of appetite suppressants are used clinically.

More recently, the discovery of new hormones such as the *obese* gene product leptin, and new roles for established hormones, such as CCKA and GLP-1, has yielded a number of novel potential mechanisms for regulating satiety and energy balance which are now rapidly being exploited. At the same time, technical advances such as time-lapse imaging of intracellular events *in vivo* by confocal microscopy (Oatey *et al.*, this meeting), are making it feasible to observe functional responses to drugs and hormones at the subcellular level. Probably at no time since the initial discovery of the role of insulin in juvenile diabetes has there been such an opportunity to develop novel treatments for this hidden epidemic.

Chan, S.L.F. et al., (1994) *Br. J. Pharmacol.* 112: 1065- 1070
Rahman A-U. & Zaman, K. (1989) *J. Ethnopharmacol.* 26: 1-55

187P EFFECTS OF TYRAMINE AND β-ADRENERGIC AGONISTS ON GLUCOSE TRANSPORT IN WHITE ADIPOCYTES *IN VIVO* AND *IN VITRO*

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Rat adipocytes exhibit a strong lipolytic response to β3-AR agonists and high capacity to oxidize tyramine.

We have investigated whether β3-AR stimulation or tyramine oxidation can affect or mimic insulin activation of glucose transport. [^{3}H]-9-deoxyglucose uptake and lipolytic activity were determined in isolated white adipocytes and monoamine oxidase (MAO) activity assayed using [^{14}C]-tyramine (Carpene *et al.*, 1993, 1995).

Neither BRL 37344, CL 316243 or tyramine stimulated glucose transport, but when adenosine deaminase was added, the β3-agonists inhibited basal and insulin-stimulated glucose transport. This inhibitory effect, also seen with noradrenaline, was blocked by propranolol or bupranolol. The addition of 0.1 mM Na orthovanadate in the presence of 1 mM tyramine induced a stimulation of glucose transport. This stimulation was comparable to that obtained by the combination of 0.1 mM vanadate with 1 mM H_2O_2.

The synergism between tyramine and vanadate was blocked by the MAO inhibitors pargyline and phenelzine, demonstrating that H_2O_2 generation and the subsequent formation of peroxovanadate was involved in this stimulation of hexose transport.

In vivo treatment of Wistar rats with CL 316943 (1 mg/kg/d) for 7 days promoted 30% fat depletion and β-AR desensitization in white adipose tissues, but also increased basal and insulin-stimulated glucose transport. The total amount of the insulin-sensitive glucose transporter GLUT4 in subcutaneous fat depots was increased. One-week treatment with tyramine plus vanadate (3.0 and 0.3 mg/kg/d) slightly promoted fat deposition in epididymal adipose tissue but did not change the insulin sensitivity of glucose transport. The acute synergistic effect of tyramine plus vanadate was unmodified.

Three weeks treatment with phenelzine (4mg/kg/d) reduced weight gain in insulin-resistant obese Zucker rats, although insulin-dependent activation of glucose transport was unchanged. Activation of hexose transport by tyramine plus vanadate was lower after phenelzine. Thus, prolonged blockade of MAO selectively impairs the tyramine-dependent activation of glucose transport.

Taken together, these data show that β3-agonists act acutely on white adipocytes as anti-insulin agents, but improve glucose transport capacity when administered chronically. In contrast, monoamines like tyramine, in combination with vanadate, acutely mimic insulin's effect on glucose metabolism via an MAO-dependent pathway. This action needs to be demonstrated *in vivo* and constitutes a new potential approach for oral diabetes therapy.

Carpene, C., *et al* (1993). *Biochem. J. 296*, 99-105.

Carpene, C., *et al (1995). J. Pharmacol.* Exp.Ther. 272, 681-688.

188P IMAGING GLUT4 TRAFFICKING IN SINGLE LIVING CELLS USING GREEN FLUORESCENT PROTEIN

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Insulin increases the rate of glucose transport into cells by promoting the translocation of a specific isoform of glucose transporter, GLUT4, from an intracellular location to the plasma membrane. Analysis of the translocation process has traditionally required cell disruption and various forms of immunostaining protocols or subcellular fractionation, procedures which provide only a static picture of GLUT4 localisation.

To circumvent this problem, we have developed an assay system in which the dynamics of GLUT4 trafficking can be followed in individual living cells. We fused the *Aequoria victoria* green fluorescent protein (GFP) to the N-terminus (GFP.GLUT4 chimera) or C-terminus (GLUT4.GFP) of GLUT4. These chimeras were expressed in CHO cells by microinjection.

Fluorescence microscopy demonstrated that both chimeras were expressed in a perinuclear compartment, and also throughout the cytosol in a punctate vesicular distribution. In the absence of insulin, both chimeras were excluded from the plasma membrane. Incubation with insulin promoted a pronounced net translocation of both of the chimeras to the plasma membrane.

The process was completely reversible for the GLUT4.GFP chimera: upon removal of insulin, this chimera was re-internalised into a vesicular compartment, and returned to the plasma membrane during a subsequent re-challenge with insulin.

In contrast, GFP.GLUT4 became trapped at the cell surface and did not appear to undergo any significant re-internalisation when insulin was removed. This strongly suggests the presence of an internalisation motif within the N-terminal cytoplasmic head of GLUT4. Time-lapse fluorescence microscopy also revealed the enrichment of GFP-GLUT4 in membrane ruffles; however, the significance of this observation is not clear.

The GFP-GLUT4 chimera was also expressed in a perinuclear location and in vesicles spread throughout the cytosol of 3T3 L1 adipocytes, as well as immediately beneath the plasma membrane ready for rapid fusion with the membrane in response to insulin. Imaging live cells incubated with Texas Red-transferrin suggested the exclusion of GFP-GLUT4 from the general recycling vesicle pool.

Time lapse confocal microscopy of 3T3 L1 adipocytes suggested that the vesicles were tightly tethered to an intracellular structure in the basal state. The first site of insulin action must, therefore, be to release of these vesicles from the tether allowing them to translocate to the plasma membrane. Indeed, insulin caused a substantial translocation to the plasma membrane: an effect which could be mimicked by over-expression of a constitutively active protein kinase B (gag-PKB).

In conclusion, this novel approach allows us to visualise, for the first time, GLUT4 movements in living cells. We have used this technique to investigate the mechanism by which insulin promotes GLUT4 translocation to the cell surface.

189P RATIONAL DESIGN OF BUTABINDIDE, THE FIRST INHIBITOR OF THE CHOLECYSTOKININ (CCK-8) INACTIVATING PEPTIDASE

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Exogenous cholecystokinin octapeptide (CCK-8) decreases food intake and causes satiety in animals and humans. However, no CCK agonist with acceptable bioavailability has yet been developed despite the interest in such an agent as a potential pro-satiating drug.

Our approach to this problem has been to design selective peptidase inhibitors to protect CCK-8, the endogenous neuropeptide, against inactivation and thereby to amplify the biological responses it triggers (Rose *et al.*, 1996).

A CCK-inactivating peptidase was purified and characterised as a serine-peptidase which cleaves CCK-8 Æ CCK-5 Æ Gly-Trp-Met. It was subsequently identified as an isoform of tripeptidyl peptidase II which is a subtilisin-like peptidase of previously unknown function.

In seeking an inhibitor of this peptidase, the enzymatic binding subsites were first characterized using a series of di- and tri-peptides from which the tripeptide Ala-Ala-Ala-OH emerged as a lead (Ki = 14,000 nM). Systematic variation of this simplified it to a dipeptide amide Nle-Nle-NHMe (Ki = 9,000 nM). This was subsequently optimised to give Abu-ProNHBu (UCL 1371, Ki = 80 nM). Finally, fusion of a benzene ring to give an indoline afforded the peptoid, butabindide (UCL 1397).

Butabindide is a potent and specific inhibitor (Ks = 7 nM) and was shown to protect endogenous CCK from inactivation. It is active *in vivo*, reducing food intake in starved mice (10 mg/kg iv). These pro-satiating effects were demonstrated to be mediated by the CCKA receptor.

Rose C, Vargas F, Facchinetti P *et al.*, *Nature*, 1996, 380, 403-409.

190P INSULIN SENSITIZERS FOR THE TREATMENT OF NON-INSULIN-DEPENDENT DIABETES

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Non-insulin dependent diabetes mellitus (NIDDM) is characterized by two interacting metabolic defects. These are: impaired insulin secretion from pancreatic β-cells, and insulin resistance in key target tissues - the liver, skeletal muscle and adipose tissue. Insulin resistance is exacerbated by obesity: more than 80% of NIDDMs are significantly overweight.

The prospect that novel antihyperglycaemic therapies, which sensitize target tissues to endogenous insulin, can prevent or delay the progression of diabetes-related complications may be realized with the advent of the thiazolidinedione class of drug. Of these, BRL 49653 is the most potent and selective insulin sensitiser in clinical development.

BRL 49653 improves glycaemic control in a number of nutritional and genetic rodent models of insulin resistance at doses of 0.1-1.0 mg/kg. Hyperinsulinaemia and hyper-triglyceridaemia are also reduced. Studies with the Zucker ratty *fa/fa* rat to determine the impact of long term BRL 49653 treatment of diabetic complications show that it prevents the development of hypertension and almost completely prevents proteinuria over a seven month period.

BRL 49653, by enhancing insulin action, may also have β-cell protective properties. Pancreatic islet insulin and insulin mRNA content of genetically diabetic *db/db* mice is increased, and BRL 49653 affords protection against the long-term deleterious morphological changes that occur in islets of these mice.

A molecular target for insulin sensitisers that may mediate some, if not all, of the antihyperglycaemic actions of BRL 49653 has been identified. This is PPARγ (peroxisomal proliferator activated receptor), a member of the nuclear receptor superfamily. Functional and radioligand binding studies using recombinant PPAR isoforms show that BRL 49653 has high selectivity for PPARγ compared to PPARα and PPARβ. Structural activity relationships show that affinity of insulin sensitisers for PPARγ is highly correlated with antihyperglycaemic activity *in vivo*.

191P THE TREATMENT OF DIABETES WITH GLUCAGON-LIKE PEPTIDE (GLP1)

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Glucagon-like peptide-1 (GLP-1) is a 30-amino acid peptide hormone encoded by the glucagon gene, arising by differential processing of the glucagon precursor in the L-cells Of the intestinal mucosa. It is secreted in response to the ingestion of food and is the most potent insulinotropic hormone known. It inhibits glucagon secretion and thus inhibits hepatic glucose production and lowers blood glucose, but this effect is self-limiting because the effects of GLP-1 on insulin and glucagon secretion are glucose dependent. The hormone, therefore, cannot cause hypoglycemia.

It is now established that GLP-1 acts as an incretin, one of the two insulinotropic hormones from the gut that augment nutrient-induced insulin secretion. Knock-out of the GLP-1 receptor is associated with severely impaired glucose tolerance. The hormone also functions in the "ileal brake" mechanism: that is, the endocrine inhibition of upper gastro-intestinal secretion and motility elicited by the presence of nutrients in the ileum. Its actions here involve centres and possibly receptors in the brain that are associated with inhibition of food intake. GLP-I may, therefore, also regulate appetite.

The actions of the hormone are preserved in patients with non-insulin-dependent diabetes, in whom infusion of the peptide normalises blood glucose, even during prolonged (up to 7 days) infusion.

However, GLP-1 is metabolized rapidly and extensively, partly by the ubiquitous enzyme dipeptidyl peptidase (DPP-IV; half-life 1-1.5 min), and partly by the kidneys (half-life 4-5 min). Following subcutaneous injection its duration of action is therefore short. Current attempts to improve the therapeutic utility of the peptide include: alternative routes of administration (transdermal, transbuccal, nasal, continuous subcutaneous infusion; cell based delivery (implantation of cells engineered to produce GLP-1); development of analogs that resist DPP-IV digestion and/or renal elimination; inhibition of DPP-IV activity; stimulation of the secretion of endogenous GLP-1; and, finally, screening of libraries for orally active GLP-1 receptor agonists with desirable pharmacokinetics.

It seems reasonable to believe that GLP-1 receptor agonism is a realistic and useful new principle in the treatment of diabetes and perhaps also obesity.