Investigations of plasma glucose control in the spontaneously hypertensive rat

Crabbe, D. S. T.

Award date:
1994

Awarding institution:
University of Bath

Investigations of plasma glucose control

in the spontaneously hypertensive rat.

Submitted by

D.S.T. Crabbe

for the degree of PhD of the University of Bath, 1994.

Simon Crabbe

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

Acknowledgements.

In the past three years I have become indebted to many people who have all helped to get my thesis to this stage.

My three supervisors, Dr. Peter Redfern, Dr. Ivor Williams, and Dr. Brian Woodward deserve special thanks for their most helpful contributions throughout the duration of this project.

The support which I have had from Ria Baguste and her technical staff has been both excellent and invaluable. Likewise the animal house staff have aided my cause tremendously, in particular the ever helpful Ewan Basterfield and Lesley Moore.

Many thanks are due, also, to the Science and Engineering Research Council for sponsoring me for the last three years.

The project has also been greatly aided by the generous gifts of captopril and epicaptopril, lisinopril, and enalaprilat from the Squibb Institute for Medical Research, Zeneca Pharmaceuticals, and Merck-Sharpe and Dohm respectively.

Finally, I would like to thank Zeneca Pharmaceuticals, and especially Jackie Wilbraham, for performing the insulin radioimmunoassay on my behalf.

Abstract.

In recent years the idea that resistance to some or all of the normal biological actions of insulin may be one of the root causes of several conditions, among them essential hypertension, has come to the fore. Moreover, although most drugs currently used in the treatment of essential hypertension do not appear to address this problem, one group, the angiotensin converting enzyme (ACE) inhibitors, does seem so to do.

The aim of this project was to investigate a small animal model of insulin resistance (based on an intravenous glucose tolerance test) and to use this model to test the assertion that ACE inhibitors are indeed able to modify insulin sensitivity. This was followed up with studies *in vitro* (measuring glucose uptake by the rat hemidiaphragm) to complement the *in vivo* results. A study of a possible link between plasma glucose and K^+ concentrations was also undertaken *in vivo*.

When compared with normotensive rats, spontaneously hypertensive rats (SHRs) showed little evidence of being insulin resistant *in vivo*. Only captopril, of the ACE inhibitors tested, had any effect on glucose tolerance, worsening it significantly. Epicaptopril and losartan also worsened glucose tolerance, but L-cysteine did not.

The hemidiaphragms of SHRs did not show any signs of insulin resistance *in vitro*, but captopril, at one of the concentrations tested, significantly reduced glucose uptake, mimicking its *in vivo* effect.

It was concluded, therefore, that glucose intolerance is not a property of skeletal muscle itself in the SHR, and that the whole animal is not insulin resistant. Captopril worsens glucose tolerance in the SHR, possibly by inhibiting the formation of angiotensin II by a specific ACE. It was concluded from the study of plasma glucose and K^+ concentrations that although the link between the two was weak, it

was, nevertheless, present. The results also indicated that there was a constitutively higher activation of Na^+/K^+ ATPase in the SHR than in Wistar rats.

Contents.

Introduction.

Hypertension.

Blood pressure, in the normal course of events, increases with age. In a healthy individual this increase represents no threat, even in old age, when systolic blood pressure may be twice what it was at birth. The control of blood pressure is finely regulated by a complex interplay of systems so that from minute to minute, or year to year, this most important of homeostatic mechanisms ensures a good blood supply to all parts of the body.

The blood pressure of an organism is dependent on two factors - cardiac output (CO) and total peripheral resistance (TPR), which are commonly linked in the formula

$$BP = CO \times TPR.$$

In healthy individuals an increase in CO would result in a reflex reduction of TPR in order to maintain a steady blood pressure, and vice versa. In the short term this is carried out mainly by neuronal mechanisms. Humoral regulation of blood pressure is generally concerned with the longer term and more gradual control of homeostasis.

The most important mechanism responsible for the maintenance of blood pressure over short time periods (a few seconds) is the baroreceptor reflex. The baroreceptors are located around the aortic arch and carotid sinuses and are essentially stretch receptors which increase their rate of firing with increases in blood pressure. Activation of the baroreceptors results in a centrally co-ordinated alteration of the balance of cardiovascular sympathetic and parasympathetic activity in favour of the parasympathetic. The major consequence of this is the reduction of sympathetic-driven vasoconstriction, and secondarily a fall in CO, caused by both a reduction of sympathetic activity and stimulation of the vagal input to the heart. The baroreceptor

reflex works to a "set point" system, altering its activity to correspond with the size of departure from that set point (Levy, 1990).

Similarly placed around the aortic arch and carotid sinuses are the chemoreceptors. These are stimulated by a reduction in blood oxygen content and pH, and an increase in blood CO_2 content. The main effect of their stimulation is the activation of a reflex which increases pulmonary ventilation, but a secondary effect is to induce bradycardia and peripheral vasoconstriction (Levy, 1990).

While most humoral mechanisms work in the longer term, some do operate over short time scales. The prime examples of these are the endothelium-derived mediators nitric oxide (Palmer *et al.*, 1987), the prostaglandins (Smith, 1986), and the endothelins (Änggård *et al.*, 1990). While these do affect blood pressure, it seems more likely, given their ubiquitous presence and short half-lives, that they act more as local regulators of blood flow than systemic regulators of pressure.

Although neural mechanisms do not play a major role in the longer term control of blood pressure, they are important in several ways. For example, sympathetic stimulation is one of the stimuli responsible for the release of renin from the juxtaglomerular apparatus in the kidney (Opie, 1992), and chronic stress is known to lead to elevated catecholamine levels in the blood and can be a factor in the development of hypertension (Herd, 1984).

It is the humoral systems of the body which are responsible for the general maintenance of vascular tone, on top of which the short term systems exert a fine tuning influence. One of the most important of these humoral mechanisms is the renin-angiotensin-aldosterone system, which will be covered in more depth later on. This system, through its action on the kidney, helps regulate the body's electrolyte content (Opie, 1992), and through the production of angiotensin II both in the plasma and the

vessel walls (for which there is now good evidence (Dzau, 1993)) maintains vascular tone (Lee *et al.*, 1992). Moreover, production of angiotensin II in the brain stimulates the drinking response in experimental animals. Several other compounds also act on the kidney. Those which stimulate Na^+ or water loss include atrial natriuretic peptide which is released from the right atrium in response to an increase in atrial filling, and inhibits water and Na^+ resorption in the nephron as well as inhibiting renin release and antagonising angiotensin II- and noradrenaline-induced vasoconstriction. This latter group also includes the kinins since a fully competent kinin system has been observed in the kidney which has both a natriuretic and diuretic action (Oparil and Katholi, 1990).

The control of blood pressure is therefore dependent upon the successful integration of short and long term mechanisms to control CO, TPR, and blood volume.

In many people, however, the control of blood pressure is less exact than in subjects which one might consider "normal". Some individuals' blood pressures are too low, which has its own complications, but in the vast majority of people with poor control, approximately 30% of the population (Ceriello *et al.*, 1993), blood pressure tends to creep up too quickly, rather than fail to rise at all. A patient is usually said to have hypertension if he or she has a diastolic blood pressure of greater than 95mmHg, although this figure is variable since blood pressure increases with age anyway. Thus a diastolic pressure of 100mmHg is considered much more serious in a person who is twenty years old than in one who is eighty.

Hypertension brings with it some well known risks, besides the detrimental effects of high blood pressure (such as glomerulonephropathy) *per se*. These include an increased risk of suffering a stroke, myocardial infarction, heart failure, other

thromboembolic episodes, blindness due to damage to the retina, and atherosclerosis. The result is that cardiovascular disease of one form or another is responsible for the greatest single number of deaths each year in Britain.

The causes of essential hypertension (as opposed to "secondary hypertension" which describes those forms of the condition for which a definite cause can be identified) are undoubtedly complex, and many different hypotheses have been put forward to explain the fundamental dysfunctions of the condition. These have variously suggested the involvement of the peripheral and central nervous systems, growth factors, electrolyte imbalance, genetic factors, environmental causes, and hormonal influences. It seems certain that some, or possibly all, of these theories are involved in the loss of good blood pressure regulation. This thesis will concentrate on some aspects of the roles of hormones and electrolytes in the aetiology of essential hypertension and generally will not be concerned with high blood pressure of known causes.

Much recent speculation has revolved around the possibility that one of the major problems in essential hypertension may be a resistance to the normal ability of insulin to reduce the concentration of glucose in the plasma (Reaven, 1988). Certainly, abnormally high concentrations of insulin have been observed in the plasma of essential hypertensives (Ferrannini *et al.*, 1987), some of the consequences of which might be to increase blood pressure. The mechanisms by which this could occur are presented in a later part of this introduction, but first the mode of action of insulin under normal conditions will be discussed.

Insulin - mode of action.

Insulin was discovered in 1922 by Banting, Best, Macleod and Collip in Toronto. In 1955 it was the first protein to be sequenced completely. These two breakthroughs gained their originators two Nobel prizes, but, in spite of the wealth of information which has now been accumulated, surprisingly little is actually known about how insulin works.

To the endocrinologist the classic function of insulin is as the primary mediator of glucose disposal in the body, and, in concert with glucagon, it is indispensably involved in the regulation of blood glucose levels. This, however, is not all. Insulin also stimulates amino acid and ion uptake, serine and tyrosine phosphorylation of a multitude of cellular proteins, the suppression of lipolysis, the inhibition of intracellular free fatty acid oxidation, the stimulation, or suppression, of transcription of certain genes, and the promotion of cell growth and expression of oncogenes (for review see Seino *et al.*, 1990). So important is it, that virtually all cells in the mammalian body can respond to the presence of insulin (Kahn and White, 1988).

Many substances alter the release of insulin from the β cells of the islets of Langerhans in the pancreas. The most important of these, and the only one able to stimulate insulin release on its own, is glucose (Espinal, 1989). All other compounds which modulate insulin secretion require the simultaneous presence of glucose. Indeed the prevailing concentration of plasma glucose is important in determining the stimulation of insulin secretion by non-glucose secretagogues. Increases in plasma glucose concentration result in a greater insulin response to, for example, arginine, a phenomenon known as "glucose potentiation" (Pfeifer and Broadstone, 1991). The non-glucose compounds which modulate insulin release include amino acids, fatty acids, sympathetic stimulation through β adrenoceptors, cholinergic stimulation, and

several hormones (secretin, gastrin, gastric inhibitory polypeptide, and cholecystokinin) (Pfeifer and Broadstone, 1991). Other factors reduce glucose-stimulated insulin secretion, in particular the other islet hormones glucagon and somatostatin, but also serotonin, and noradrenaline working through α adrenoceptors on the β cells (Espinal, 1989).

When insulin stimulates glucose uptake, by far the largest proportion of the glucose is taken up into the skeletal muscle mass. In man, this proportion has been estimated to be approximately 85% of total insulin-stimulated glucose metabolism (De Fronzo *et al.*, 1981), another 8% being removed by the splanchnic region (Ferrannini *et al.*, 1987). Skeletal muscle is also by far the most insulin-sensitive tissue in rats (James *et al.*, 1985), accounting for more than 90% of insulin-stimulated glucose disposal (Kraegen *et al.*, 1985). The other insulin-sensitive tissues in this species include adipose tissue, heart, and lung. The liver, brain, and other organs (e.g. kidney, spleen, pancreas) are not sensitive to the ability of insulin to promote glucose uptake, and together with adipose tissue, the heart, and the lung account for less than 10% of the glucose uptake of an intravenous load (Kraegen *et al.*, 1985).

Circulating insulin mediates its effects through the insulin receptor - the product of a gene found near the end of the short arm of chromosome 19. The 22 exon gene codes for an α-β subunit dimer, two of which are linked by a disulphide bridge between the two α subunits to make the active receptor (figure I 1). Exon 11 may be omitted during the splicing process which produces the translatable messenger RNA (mRNA), thus producing an α subunit which is smaller by 12 amino acids than the unspliced alternative. The receptor with the larger α subunit is known as the type B receptor, and that with the smaller one is type A (Yarden and Ullrich, 1988). The 3brain and spleen express type B subunit almost exclusively, whereas the other tissues of the body express both (Seino *et al.*, 1990).

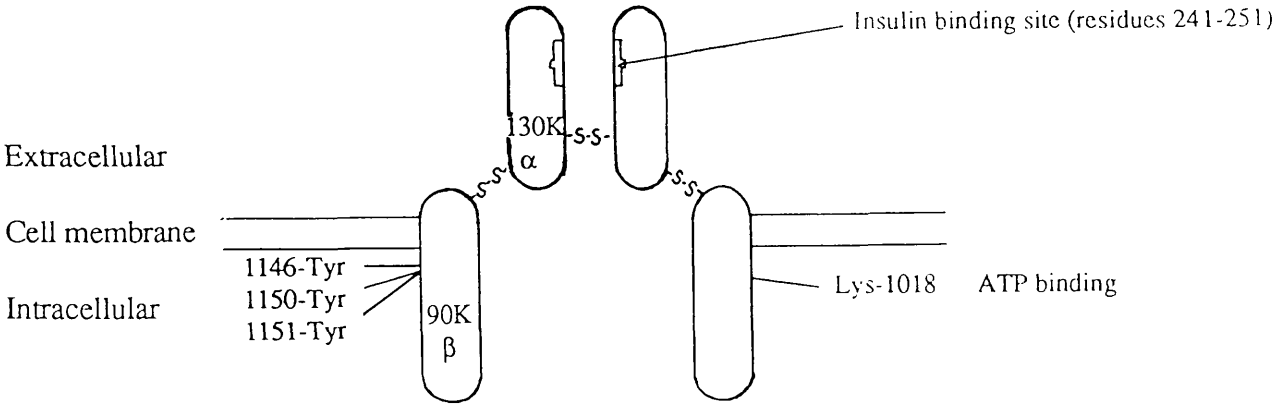

Figure I 1. Diagram to show the subunit structure of the insulin receptor and some important points of the primary amino acid sequence.

Key.
K (=KDa) - molecular mass of the subunit; Tyr-11xx - position in the primary amino acid sequence of a tyrosine residue which is phosphorylated on stimulation of the receptor by insulin; -S-S- disulphide bridge linking two subunits; Lys-1018 - lysine residue in position 1018 in the primary amino acid sequence, thought to be important for ATP binding.

References.
Insulin binding site: Carpentier, 1989; Yip *et al.*, 1990.
Molecular mass, disulphide bridges: Carpentier, 1989.
ATP binding site: Kahn and White, 1988.
Tyrosine autophosphorylation sites: Zhang *et al.*, 1991; Sung, 1992.

Stimulation of the receptor by insulin activates the β subunit which is able to autophosphorylate, and phosphorylate other proteins, on tyrosine residues (Zhang *et al.*, 1991). There is, in addition, good evidence that, through the insulin receptor, insulin is able to activate at least one (Eckel *et al.*, 1990; Kellerer *et al.*, 1991; Mortensen *et al.*, 1992) and possibly two (Kahn and White, 1988) G-proteins, and that a protein known as "insulin receptor substrate 1" (IRS-1) interacts with the insulin bound receptor to cause several intracellular changes (Sun *et al.*, 1991; Shoelson *et al.*, 1992). Through the activation of the oncogene product $p21^{ras}$ insulin also appears to be able to stimulate gene expression (Burgering *et al.*, 1991). This is by no means an exhaustive list of the first steps of insulin signalling. Figure I 2 gives an idea of the complexity of insulin's intracellular influences.

The ultimate consequences of insulin stimulation of a cell must be equally convoluted. At the plasma membrane the classic cellular response is the translocation of glucose transporters from intracellular stores to the cell membrane (Bornemann *et al.*, 1992; Pessin and Bell, 1992). Na^+/H^+ antiport activity is also stimulated by insulin (Vara and Rozengurt, 1985) as is the translocation to the plasma membrane of an α and β subunit pair of the Na^+/K^+ ATPase in both the frog (Omatsu-Kanbe and Kitasato, 1990) and the rat (Hundal *et al.*, 1992). Intracellularly, this almost omnipotent hormone sets off a cascade of phosphorylation and dephosphorylation reactions, the consequences of which include stimulation of glycogen synthase and pyruvate dehydrogenase (Roach, 1990; Lawrence, 1992). It is due to these highly complex interactions that the pathways through which insulin has effects such as the stimulation of glucose and amino acid uptake still remain uncertain.

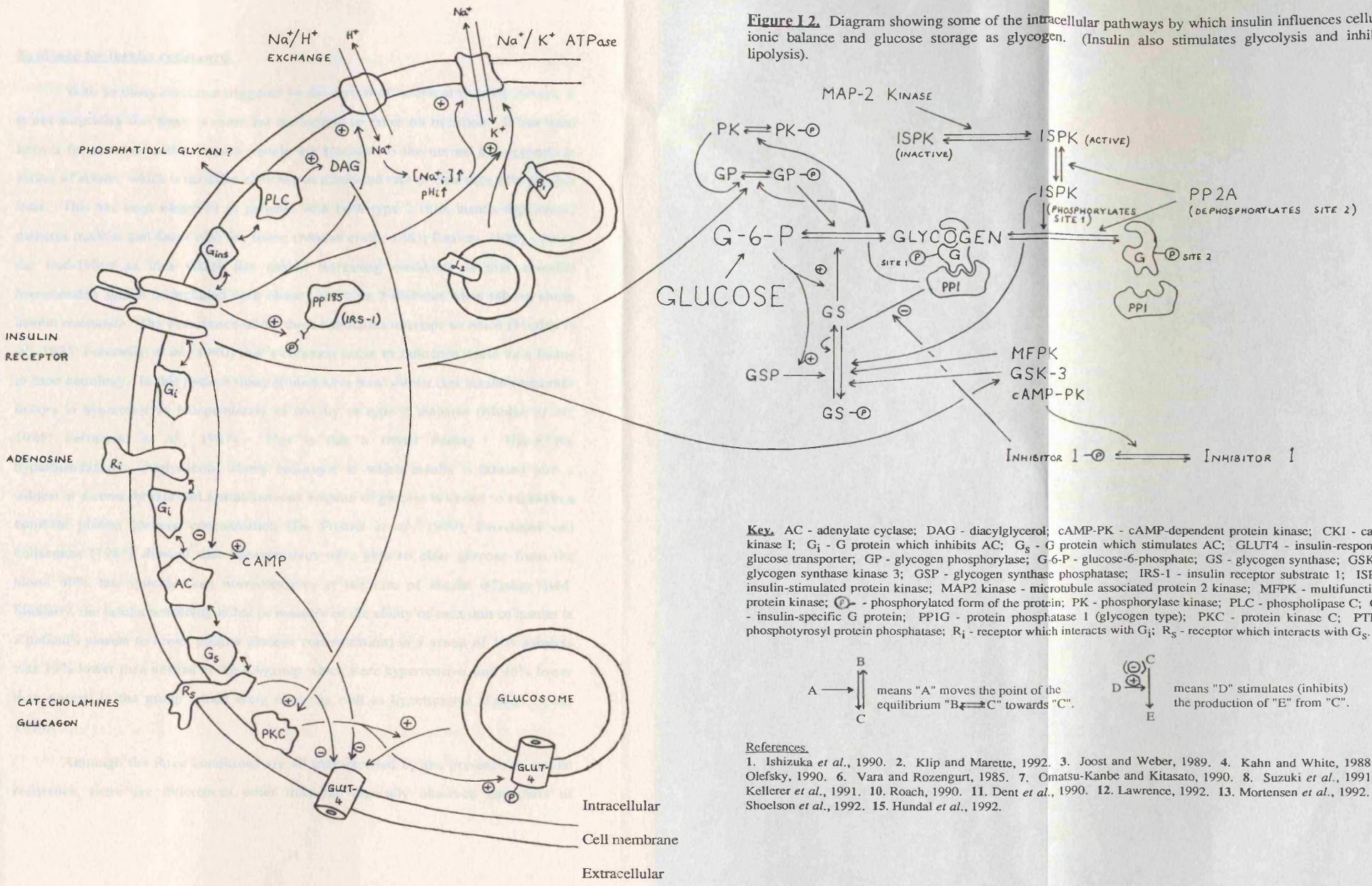

Figure I 2. Diagram showing some of the intracellular pathways by which insulin influences cellular ionic balance and glucose storage as glycogen. (Insulin also stimulates glycolysis and inhibits lipolysis).

Key. AC - adenylate cyclase; DAG - diacylglycerol; cAMP-PK - cAMP-dependent protein kinase; CKI - casein kinase I; G_i - G protein which inhibits AC; G_s - G protein which stimulates AC; GLUT4 - insulin-responsive glucose transporter; GP - glycogen phosphorylase; G-6-P - glucose-6-phosphate; GS - glycogen synthase; GSK-3 - glycogen synthase kinase 3; GSP - glycogen synthase phosphatase; IRS-1 - insulin receptor substrate 1; ISPK - insulin-stimulated protein kinase; MAP2 kinase - microtubule associated protein 2 kinase; MFPK - multifunctional protein kinase; Ⓟ - phosphorylated form of the protein; PK - phosphorylase kinase; PLC - phospholipase C; G_{ins} - insulin-specific G protein; PP1G - protein phosphatase 1 (glycogen type); PKC - protein kinase C; PTPP - phosphotyrosyl protein phosphatase; R_i - receptor which interacts with G_i; R_s - receptor which interacts with G_s.

References.

1. Ishizuka *et al.*, 1990. 2. Klip and Marette, 1992. 3. Joost and Weber, 1989. 4. Kahn and White, 1988. 5. Olefsky, 1990. 6. Vara and Rozengurt, 1985. 7. Omatsu-Kanbe and Kitasato, 1990. 8. Suzuki *et al.*, 1991. 9. Kellerer *et al.*, 1991. 10. Roach, 1990. 11. Dent *et al.*, 1990. 12. Lawrence, 1992. 13. Mortensen *et al.*, 1992. 14. Shoelson *et al.*, 1992. 15. Hundal *et al.*, 1992.

Evidence for insulin resistance.

With so many reactions triggered by the arrival of insulin at the cell surface, it is not surprising that there is room for the system to falter on occasion. It has been known for some time that certain people are resistant to the normal hypoglycaemic action of insulin, which is manifest clinically as a reduced rate of clearance of a glucose load. This has been observed in patients with both type 2 (non insulin-dependent) diabetes mellitus and those who are obese (Modan *et al.*, 1985; Reaven, 1988). Since the mid-1980s an idea which has gained increasing credibility is that essential hypertension should be included with obesity and type 2 diabetes when talking about insulin resistance. The occurrence of the three conditions overlaps so much (Modan *et al.*, 1985; Ferrannini *et al.*, 1990) that a common cause or influence could be a factor in their aetiology. In this respect many studies have now shown that insulin resistance occurs in hypertensives independently of obesity or type 2 diabetes (Modan *et al.*, 1985; Ferrannini *et al.*, 1987). Nor is this a trivial finding. Using the hyperinsulinaemic, euglycaemic clamp technique in which insulin is infused into a subject at a constant rate and a simultaneous infusion of glucose is varied to maintain a constant plasma glucose concentration (De Fronzo *et al.*, 1979), Ferrannini and colleagues (1987) showed that hypertensives were able to clear glucose from the blood 40% less quickly than normotensives at the rate of insulin infusion used. Similarly, the insulin sensitivity index (a measure of the ability of each unit of insulin in a patient's plasma to lower plasma glucose concentration) in a group of 194 subjects was 19% lower than normal in the subgroup which were hypertensive, and 46% lower than normal in the group which were obese as well as hypertensive (Lithell *et al.*, 1990).

Although the three conditions are all characterised by the presence of insulin resistance, there are differences other than the clinically observed endpoints of

hypertension, obesity or type 2 diabetes (Ferrannini *et al.*, 1987). A summary by these authors of metabolic changes in the three conditions showed interesting "variations on a theme". All three diseases incorporate a reduced whole body glucose uptake rate and decreased non oxidative glucose disposal. Hypertension showed no further complications, unlike type 2 diabetes where glucose oxidation and suppression of lipolysis were both compromised, and obesity which showed reduced suppression of glucose output and less effective promotion of K^+ uptake while lipid oxidation was increased (suppression of lipolysis was normal). On the one hand this may suggest that insulin resistance is a root cause of all three conditions, and the clinical outcome depends on the presence or absence of other genetic or environmental factors. Conversely, however, the different metabolic disorders may be different routes to the same end - insulin resistance - which has resulted in an incorrect association of the three conditions (Ferrari and Weidmann, 1990). Care must therefore be taken when extrapolating results from studies of one disease to considerations of another. Unfortunately the hand of the investigator of this aspect of essential hypertension is somewhat forced since most of the data on insulin resistance collected so far refer to type 2 diabetes and obesity. It is necessary, therefore, to take a calculated risk based upon the evidence of similarity, and make such extrapolations. The most widely accepted case is that the three diseases have a common cause. One can see, therefore, that essential hypertension appears to represent the simplest case of insulin resistance, and as such could offer a unique opportunity to study the root cause of the disorder without too many complicating factors.

As a result of the conflicting data produced by experiments with humans, other researchers have turned to animal models of essential hypertension to address the problem. An early study by Yamori and co-workers (1978) showed the stroke prone spontaneously hypertensive rats (SHRs) exhibited a raised basal plasma glucose

concentration, and a greater increase in plasma glucose concentration during an intravenous glucose tolerance test compared with Wistar Kyoto (WKY) controls, while insulin concentrations were no different. In contrast, in another study glucose concentrations during an oral glucose tolerance test were similar in SHRs and WKY rats, but insulin concentrations were higher in the hypertensive strain (Mondon and Reaven, 1988). These early findings suggested that SHRs are insulin resistant. However, as the more sophisticated hyperinsulinaemic euglycaemic clamp technique has become more widely used, other studies have failed to show the same trends. Hulman and colleagues (1991) were unable to provide evidence of insulin resistance in SHRs compared with WKY rats, and both Gaboury and associates (1991) and Frontoni and co-workers (1992) found evidence that the WKY rat is less sensitive to insulin than the SHR. In particular, during an intravenous glucose tolerance test the area under the glucose extinction curve was greater in WKY rats than in SHRs, and during an oral glucose tolerance test the same was true of the insulin response curve (Gaboury *et al.*, 1991). Moreover, at submaximal plasma insulin concentrations during a clamp experiment, the rates of glucose uptake and glycogen synthesis were 1.5 and 1.8 times greater in SHRs than WKY rats (Frontoni *et al.*, 1992). The most recent results, however, do appear to show insulin resistance using the insulin clamp technique (Rao, 1993). In this investigation, the relationship of plasma insulin concentration to glucose disposal rate showed very significant insulin resistance in SHRs. In the SHR, therefore, it is not yet firmly established that insulin resistance exists, and more work needs to be done to find out whether the SHR represents a good model of human insulin resistance.

It has become one of the established tenets of this area of research that the resistance is almost entirely due to a reduction of skeletal muscle glucose uptake, in itself a result of a lower flux through the glycogen synthesising system (Cusin *et al.*,

1990; Ferrannini *et al.*, 1987; Häring and Mehnert, 1993). Other tissues may also contribute to insulin resistance, in particular white adipose tissue (WAT), but estimates of the importance of WAT vary widely. Adipocytes from SHRs have been shown to be insulin resistant *in vitro* (Reaven *et al.*, 1989) as have those of patients with type 2 diabetes (Kashiwagi *et al.*, 1983) and those from Sprague-Dawley rats fed on a high fat diet, another recognised route to whole body insulin resistance (Pedersen *et al.*, 1991). In contrast, Cusin and co-workers (1990) caused the opposite effect in rats which were infused with insulin for four days to induce hyperinsulinaemia, as did Takao and colleagues (1990) when they induced hyperglycaemia in rats for four days. Current research therefore tends to concentrate on skeletal muscle as the likely site of insulin resistance. This is because it is by far the largest glucose sink, and because it is the largest of the three main insulin-responsive tissues and as such has the greatest potential for causing the substantial decreases in insulin-stimulated glucose uptake which have been observed.

The case for the existence of insulin resistance in essential hypertension is not absolute, though. One study, especially, was unable to find insulin resistance in essential hypertensives for which obesity or type 2 diabetes did not provide an adequate explanation (Bonora *et al.*, 1993). In this study five groups of subjects were used: lean, non-hypertensive non-diabetic controls, obese subjects, obese subjects with hypertension, type 2 diabetic subjects, and type 2 diabetic subjects with hypertension. Compared with the controls, the obese and diabetic groups were insulin resistant. However, in neither disease did simultaneous hypertension appear to cause a further worsening of insulin resistance. As well as implying that insulin resistance and essential hypertension are not causally linked, this study also illustrates the great importance of controlling experiments for the confounding factors of type 2 diabetes and obesity, which can cause up to a 50% reduction in insulin-stimulated glucose

uptake in their own right (Bonora *et al.*, 1993).

Causes of insulin resistance.

The evidence in favour of a role for insulin resistance in the aetiology of essential hypertension, as well as type 2 diabetes and obesity, seems convincing, and certainly worth further investigation. The cause of insulin resistance itself, however, is still more or less a complete mystery. This is perhaps not surprising given the number of elements involved in the insulin signalling pathways. The only way to be sure of finding the fundamental fault within the cell is to sequence every protein involved in any way with insulin, and determine which of these has or have an altered sequence or processing. Unfortunately, this is impractical since it would involve an inordinate cost in personnel and resources. The human genome project aims to sequence all 23 chromosomes, but even when this has been done most of the genes so discovered will be of unknown function for a long time afterwards.

There is good evidence that this approach would turn up some valuable answers. This evidence comes from studies of monozygotic twins suffering from type 2 diabetes or from family histories of type 2 diabetics such as that of Cook and associates (1993). These workers concluded that type 2 diabetes is likely to occur in response to the inheritance of several genes, which is a similar finding to that of the many investigations of genetic causes of essential hypertension. With direct reference to essential hypertension in humans, Lever and Harrapp (1992) have suggested that the influence of genetic make-up on blood pressure is approximately twice as strong as environmental influence.

Investigations of the actual genes which co-segregate with essential hypertension have been carried out in animals. The number of genes involved in

essential hypertension is thought to be small - two to three major ones plus some less important loci (Lindpainter *et al.*, 1990). Currently about six different genes have been implicated in the hypertension seen in SHRs (Ganten *et al.*, 1991; Jacob *et al.*, 1991; Nara *et al.*, 1991; Nabika *et al.*, 1993; Hilbert *et al.*, 1991). However, since the functions of these genes are unknown (except in the cases of Lindpainter and colleagues (1990) where a mutation of the renin gene of the stroke-prone SHR (SHRSP) was identified, and Nara and co-workers (1991) who demonstrated that angiotensin converting enzyme (ACE) in the SHRSP was different from the WKY rat version), and since the signalling pathways are incompletely understood, an "experimental" (as opposed to "observational") approach to dissecting out the causes of insulin resistance must be adopted.

The most obvious place to start is with the insulin receptor. Although Mosthaf and colleagues (1991) found that the type B (low affinity) receptor mRNA was expressed at levels equivalent to 50-100% of those of the type A receptor mRNA in skeletal muscle from type 2 diabetics compared with tissue from normal subjects, which express only type A receptor mRNA, most reports have found no alteration of receptor affinity or activity. Thus insulin binding was normal in insulin resistant adipocytes from type 2 diabetics (Kashiwagi *et al.*, 1983), and total receptor number, affinity and tyrosine kinase activity (as estimated by the ability of partially purified receptors to phosphorylate poly-(Glu.Tyr)) were similar in SHR and WKY rat adipocytes (Reaven *et al.*, 1989). In addition, the latter study also revealed similar basal and maximal (insulin-stimulated) tyrosine kinase activities with similar sensitivities. Where insulin receptor number has been found to be reduced in several tissues, estimates have suggested that this is not enough to account for the whole of the insulin resistance in the patients in question (Moller and Flier, 1991).

If insulin receptor number and function is unaffected by insulin resistance,

perhaps the same is not the case for the expression of active glucose transporters at the cell surface. In both fat and muscle the insulin-responsive glucose transporter is GLUT4, one of five glucose transporter types so far characterised in man and rat (for review see Pessin and Bell, 1992). Measurements of GLUT4 and GLUT4 mRNA showed that there was no difference which could explain insulin resistance between normals and type 2 diabetics with respect to these parameters (Eriksson *et al.*, 1992a). Indeed, there was an over-expression of GLUT4 mRNA in subjects with diabetes.

Results in experiments where hyperinsulinaemia has been artificially induced are somewhat contradictory. Infusion of $60mg.Kg^{-1}.min^{-1}$ glucose into Sprague-Dawley rats for three days induces a hyperinsulinaemia associated with an increase of GLUT4 mRNA of approximately 200% but no increase in GLUT4 protein (Hager *et al.*, 1991). This mimics the human situation. However, feeding rats with a high fat diet induced hypoinsulinaemia with insulin resistance that stemmed from a reduction in GLUT4 protein in the adipocyte membrane (Pedersen *et al.*, 1991). In another study it was shown that four days of hyperinsulinaemia in lean Zucker rats was able to increase GLUT4 mRNA expression in adipocytes by about fifteen-fold while reducing its expression in diaphragm and tibialis muscle by approximately 60% (Cusin *et al.*, 1990). Clearly the results obtained are highly dependent upon species, tissue, and the method of achieving insulin resistance. The consensus is that in the human a reduction in GLUT4 number or function in the plasma membrane is not a major contributor to insulin resistance (Moller and Flier, 1991). Nor is it necessary that one should be. In a study of the mechanics of glucose transport and metabolism, Furler and colleagues (1991) were able to show that glucose transport into the cell is the rate limiting step of glucose metabolism under normal conditions. When brain and red muscle are under conditions of maximal glucose metabolism, however, the rate limiting step moves towards the phosphorylation of glucose. This illustrates the potential for the slightest

defect in a glucose-processing enzyme to cause insulin resistance by altering the position of the rate limiting step of glucose metabolism. There is, in fact, good evidence that glycogen synthase (GS) is just such an enzyme (Ferrannini *et al.*, 1990). The findings of Bak and colleagues (1992), namely that in type 2 diabetes levels of GS in vastus lateralis muscle were normal but that its activation by insulin was markedly reduced, are complemented by a series of studies carried out by Beck-Nielsen's group. These investigators also showed that type 2 diabetes and obesity separately affect GS activity by reducing its insulin-stimulated activation while not actually having any effect on allosteric activation of the enzyme by glucose-6-phosphate (Damsbo *et al.*, 1991). Very similar results were found in first degree relatives of type 2 diabetics (Vaag *et al.*, 1992a) where the insulin resistance was shown to correlate positively with a defect in GS activation. The same group additionally demonstrated that the hyperglycaemia associated with insulin resistance overcomes the resistance in skeletal muscle by acting in a permissive role to allow greater stimulation of GS by insulin. Thus basal GS flux is similar in normals and diabetics, but the diabetics need the greater stimulus to achieve parity (Vaag *et al.*, 1992b).

It is probable, therefore, that the control of GS and other enzymes is faulty. It seems unlikely that this is caused by an inhibitory factor at the level of the insulin receptor, as Sbraccia and colleagues (1991) found in their study of a particular patient with type 2 diabetes and insulin resistance, since other reports conclude that tyrosine kinase activity (Reaven *et al.*, 1989; Groop *et al.*, 1991; Bak *et al.*, 1992) and insulin binding to its receptor (Kashgiwagi *et al.*, 1983) are normal. A more likely candidate is a protein kinase or phosphatase in the insulin signalling cascade. The activity of protein tyrosine phosphatase (PTPase), which dephosphorylates tyrosine residues, is inadequately suppressed by insulin in skeletal muscle of diabetics (McGuire *et al.*, 1991). This would dampen down the effects of insulin signalling through tyrosine

phosphorylation. Phosphorylase phosphatase is an enzyme which activates GS. Its activity in quadriceps femoris muscle was shown to be reduced by approximately 25% in insulin resistant man (Kida *et al.*, 1992) which could account for the defect in GS activity in the condition. On the other hand, it could equally well be the activation of phosphorylase phosphatase which is abnormal. These examples serve as clues to possible intracellular causes of insulin resistance, but need confirmation by other workers and by other approaches.

Disturbed ionic balance across the plasma membrane is often thought to be a consequence of insulin resistance, but there is evidence that it may be a cause of it. Magnesium (Mg^{++}) has received some attention in this respect, particularly since it is known that type 2 diabetes is associated with Mg^{++} depletion of the plasma and erythrocytes (Paolisso *et al.*, 1990). Several glucose metabolising enzymes require Mg^{++} as a cofactor, and erythrocytes from essential hypertensives have an impaired ability to accumulate Mg^{++} (Paolisso *et al.*, 1990). Especially interesting is the fact that abnormalities of Mg^{++} handling occur very early in the development of diabetes (Paolisso *et al.*, 1990). Calcium (Ca^{++}) has also received some attention. The concentration of Ca^{++} in platelets has been found to correlate negatively with insulin sensitivity and to be an early marker of it in normotensive offspring with a family history of essential hypertension (Ohno *et al.*, 1993). The mechanism by which impaired Ca^{++} homeostasis could cause insulin resistance is unknown, but could conceivably work through activation of enzymes which inhibit the action of insulin.

Another normal physiological response to insulin is an increase in blood flow through skeletal muscle (Gans, 1992). Once again the efficacy of insulin in causing this reaction appears to be reduced in insulin resistance (Laasko *et al.*, 1992). Clearly, if the normal increase in blood flow in response to insulin is lacking in type 2 diabetes and obese subjects, insulin resistance may be due in part to a reduced delivery of

substrate to the glucose transporters.

In theory, then, there are many opportunities for insulin resistance to develop within the cell. A factor under genetic control is not, though, the only way to cause insulin resistance. The over-production of a physiological factor could also reduce the sensitivity of the body to insulin. One such factor is amylin (or islet amyloid polypeptide).

Amyloid deposits are a well known feature of the islets of Langerhans in type 2 diabetic patients. They occur in the β cells of the islets and contain large quantities of the 37 amino acid peptide amylin. Synthetic amylin has been shown to reduce insulin-stimulated glucose uptake and GS activity in rat skeletal muscle (Cooper *et al.*, 1988) and similar results have been obtained with native amylin (Leighton and Cooper, 1988). Frontoni and co-workers (1991) have also shown that GS activity is reduced in rats infused with amylin. In their study this reduction amounted to 77% in soleus muscle. The results have been extended to rat diaphragm, where amylin caused a 60% reduction in insulin-stimulated glucose uptake (Hothersall *et al.*, 1990). A second action of amylin is the ability to stimulate glycogenolysis. In fact it is reported to be more potent and more effective in this respect than glucagon (Wang *et al.*, 1991).

These reports would be convincing were it not for the fact that they were all carried out with very high levels of amylin (10^{-9}M upwards). Physiological levels of the polypeptide *in vivo* are closer to 10^{-14}M (Eriksson *et al.*, 1992b), and concentrations as high as 10^{-10}M have no discernible effect *in vitro* (Zierath *et al.*, 1992). Erikson and colleagues (1992) did, however, make the observation that the release of amylin into the plasma of glucose-intolerant first degree relatives of type 2 diabetics was greater than in normals or in glucose tolerant relatives. Perhaps in the longer term less amylin is required to induce insulin resistance than in the acute

experiments reviewed here.

There are also several other factors which have the potential to reduce insulin sensitivity, of skeletal muscle in particular. Glucocorticoids, for example are constantly present in the blood, and the inhibition of their effects with the glucocorticoid receptor antagonist RU 486 has been shown to increase the sensitivity of tissues to insulin (Leighton *et al.*, 1991). Furthermore, the administration of a glucocorticoid, dexamethasone, to rats is well known to raise blood pressure and induce insulin resistance (Yin *et al.*, 1992). Glucagon, present at raised concentrations in the blood of type 2 diabetics (Lefèbvre, 1991) and released in excess in response to stimulation of α cells with arginine in similar patients (Hamaguchi *et al.*, 1991), provides classical functional antagonism to the action of insulin. Gastric inhibitory polypeptide has also been shown to have the capacity to reduce the insulin sensitivity of muscle in rats (Dupre, 1991) and is found to be increased in obese humans (Dupre, 1991). Finally, adrenaline is also capable of acting similarly to glucagon in that it inhibits skeletal muscle glycogenesis and promotes glycogen breakdown by muscle and liver (Christopher *et al.*, 1992) and is present in increased amounts in the SHR (Frontoni *et al.*, 1992).

The data reviewed here therefore indicate that there are several approaches to investigating the causes of insulin resistance which are under consideration in the scientific community, and that, besides the fact that the cause is probably a modification of insulin signalling at a point beyond the insulin receptor, little progress has been made towards finding out what makes the majority of patients insulin resistant.

Consequences of insulin resistance.

There are two immediately apparent consequences of insulin resistance. These are hyperglycaemia, and, in order to try to compensate for this, over-secretion of insulin by the β cells leading to hyperinsulinaemia. These are the two most frequently measured parameters of insulin resistance.

The increase in plasma glucose may itself be a risk factor which exacerbates insulin resistance. The production of oxygen free radicals through, for example, glycation of proteins could compromise endothelial cell functions such as the production of endothelium derived relaxant factor (Ceriello *et al.*, 1993). Within the cell, at least in rat adipocytes, glucose has been reported to increase insulin binding to its receptor, and the sensitivity of the glucose transport response (Traxinger and Marshall, 1990). In contrast, other experiments, also carried out in rat adipocytes, have led to the opposite conclusion. Incubation of adipocytes with insulin, glucose, or both over 24 hours caused a marked reduction in glucose uptake in response to a dose of insulin. The ED_{50} value of insulin was raised by 183% when cells had been pre-incubated with both insulin and glucose (Lima *et al.*, 1991). Clearly these data conflict with one another and are therefore inconclusive. They do show, though, that glucose can have an effect of its own on its target cells, and is not just a source of energy.

Hyperinsulinaemia is more likely, however, to be the main offender in the scenario, although once again the evidence is not absolute. One can hypothesise many routes by which hyperinsulinaemia might cause hypertension (some of which will be dealt with hereafter) and much of the evidence collected from investigations support elements of these hypotheses. However, it has proven difficult to show that hyperinsulinaemia does cause hypertension. Brands and colleagues (1991) have had some success. They caused hypertension (an increase of 8mmHg) in Sprague-Dawley

rats by infusing insulin for five days at a rate which caused moderate hyperinsulinaemia. These data are contradicted by other findings, though. In a study of patients with confirmed insulinomas, and consequent hyperinsulinaemia, Pontiroli and co-workers (1992) were unable to find any correlation between blood insulin concentration and blood pressure, even though insulin concentration did correlate with insulin resistance. In SHRs, while one report has provided evidence for hyperinsulinaemia (compared with WKY rats) (Mondon and Reaven, 1988), another (Buchanan *et al.*, 1992) has shown hypertension without insulin resistance in the same strain of rat. These latter studies illustrate that though the SHR is probably regarded as the best model of human essential hypertension to date, its similarity to the human condition cannot be taken for granted and it is still being assessed with respect to insulin resistance and the consequences thereof.

One of the mechanisms by which hyperinsulinaemia may cause hypertension is very simple. The various arms of insulin signalling are largely separate (Ferrannini *et al.*, 1988). If it is only glucose metabolism that becomes resistant, the resulting hyperinsulinaemia will over-stimulate other branches of the insulin signalling cascade. For example, one might expect to see over-stimulation of Na^+/K^+ ATPase.

A variety of approaches have provided data which imply an ability of insulin to raise blood pressure including the augmentation of sympathetic nervous system (SNS) activity, the stimulation of vascular smooth muscle cell (VSMC) hypertrophy and hyperplasia, the alteration of blood flow, and the stimulation of certain ionic homeostatic mechanisms.

Both in normal humans (Gans *et al.*, 1991b) and in rat isolated mesenteric beds (Townsend *et al.*, 1992) the pressor activity of exogenous NA was enhanced by physiological levels of insulin (10-100$\mu U.ml^{-1}$), although these same concentrations

were unable to enhance responses to endogenous NA released as a result of periarterial nerve stimulation (Townsend *et al.*, 1992). It seems probable that, if insulin does enhance responses to SNS activity, it is through a post-synaptic effect because the infusion of insulin at physiological levels into healthy subjects was without effect on plasma adrenaline (Adr) and NA concentrations even after 12 hours (Mitrakou *et al.*, 1992). This does not exclude the possibility that hyperactivity of the SNS has a role to play in essential hypertension. Indeed evidence both for this and hyperinnervation of blood vessels (including resistance vessels) in the SHR has been forthcoming (for review see Head, 1989), which implies a positive role for the SNS in the development of hypertension. Any effect which insulin may have on this system, however small, could be important because of the chronic nature of the disease. The same can be said of insulin's other effects. The differences made may be so small in the short term that they are not readily measurable with current techniques.

It is also possible that insulin has central actions. Although it is not known whether the hormone can cross the blood brain barrier, even where the blood brain barrier is less selective around the circumventricular organs, it has been shown to have activity in the brain (Shian and Lin, 1991). These investigators found that injection of insulin into the anterior or lateral hypothalamic regions stimulated a dose dependent increase in glucose and insulin concentrations in the blood. However, the doses given to the rats appear to have been very high - roughly 1000-fold greater than the normal plasma content per millilitre. This may not be important since the injections into the ventromedial hypothalamus did not have the same effect.

It is well known that insulin can stimulate certain ion transporters in the plasma membrane. The two most important of these are the Na^+/K^+ ATPase which is translocated to the plasma membrane (Hundal *et al.*, 1992; Omatsu-Kanbe and Kitasato, 1990) and the Na^+/H^+ antiporter (Vara and Rozengurt, 1985). The

consequences of this action are twofold. Firstly, the cells in which it occurs (the experiments were carried out using rat and frog skeletal muscle for the Na^+/K^+ ATPase) will become hyperpolarised, making them less immediately reactive. Secondly the stimulation of the Na^+/H^+ antiporter will raise intracellular pH, which tends to promote cell division. This is a property which insulin shares with epidermal growth factor (EGF) (Vara and Rozengurt, 1985).

These hypotheses do in fact correspond with what is found experimentally. Three groups have shown that Na^+/K^+ ATPase activity is increased in the SHR and essential hypertensive man compared with their controls. Touyz and associates (1992) showed this difference in human platelets and red blood cells, whereas Syme and colleagues (1990b) and Orlov and co-workers (1992) demonstrated it in SHR skeletal muscle and VSMC respectively. One report of unchanged skeletal muscle Na^+/K^+ ratio in man (Landin *et al.*, 1991) implies that the Na^+/K^+ ATPase is functioning normally in essential hypertension, but such findings are outnumbered by the previously quoted evidence.

Likewise the findings with the Na^+/H^+ antiport system have been revealing. The antiporter generates a greater H^+ extrusion flux in SHR VSMC cultures than in WKY rat VSMC cultures (Davies *et al.*, 1991) and this was associated with an increased V_{max}. These investigators also noted that the basal intracellular pH of SHR VSMC was significantly higher than that of corresponding cells from WKY rats. In contrast, another report also found an increased rate of Na^+/H^+ exchange, but these authors came to the conclusion that this was due to an altered affinity of the carrier for intracellular H^+ ions (Syme *et al.*, 1990a). The promotion of Na^+/H^+ exchange and the observation of raised intracellular pH in VSMC cultures from SHRs highlights another possible role of insulin in causing hypertension, that of stimulation of cell division. This typically manifests itself as an increased medial thickness in the artery

wall.

These are the two major changes, but not the only ones in essential hypertension. Both Ca^{++} ATPase and Mg^{++} ATPase show reduced activity in SHRs (Touyz *et al.*, 1992). The reduction of Ca^{++} ATPase turnover in particular would lead to an increased intracellular free Ca^{++} concentration which would in turn enhance muscular contractility, if the results from platelets can be extrapolated to VSMC.

Folkow proposed in 1958 that a thickening of the media of hypertensive arteries and resistance vessels would decrease the lumen of these vessels due to encroachment of the extra muscle mass. This has been observed by many investigators and leads to the greater passive resistance of SHR vascular beds as compared with those from Wistar rats (Korner *et al.*, 1991). It also accounts for the frequently observed increase in absolute response of blood vessels to pressor substances while sensitivity remains the same (Mulvany, 1991).

There are two possible theories about the relationship between medial thickening and hypertension. The first is simply as an adaptive process to reduce vessel wall stress in response to increased pressure (Gibbons, 1991). The second suggestion is that vascular hypertrophy or hyperplasia is a cause of hypertension. The former suggestion gains support from Bund and his colleagues (1991) who demonstrated that reducing pressure in the femoral vascular bed of the SHR by tying a ligature around the iliac artery was sufficient to prevent the development of medial thickening. Between five and 12 weeks of age the protected arteries in the SHRs were structurally indistinguishable from unprotected arteries in WKY rats. The latter hypothesis gains credence from the finding that media thickness is already increased in young SHR, even before they have raised blood pressure (Mulvany, 1990).

The oncogene product $p21H^{ras}$, one of a family of similar guanine nucleotide

binding proteins with low GTPase activity, has been shown to be linked with many growth promoting systems, for example signal transduction of nerve growth factor and EGF stimuli (Burgering *et al.*, 1991). In the presence of $p21H^{ras}$ insulin is able to stimulate the expression of c-fos, c-jun, and p33 in various transformed cell lines. In fact, as an inducer of c-fos expression and DNA synthesis insulin is as potent as insulin-like growth factor 1 (IGF-1) in 3T3-L1 adipocytes (Weiland *et al.*, 1991). (c-fos and c-jun are proto-oncogene products which are formed rapidly and transiently when, for example, growth hormone stimulates cell surface receptors. They bind to specific DNA sequences to activate gene transcription and cell growth or differentiation.) In addition to hyperinsulinaemia (as with plasma membrane ATPases, growth promoting mechanisms do not appear to be insulin resistant in the same way that glucose uptake is) other driving forces exist to enhance cell proliferation in response to insulin. Not only can high concentrations of insulin stimulate the IGF-1 receptor, but so also can activated insulin receptors (Tartare *et al.*, 1991). Since the insulin receptor is not the cause of insulin resistance, this represents a second route by which insulin can stimulate cell proliferation.

Insulin-stimulated cell proliferation could be exacerbated by other properties of cells from hypertensive rats. Mesenteric artery myocytes from rats will grow in response to EGF provided either foetal calf serum (FCS) or insulin is also present. Myocytes from SHR need less insulin than cells from WKY rats in order to be competent to respond to EGF (Bukoski *et al.*, 1991). The general trend towards more rapid division than normal cells has also been noted in aortic VSMC from SHR (Miller *et al.*, 1990). As the experiments of Miller and colleagues suggest, the over-proliferative response seems to be a generalised feature of VSMC from SHR. Excessive responses have also been seen upon stimulation with angiotensin II (AII) (Miller *et al.*, 1990; Baudouin-Legros *et al.*, 1990), EGF, bFGF and three platelet-

derived growth factor variants (Saltis *et al.*, 1993).

Medial thickening is not due only to muscle cell growth. Another very important factor is the laying down of more extracellular matrix, principally elastin and collagen (Keeley *et al.*, 1991). It is this, rather than the increased VSMC bulk, which reduces vascular compliance and results in high systolic pressures. The half life of these proteins is very long (60-90 days for collagen, and longer than the lifetime of experimental animals for elastin) and this could account for the very lengthy therapy which is required before visible beneficial effects are seen in the structure of resistance vessels in man (Keeley *et al.*, 1991). Although it is not known whether insulin is able to stimulate extracellular matrix deposition, this could be an important factor in the maintenance of the disease.

Insulin is also able to stimulate the production of the pressor substance endothelin 1 (ET-1) by endothelial cells (Oliver *et al.*, 1991). However, thoughts that insulin might increase tubular resorption of sodium in the kidney, thereby inducing plasma volume expansion, appear to have been refuted by the discovery that insulin-stimulated sodium resorption is normal in type 2 diabetic patients (Skott *et al.*, 1991).

A good case for insulin playing a role in the genesis of essential hypertension can therefore be made, but all may not be as seems. In a paper entitled "In defence of insulin - a critique of syndrome X", Jarrett (1992) set out to question many of the foundations upon which the pathological role of insulin resistance is based. Syndrome X was proposed by Reaven in his 1988 Banting lecture. It consists of an association of raised very low density lipoprotein (VLDL), lowered high density lipoprotein (HDL), glucose intolerance, insulin resistance and hypertension. Jarrett points out that a number of studies which found no, or only poor, correlation of indices of insulin resistance with blood pressure have been reported. Obesity, if not properly taken into

account, is likely to be the most significant confounding factor in studies of this relationship, and the point that evidence is difficult to compare due to differing and unproved methodologies is a good one. Nevertheless, at least as much evidence exists to support the claims that Reaven and many others make as can be found to challenge them.

Treatment of hypertension.

Since the 1940s great advances have been made in the drug treatment of hypertension. The battery of chemical weapons now available to the medical profession is both varied and sophisticated. Indeed, there are eight or nine different classes of drug which have been developed (although some are not now used because of their side effects) with several others in the laboratory undergoing evaluation. As a result, the control of blood pressure has, in nearly all cases, become very good, and the number of cardiovascular incidents which are known to stem from high blood pressure, such as strokes, have been reduced in direct proportion with the quality of blood pressure control. However, the improvement in antihypertensive therapy has had little effect on the incidence of heart failure and myocardial infarction (Sever, 1986). Indeed, data from the Australian Therapeutic Trial in Mild Hypertension (1980) suggested that at each level of (average diastolic) blood pressure, the incidence of stroke, myocardial infarction and related conditions was higher in the drug-treated group of patients, implying that the drugs used were actually an added risk factor. The reason for this is not known, but one of the most widely proposed theories is that while reducing the risk factors of high blood pressure they also worsen other risk factors. Chief among these are glucose and lipid metabolism (Shionoiri *et al.*, 1990; Lithell *et al.*, 1990). For example, both metoprolol and atenolol have been reported to

decrease the insulin sensitivity index, by 27% and 23% respectively, in essential hypertensive patients, as well as increasing VLDL cholesterol and triglyceride concentrations, increasing low density lipoprotein (LDL) triglycerides, and reducing HDL cholesterol levels (Lithell *et al.*, 1990). Similarly hydrochlorothiazide reduced the insulin sensitivity index by 16% and raised serum cholesterol and triglyceride levels, especially LDL cholesterol in essential hypertensives (Pollare *et al.*, 1989).

This may not be the case for all antihypertensive drugs. The calcium channel blocker diltiazem was found not to worsen the insulin sensitivity index and had no effect on triglyceride and cholesterol levels (Lithell *et al.*, 1990). In contrast, the angiotensin converting enzyme (ACE) inhibitor captopril was shown to improve the insulin sensitivity index by 18% without affecting lipoprotein concentrations (Pollare *et al.*, 1989). Insulin sensitivity has also been determined by Torlone and co-workers (1991) who showed that both hepatic and extrahepatic insulin sensitivity in type 2 diabetics with hypertension were greater after captopril than placebo. Gans and colleagues (1991a) were likewise able to determine a greater effect of an insulin infusion after treatment of normal volunteers with enalapril than after saline treatment. The results have not been entirely consistent, though. In two studies of the actions of ACE inhibitors on glucose tolerance and insulin sensitivity, Shionoiri and colleagues were unable to demonstrate an action, on these parameters, of captopril (Shionoiri *et al.*, 1987) or lisinopril (Shionoiri *et al.*, 1990) in hypertensive patients. Consequently the literature does not yet direct one to draw any firm conclusions concerning the efficacy of ACE inhibitors in improving insulin sensitivity, but it does seem that β blockers and diuretics may have adverse effects upon insulin sensitivity. In this respect both ACE inhibitors and Ca^{++} channel blockers may have an edge over more conventional drugs in the treatment of hypertension, and further investigation of their properties in affecting metabolic functions is clearly warranted.

The renin angiotensin aldosterone system.

Although the renin angiotensin aldosterone system (RAAS) will not feature greatly in the discussion of the results presented hereafter, an overview of it will now be set out in order to place the main actions of the ACE inhibitors used in the project in their proper context. (For review see Opie, 1992)

The RAAS is a feedback system which has evolved to regulate the body's electrolyte balance and blood pressure. Its function is therefore intricately involved with the kidney.

Its activity is increased when renin is released from the juxtaglomerular cells of the nephron in the kidney. This occurs in response to one or more of three main stimuli, which are impaired renal blood flow, reduced plasma Na^+ content, or β adrenergic stimulation. Renin proteolytically cleaves the plasma borne factor angiotensinogen to release angiotensin I (AI), a decapeptide, which is itself acted upon by ACE to form the octapeptide angiotensin II (AII). AII is the active constituent of the system, and has three main actions. It causes vasoconstriction by increasing Ca^{++} entry into VSMC, it enhances sympathetic activity which also leads to vasoconstriction, and it causes the adrenal cortex to release the Na^+ retaining hormone aldosterone. AII is then broken down to angiotensin III which has no vasoconstrictor activity, but retains some aldosterone releasing activity (Opie, 1992) (figure I 3).

ACE inhibitors, therefore, have three potential angles of attack on hypertension in the short term, provided the RAAS plays a reasonably important role in the maintenance of high blood pressure. By inhibiting the formation of AII they remove elements of vasoconstriction, adrenergic facilitation, and plasma expansion through the Na^+ retaining properties of aldosterone. In the long term, by contrast, AII may act as a growth factor and thereby promote vascular wall hyperplasia and hypertrophy

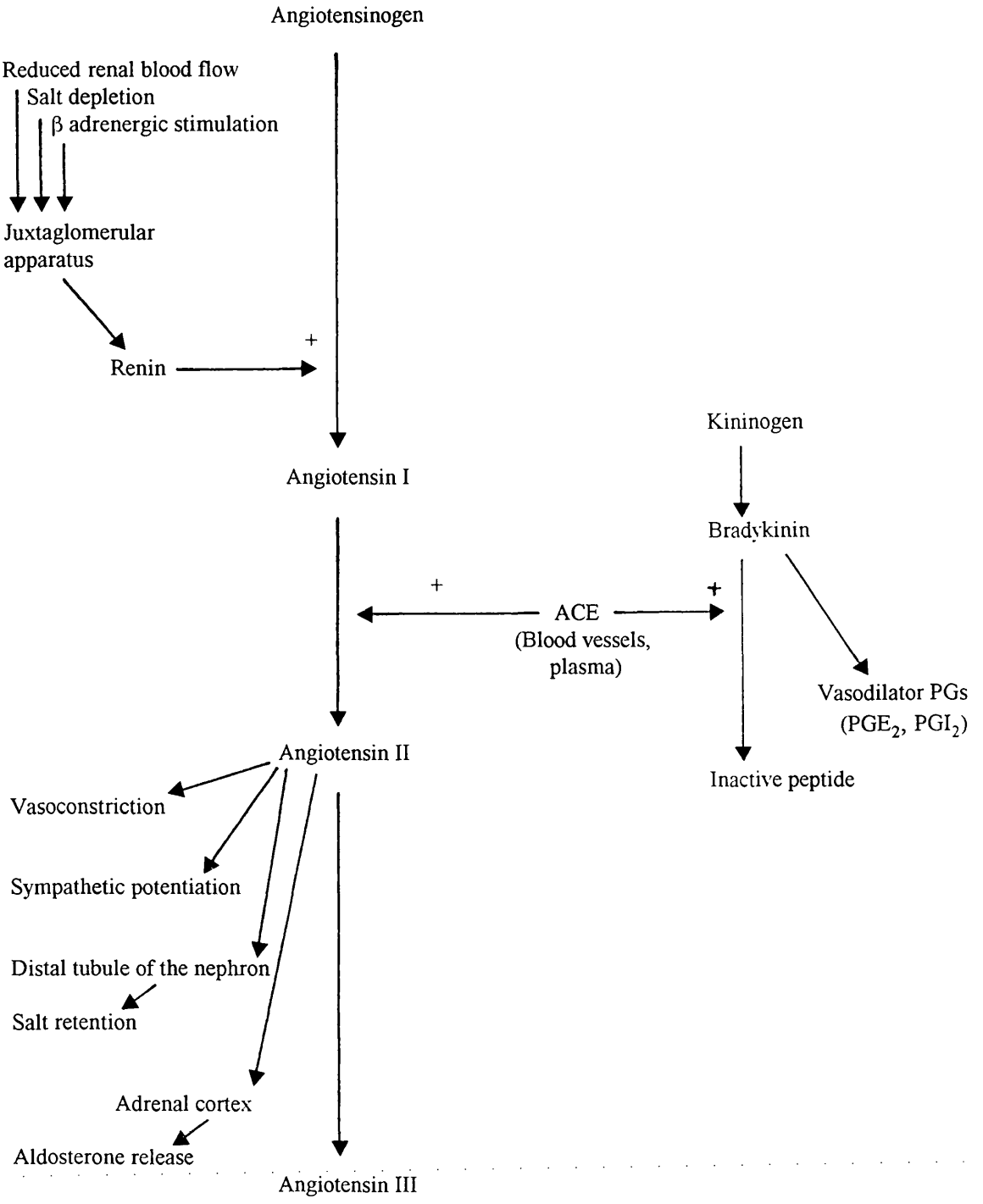

Figure I3. Schematic diagram of the generation of angiotensin II and the breakdown of bradykinin. ACE - angiotensin converting enzyme.

(Baudouin-Legros *et al.*, 1990; Dzau and Gibbons, 1991).

The measured plasma concentration of renin in most essential hypertensives is normal (Opie, 1992) as it is in the SHR (Fujito *et al.*, 1992). (Plasma renin concentration is used as a marker of the degree of RAAS activation). This would suggest that renin release is not a cause of hypertension and that ACE inhibitors would not have a great hypotensive effect. This is not the case, however. It has become accepted that there is a tissue RAAS which is important both to regional blood flow control and the development of essential hypertension. The expression of genes for renin, angiotensinogen, and ACE have all been demonstrated in brain, kidney, and the vasculature (for review see Lee *et al.*, 1992), and data presented in the literature strongly imply the presence of a fully competent and functional RAAS in the vascular walls of the rat (Eglème *et al.*, 1990; Mizuno *et al.*, 1988). Actions on the tissue RAAS must, therefore, also be taken into account when studying the site of action of ACE inhibitors.

Besides these conventional actions of ACE inhibitors, however, are two other activities of drugs such as captopril which may have an important bearing on their effect in hypertension. The first of these is dependent on the fact that ACE is also known as kininase II, the enzyme responsible for the breakdown of bradykinin (Bk) (Soffer *et al.*, 1974). ACE inhibitors, therefore, prevent the catabolism of Bk and thereby increase its concentration in the blood, and, due probably to a local Bk generating system, in the vessel wall also (Wiemar *et al.*, 1991). Bk is a direct vasodilator, and a substance which induces the release of EDRF and vasorelaxant prostanoids from the endothelium (Wiemar *et al.*, 1991; Hoffmann *et al.*, 1990). Additionally, it appears that it could be at least partially responsible for the regression of the cardiac hypertrophy commonly seen with long term ACE inhibitor therapy (Linz and Schölkens, 1992), and the similar vascular hypertrophy (Farhy *et al.*, 1992) since

antagonists of Bk have been used to prevent ACE inhibitor-induced regression.

The second property of ACE inhibitors is the ability, at least, it seems, in some circumstances, to improve glucose tolerance and insulin resistance, as has been mentioned already. The mechanism by which they have this effect is completely unknown, the research to date having focused on the simple observation that these drugs do have this property. It is not clear how the RAAS might interact with insulin-stimulated glucose uptake. The only major connection between the two systems is through the control of electrolytes, since both insulin (Omatsu-Kanbe and Kitasato, 1990; Hundal *et al.*, 1992) and aldosterone (Bia and De Fronzo, 1981) are able to stimulate Na^+/K^+ ATPase and play an important part in Na^+/K^+ regulation. Plasma K^+ concentration in hypertension is lower than in normotension, and this may be due to the high concentrations of insulin and aldosterone present in the plasma (Kim *et al.*, 1991). A self-compounding aspect of low plasma K^+ concentrations is that this stimulates renin release, and the subsequent rise in AII stimulates further K^+ excretion via aldosterone release (Ferrannini *et al.*, 1992). It could be, then, that the excessive amounts of these two hormones combine to stimulate cell growth and division through the stimulation of Na^+/K^+ ATPase and the consequent activation of the Na^+/H^+ exchanger which raises intracellular pH. Allied to the fact that VSMCs from SHRs are generally over-responsive to mitogenic stimuli anyway (Miller *et al.*, 1990, Baudouin-Legros *et al.*, 1990) such circumstances provide a powerful reason for believing that vascular hypertrophy and hyperplasia is a cause of essential hypertension, and not an adaptation to it. The action of ACE inhibitors, therefore, would reduce one of these stimuli. Indeed, one of the most vaunted properties of ACE inhibitors is the ability to reduce both cardiac and vascular hypertrophy (Clozel *et al.*, 1991, 1992; Frelson and Giudicelli, 1983).

This theory might explain why ACE inhibitors have such a marked effect on

vascular growth, but it does not explain why they appear to have an effect on glucose tolerance and insulin resistance. Glucose tolerance may be reduced in essential hypertension as a result of the lower plasma K^+ concentration, which provides a reduced stimulus for insulin secretion (Ferrannini *et al.*, 1992), but this does not account for insulin resistance and it is difficult to see how it might do so.

Aims.

Aims.

The aims of this project were to cover two main areas in hypertension research. The first of these was to investigate a model of insulin resistance and use it to determine whether the properties claimed for ACE inhibitors in humans (amelioration of insulin resistance) could be achieved in animal models both *in vivo* and *in vitro*. The second thread of the investigation was to carry out some work to elucidate any differences in K^+ metabolism *in vivo* by using plasma K^+ concentration as an indicator of K^+ status, and how this might relate to glucose metabolism.

From these approaches it was hoped that some conclusions about the cause of insulin resistance might be drawn, and further evidence for or against ACE inhibitors in its treatment established.

Methods.

Methods.

Animals.

All animals were male Wistar or Japanese Okamoto spontaneously hypertensive rats bred in the colony held at the University of Bath. They were housed in the University of Bath animal unit, and subjected to a twelve hour light:dark cycle, with the lights coming on at 6am. They drank tap water and ate SDS animal chow (Special Diet Services, Manea, Cambridgeshire), both supplied *ad libitum*.

Rats were fasted overnight (16-20 hours) prior to an experiment, and weighed 250-350g before fasting if they were to be used for glucose tolerance experiments, and 200-250g for isolated diaphragm experiments.

Anaesthesia, cannulations, injections and samples.

Rats were anaesthetised by intraperitoneal injection of anaesthetic. When surgical anaesthesia had been achieved the trachea was cannulated to allow artificial ventilation at 48 strokes.min^{-1} with a stroke volume of 0.75ml.100g^{-1} body weight if necessary. The two internal common carotid arteries were then cannulated with polythene cannulae (Portex cannula, external diameter 1.02 mm) as was a femoral vein (Portex cannula, external diameter 0.63 mm). Cannulae were tied in place with cotton ties.

One carotid cannula was connected, through a three way tap, to a blood pressure transducer (Bell and Howell, type 4-422-0001) the signal of which was processed by a pre-amplifier (Lectromed, model 3576) and recorded on a chart recorder (Lectromed, model MX2). Phasic blood pressure was recorded. The second carotid cannula was also attached to a three way tap, and this was used to remove

0.2ml blood samples for analysis of blood glucose, potassium, and insulin concentrations, as appropriate. It was also used to replace the volume of blood removed with an equal volume of 0.9% (w/v) saline solution. On some occasions it was only possible to cannulate one carotid artery. When this happened the single cannula was be used to record blood pressure before and after glucose tolerance tests, but during the tests it was used for sample withdrawal and volume replacement.

The femoral vein cannula was used for administration of drugs and glucose. Each dose was washed through the cannula with 0.1ml 0.9% saline solution which was roughly equal to the dead space of the cannula.

The animals were kept warm throughout the experiment on a small animal heating table (Scientific Research Instruments), their rectal temperature being maintained between 35.5 and 38.5°C, with an ideal temperature of 37°C.

Before all protocols with anaesthetised animals were started, but after they had been cannulated, the animals received 600units.Kg^{-1} heparin intravenously. Cannulae were also initially filled with heparin at a concentration of 1000 units.ml^{-1}.

Comparison of plasma glucose concentration in freshly killed Wistar rats and SHRs.

A group of six Wistar rats (300-350g) was compared with a similar group of SHRs. Each animal was sacrificed by cervical dislocation, and immediately decapitated. Approximately 1.5ml of thoracic blood was collected from each rat. Each of the six samples for each group was then treated as detailed later, and analysed for glucose concentration.

Development of the intravenous glucose tolerance test model.

Assessment of anaesthetic suitability.

Two anaesthetics were initially tested in Wistar rats. These were urethane ($1400mg.Kg^{-1}$) and hypnorm cocktail ($0.4ml.100g^{-1}$). Both were injected intraperitoneally. After induction of anaesthesia and cannulation of blood vessels and trachea, a blood sample was taken at t=0 and every 15 minutes after that, up to and including a two hour sample. The samples were treated as detailed later, and analysed for glucose concentration. Figure M1 shows the result of this experiment. As can clearly be seen, the control of plasma glucose concentration varied considerably less in the hypnorm cocktail-injected rats than in those which had been injected with urethane to induce anaesthesia. As a result of this study, hypnorm cocktail was chosen as the anaesthetic for further studies.

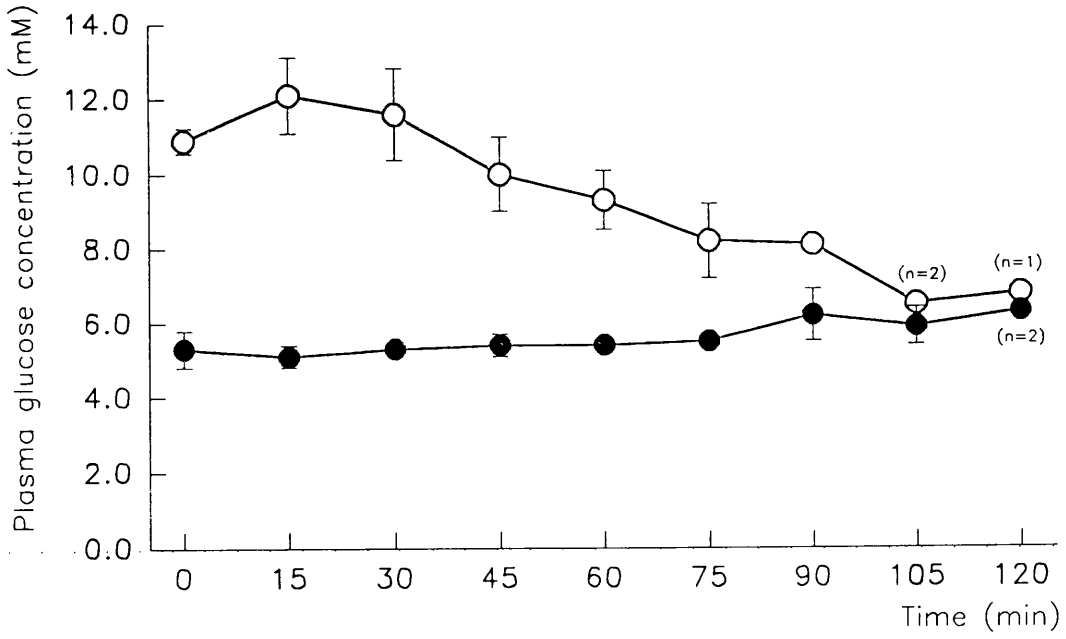

Figure M1. Effect of anaesthetic on basal plasma glucose concentration in Wistar rats. Symbols are: O - urethane anaesthesia (n=3 except where indicated), or ● - hypnorm cocktail (n=3, except where indicated). Urethane ($1400mg.Kg^{-1}$) or hypnorm cocktail ($4.0ml.Kg^{-1}$) were injected intraperitoneally. Points represent mean ±sem, here and in all following legends.

A comparison was then made of the quality of the control of plasma glucose concentration in Wistar rats and SHRs. Figure M2 shows that SHRs had a significantly higher basal plasma glucose concentration than Wistar rats, and that this difference was maintained at all time points except t=105 minutes. The narrowing of the gap over the two hours appeared to be due to an increase in plasma glucose concentration in Wistar rats, but this increase was not significant.

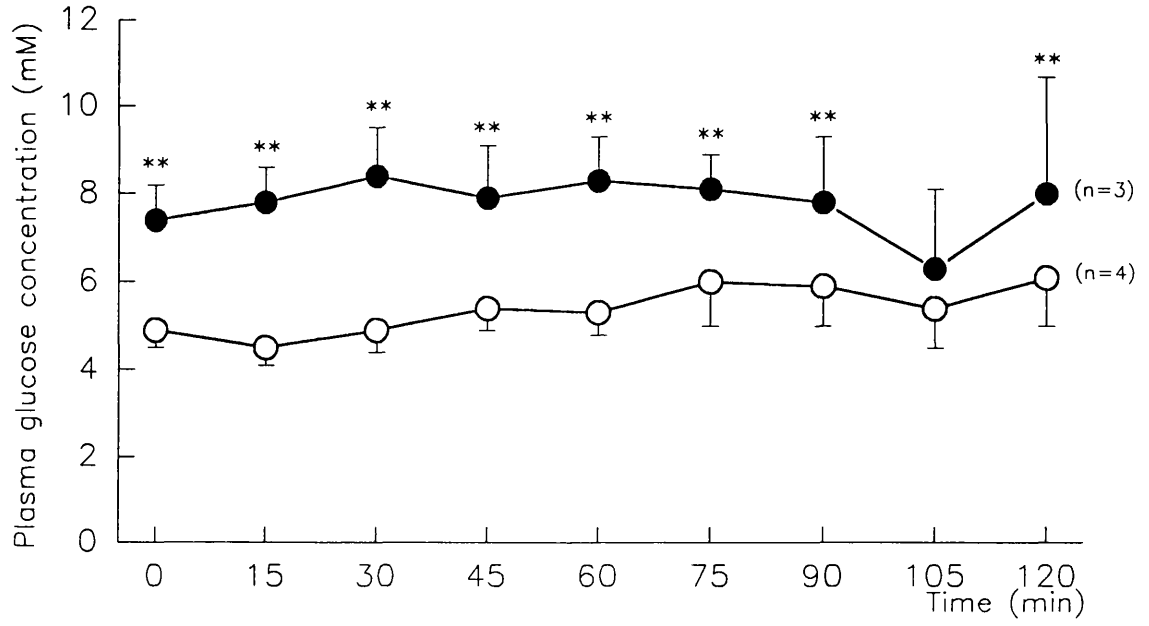

Figure M2. Effect of rat strain on basal plasma glucose concentration in hypnorm cocktail-anaesthetised rats. Symbols are O - Wistar rat (n=5, except where indicated), or ● - SHR (n=5, except where indicated). ** P<0.01, compared with the concentration in Wistar rats, by ANOVAR and post hoc Studentised range test

However, particularly in SHRs, it proved very difficult to maintain surgical anaesthesia without risking overdosing the animals. Therefore when a third anaesthetic, inactin, became available, it, too, was investigated. Hypnorm cocktail was compared with inactin in a similar protocol to the comparison with urethane. Inactin was injected intraperitoneally at a dose of 150mg.Kg^{-1}. As well as proving much more

reliable in inducing anaesthesia, it had the additional advantage that it did not need topping up in the majority of animals, so depth of anaesthesia, judged by the paw withdrawal and blink reflexes, was much more constant than with hypnorm cocktail. The result of this comparison is shown in figure M3. This figure demonstrates that there is no difference in the quality of control of basal plasma glucose concentration between rats anaesthetised with hypnorm cocktail or inactin. In hypnorm cocktail-anaesthetised rats the basal plasma glucose concentration was approximately 1mM higher than in inactin-anaesthetised rats. The possible reasons for this difference will be discussed later. Inactin was therefore chosen to continue the studies, and some experiments were repeated using the new anaesthetic. The results represented by figure M4, and all of the *in vivo* results in the results section (except the assessment of epicaptopril activity and the assessment of the ability of losartan to inhibit AI pressor responses) were carried out using inactin anaesthesia.

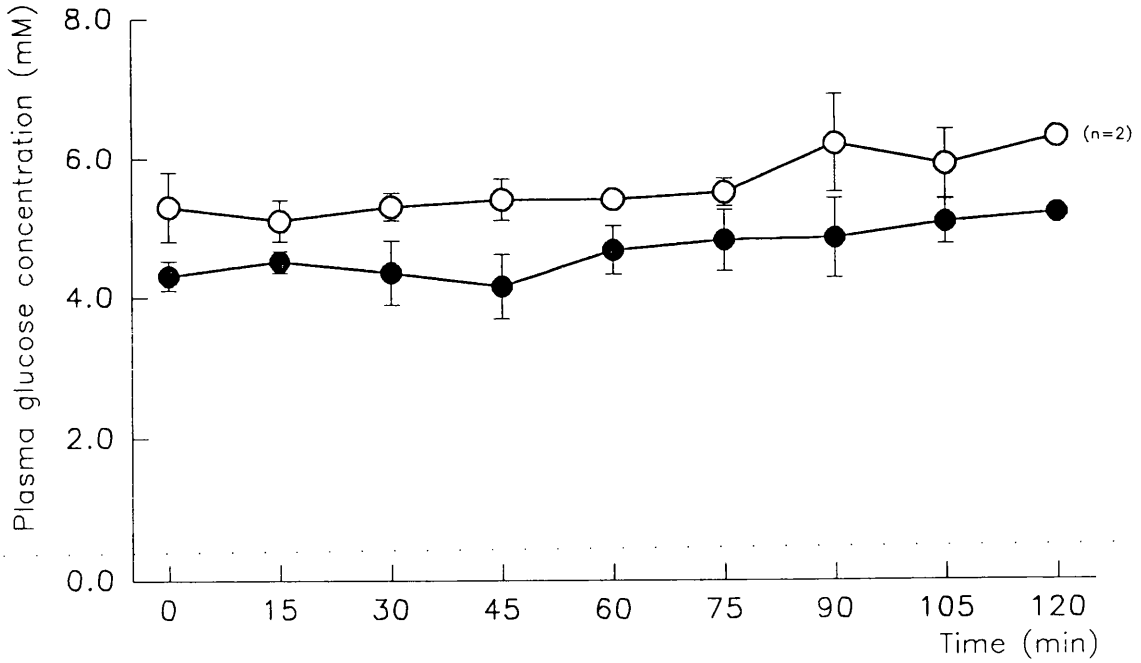

Figure M3. Basal plasma glucose concentration in SHRs anaesthetised with different anaesthetic agents. Symbols are: ○ - hypnorm cocktail anaesthesia (n=3, except where indicated), or ● - inactin anaesthesia (n=4). Hypnorm cocktail (4.0ml.Kg^{-1}) or inactin (150mg.Kg^{-1}) were injected intraperitoneally.

Glucose tolerance tests.

Intravenous glucose tolerance tests (IVGTTs) were performed in the following manner. At t=-2 minutes a basal blood sample was taken, followed at t=0 by intravenous (iv) administration of 250mg.Kg^{-1} (1.39mmol.Kg^{-1}) glucose in solution. Then, at t=2, 5, 10, 15, and 30 minutes further blood samples were taken.

Where two IVGTTs were carried out in the same animal, either 30 or 60 minutes was allowed between the two tests for the animals to re-establish baseline glucose and insulin concentrations. This time period was constant within an experiment. Drugs were administered 27-30 minutes before the second IVGTT, except in the case of lisinopril which was administered 57 minutes before the second test.

Assessment of the effect of time between two IVGTTs on the second IVGTT.

In this study two IVGTTs were carried out in each animal, the time between the two tests being either 30 minutes or one hour. No other treatment was given to either group of SHRs used for this investigation.

The results of this experiment, shown in figure M4, demonstrate that when the second IVGTT was performed one hour after the end of the first, the clearance of the glucose load was no different from that in animals in which only half an hour had been allowed between the two IVGTTs. This test was carried out to ensure that studies which had different times between the two IVGTTs could be considered similar, and could therefore be compared.

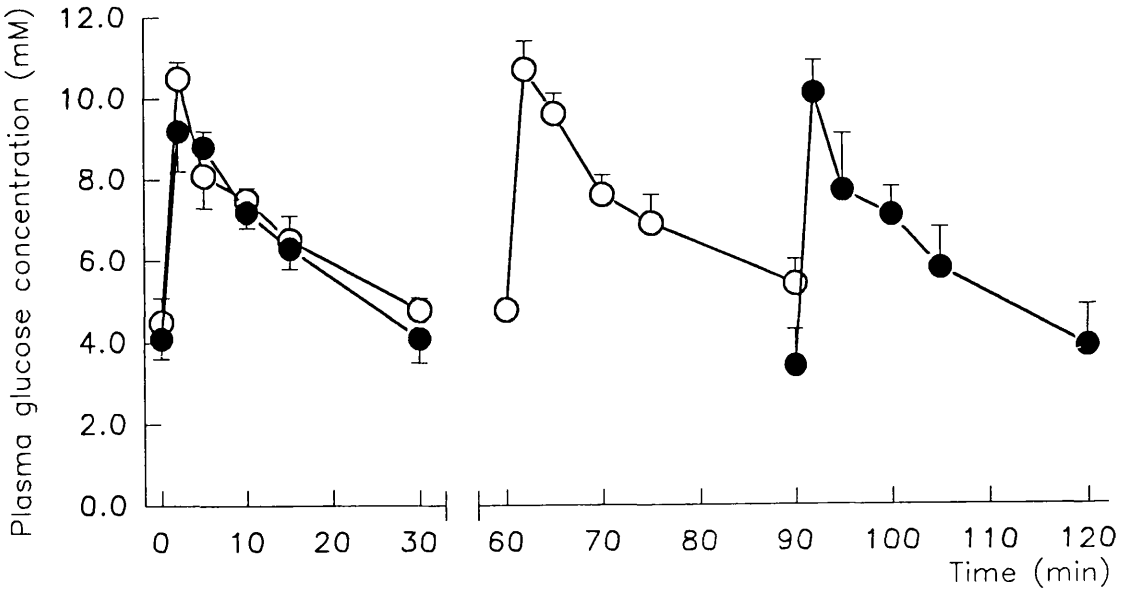

Figure M4. The effect of time between two IVGTTs on the second IVGTT in inactin anaesthetised SHRs. Symbols are: O - half an hour (n=5), or ● - one hour between IVGTTs.

Chronic treatment of SHRs with captopril.

The investigations were also extended to cover a possible chronic action of captopril. In this case the drug was injected subcutaneously twice a day (at 9am and 4pm) in two equal doses so that each animal received 230μmol.Kg^{-1} per day for two weeks. After this period the animals were subjected to an IVGTT as for acute experiments. Animals were tested in pairs of one each of the control and treated groups. On the experimental day only animals to be used in the afternoon received the morning injection. The captopril was dissolved in saline and a similar volume of saline was injected into the control animals as vehicle.

Assessment of the efficacy of lisinopril and losartan.

For the assessment of angiotensin II (AII) antagonism by losartan SHRs were anaesthetised with hypnorm cocktail and cannulated as previously described, except that only one carotid cannula was used. Blood pressure was recorded throughout the

experiment. The effect of a bolus of saline ($0.5ml.Kg^{-1}$, iv) on blood pressure was assessed, and any increase in blood pressure that this caused was subtracted from subsequent responses to angiotensin II. A dose:response curve of increases in blood pressure in response to bolus doses of AII (iv) was then constructed, after which a dose of losartan was injected intravenously. Thirty minutes was allowed for the drug to take effect, after which a second dose:response curve was performed.

The assessment of lisinopril activity was similar, except that inactin was used to anaesthetise the rats.

Treatment and analysis of samples.

Blood samples were immediately transferred to 0.4ml Sarstedt tubes and centrifuged (Beckman Microfuge II) for one and a half minutes to remove erythrocytes and white blood cells. The plasma was collected and stored on ice, either until it was analysed after the experiment, or until the end of the experiment when it was frozen to -20°C.

Samples were routinely analysed for glucose concentration. and on occasions also for K^+ and insulin concentrations.

Glucose concentration was determined using a glucose oxidase-based colourimetic assay (GOD-Perid kit, Boehringer-Mannheim). This was carried out according to the supplied instructions, with three alterations. The enzyme solution was made up at $10mg.ml^{-1}$, of which 2ml per assay was used, and the volume of plasma sample used for each replicate was 5µl. Briefly, 5µl of sample was pipetted into a test tube and 2ml of enzyme solution added to it. After whirlimixing, it was incubated at room temperature for 30 minutes while it developed a green colour, at which time the intensity of the colour was measured in a spectrophotometer (Pye-

Unicam, model 8610) at 436nm. Assays were performed in duplicate. A standard curve was constructed for each set of assays using known glucose concentrations. The standard curve assays were done in triplicate.

K^+ concentration was measured using flame photometry (Corning 405 flame photometer). 1.98ml of Corning 405 diluent solution was added to 20µl of plasma sample. After whirlimixing, the sample was read straight away. The flame photometer was re-calibrated after every fourth sample reading.

Insulin concentrations were measured by radioimmunoassay. This was kindly done by staff at Zeneca Pharmaceuticals PLC (Alderley Park, Cheshire), and was based on a method by Albano and colleagues (1972).

The assay depends on competition for a limited number of binding sites on a fixed amount of antibody (Ab). Radiolabelled insulin competes with plasma insulin for the Ab binding sites. After equilibrium is reached, the free and bound radioactive insulin are separated by charcoal absorption.

The quantities indicated in columns 1 to 6 of table M1 were added to LP3 tubes, mixed, and incubated at 4°C for five hours. Then the quantities of buffer solution or charcoal solution in columns 7 and 8 were added to the contents of the tubes, mixed in, and left to incubate at 4°C overnight. The tubes were then centrifuged at 2500 rpm (Sorvall RC-3B centrifuge) for 30 minutes at 4°C, and 500µl of the supernatant transferred to LP3 tubes for counting of the radioactivity (LKB-WALLAC universal

Table M1. This table represents the constituents, and quantities of those constituents, which were used in the insulin radioimmunoassay. Refer to text (page 47) for order and timing of mixing.

All volumes are in µl. Abbreviations are: TC - total count of radiolabelled insulin; CB - charcoal blank; CTP - charcoal-treated plasma; AIS - anti-insulin serum; A - standard buffer A solution;; B - standard buffer B solution; ^{125}I - radioactive insulin; Std - lyophylised rat insulin standard; char - activated charcoal solution.

	1	2	3	4	5	6	7	8
	B	Std	CTP	A	AIS	^{125}I	A	Char
TC						100	700	
CB	50		50	200		100		400
Zero	50		50		200	100		400
Std		50	50		200	100		400
Sample	50				200	100		400

gamma counter (window 035-102, count time 60 seconds)). A standard curve was drawn, from which sample concentrations were interpolated.

Charcoal treated plasma was prepared as follows: the plasma samples were defrosted, and 10mg.100μl^{-1} charcoal was added, and mixed at room temperature for 30 minutes. Next the samples were centrifuged at 18,000 rpm for 20 minutes. The supernatant was then filtered and used in the radioimmunoassay.

Measurement of glucose uptake by rat hemidiaphragm.

This method is an adaptation of the method used by Kirby and Turner (1975). Rats were killed by stunning and then cervical dislocation. Diaphragms were rapidly excised, given three washes in ice cold, glucose-free Krebs solution, and rested in ice cold, glucose-free Krebs solution for ten minutes. The dissection and washing took about 90 seconds. They were then divided into halves that were trimmed, blotted, and weighed, and placed into the incubation flasks. The flasks contained 3ml of previously oxygenated incubation mixture which was glucose-containing Krebs solution with the appropriate concentrations of human insulin and drug. A constant stream of water-saturated 95%O_2/5%CO_2 was passed through each flask to ensure adequate oxygen supply. In experiments where a concentration:effect curve for insulin was constructed, each hemidiaphragm was randomly assigned to one of the concentrations of insulin being tested. When the effect of captopril on insulin-stimulated glucose uptake was being assessed one of each pair of hemidiaphragms was used as control and received vehicle while the other received drug. These were also randomly assigned to drug or control treatment.

The flasks were placed in a heated (37°C), shaking (150 strokes.min^{-1}) water bath for two hours. 50µl samples were taken of the initial incubation medium, and of

the flask incubation medium at t=10 and 20 minutes, and every 20 minutes thereafter up to and including a t=120 minutes sample. Samples were kept on ice until analysed, or until frozen to -20°C to be analysed on another day. At the end of the experiment the remaining volume of incubation medium was measured and an estimation of the rate of evaporation due to the continuous gassing was made.

The glucose concentrations were determined, and estimates of cumulative glucose uptake by each hemidiaphragm were made by subtracting the measured concentration from the initial concentration, taking into account sample volume loss and the concentrating effect of evaporation. The mass of glucose taken up by each diaphragm was expressed as μmol uptake.g^{-1} tissue wet weight.

Assessment of aldosterone and canrenoate potassium effects on plasma glucose and potassium concentrations.

SHRs or Wistar rats weighing 250-300g were anaesthetised and prepared, as previously described, after being fasted overnight. After approximately 30 minutes, during which the animals stabilised, the experiment was started.

In the aldosterone assessments, three basal blood samples were taken, at t=0, 10, and 20 minutes. Immediately after the 20 minute sample a dose of aldosterone (1.39nmol.Kg^{-1}), which would produce an incremental increase in plasma aldosterone concentration of approximately 15- to 70-fold, was administered iv. Further samples were taken at 10 minute intervals until the termination of the experiment at t=180 minutes. Samples were treated, and assayed for glucose and K^+content as described previously.

The assessment of canrenoate K^+ action was carried out similarly. At t=0 and 20 minutes basal blood samples were taken, immediately after which a dose of 7.5mol.Kg^{-1} canrenoate K^+ was given iv. Samples were taken, for estimation of plasma glucose and potassium concentration, every 10 minutes until t=60 minutes, after which they were taken every 20 minutes until t=180 minutes when the experiment was ended.

Materials and solutions.

All salts for Krebs' solution were supplied by BDH Chemicals Ltd., Poole, Dorset, as was the D-glucose used throughout the studies. L-cysteine, aldosterone and canrenoate K^+ were supplied by Sigma Chemicals Ltd., Poole, Dorset. Human insulin was "Actrapid" (100IU.ml^{-1}) from Farillon Ltd., Romford, Essex.

The ACE inhibitors were all generous gifts of industrial companies. Both captopril and epicaptopril were supplied by the Squibb Institute for Medical Research, Princetown, New Jersey, enalaprilat was given by Merck Sharpe and Dohm Ltd., Rahway, New Jersey, and lisinopril was from Zeneca Pharmaceuticals, Alderley Park, Cheshire.

The glucose oxidase kit (GOD-perid method) was obtained from Boehringer-Mannheim Diagnostica, Lewes, East Sussex.

Urethane was obtained from Sigma Chemicals Ltd. Inactin was purchased from from Byk-Gulden, Konstanz, Germany. Hypnorm was supplied by Janssen Pharmaceuticals Ltd., Grove, Oxford, and Hypnovel by Roche Products Ltd., Welwyn Garden City, Herts.

Heparin was supplied by Sigma Chemicals Ltd, as were human angiotensins I

and II.

The saline to make up the captopril solution during the chronic captopril treatment was from Steripak.

Urethane (crystalline form) was made up as a 1400mg.ml^{-1} solution in distilled water, and kept at 0-4°C for two weeks. "Hypnorm cocktail" was made up by mixing one volume of hypnorm, one volume of hypnovel, and two volumes of sterile water. The final concentrations of the active constituents were 78.8μg.ml^{-1} fentanyl citrate, 2.5mg.ml^{-1} fluanisone, and 1.25mg.ml^{-1} midazolam. It was stored at room temperature in the dark for up to one month. Inactin, supplied as a powder, was dissolved in distilled water to a final concentration of 50mg.ml^{-1}. It was divided into aliquots and these were frozen (-20°C) until used. Heparin was dissolved in 0.9% saline solution and made up to a final concentration of 1000U.ml^{-1} before being aliquotted and kept at 0-4°C. The saline solution (0.9% w/v) was made up with distilled water.

D-glucose solution was made up to 250mg.ml^{-1} (1.39M) in distilled water. It was kept at 0-4°C between experiments, for not longer than two weeks.

Captopril (2.3x10^{-6}mol.ml^{-1}), epicaptopril (2.3x10^{-6}mol.ml^{-1}), enalaprilat (1.3x10^{-6}mol.ml^{-1}), lisinopril (2.75x10^{-7}mol.ml^{-1}), losartan (2.17x10^{-5}mol.ml^{-1}), and L-cysteine (2.3x10^{-6}mol.ml^{-1}) were all dissolved in 0.9% saline solution. Likewise, solutions of aldosterone (1.39x10^{-9}mol.ml^{-1}) and canrenoate K^{+} (7.5x10^{-5}mol.ml^{-1}) were made up in saline. In the study of the effects of chronically administered captopril, the drug was dissolved in sterile saline to a concentration of 1.15x10^{-4} mol.ml^{-1}. The solution was freshly made up for each injection time.

Krebs' solution contained the following compounds in the designated concentrations: NaCl 118.4mM, KCl 4.7mM, $CaCl_2$ 2.5mM, $NaHCO_3$ 25mM, $MgSO_4$

1.2mM, KH_2PO_4 1.2mM, and D-glucose 15mM. The insulin solution used to stimulate glucose uptake by hemidiaphragms was diluted with this Krebs' solution to the required concentrations, which were $10\mu U.ml^{-1}$, $100\mu U.ml^{-1}$, $1mU.ml^{-1}$, $10mU.ml^{-1}$, or $100mU.ml^{-1}$.

Corning 405 diluent was made up by diluting a volume of the concentrated diluent 1000-fold with distilled water.

Statistics.

Graphs show mean values ± standard error of the mean (sem) throughout (except figure R 2). The data quoted in the text are also mean values ±sem.

The significance of differences in blood pressure were tested using Student's t-test for unpaired data. The comparison of glucose tolerance curves was made with two way analysis of variance (ANOVAR) with replications in order to determine whether a difference existed between glucose extinction curves. Comparisons were made between the groups either before or after treatment with saline or drug, or between the two IVGTTs done in the same group before and after treatment. Similarly, comparisons of insulin-stimulated glucose uptake and the effect of captopril on this parameter were tested using the same test, as were comparisons of the effect of aldosterone and canrenoate potassium on plasma glucose and potassium concentrations. Where a significant difference was indicated by the value of F in the ANOVAR, a post hoc studentised range test was carried out to determine where the significant difference lay. When the groups being tested were of equal size, the post hoc studentised range test was undertaken using the Tukey method, whereas for groups of unequal size the Tukey/Kramer method was used (Hinkle *et al.*, 1988). Values of P of 0.05 or less were considered statistically significant.

Results.

Results.

Blood pressure in Wistar rats and SHRs.

Figure R 1 shows the mean systolic, diastolic, and pulse pressures recorded from the anaesthetised rats used in the comparison of intravenous glucose tolerance and insulin action in Wistar rats and SHRs (see below). As can be seen, there was a statistically significant difference between the two strains with respect to each parameter. These values were typical of the pressures recorded during the studies detailed in this thesis.

Glucose oxidase-based glucose assay.

The glucose oxidase-based assay used for measuring plasma glucose concentrations was highly reliable and reproducible. A sample of six standard curve assays, each performed in triplicate, had a positive gradient of 0.06882 ±0.00529 absorbance units per millimolar increase in concentration (mean ±95% confidence intervals). These data are shown in figure R 2.

Plasma glucose concentration in freshly killed rats.

Plasma glucose concentration in freshly killed Wistar rats and SHRs rats did not differ significantly. The values were 8.0 ±0.1mM (mean ±sem, n=6) for Wistar rats and 8.6 ±0.3mM (n=6) for SHRs. These values are significantly higher than for inactin anaesthetised rats of similar strain (Wistar - 3.4 ±0.1, n=8, SHR - 4.7 ±0.4mM, n=6, $P<0.01$).

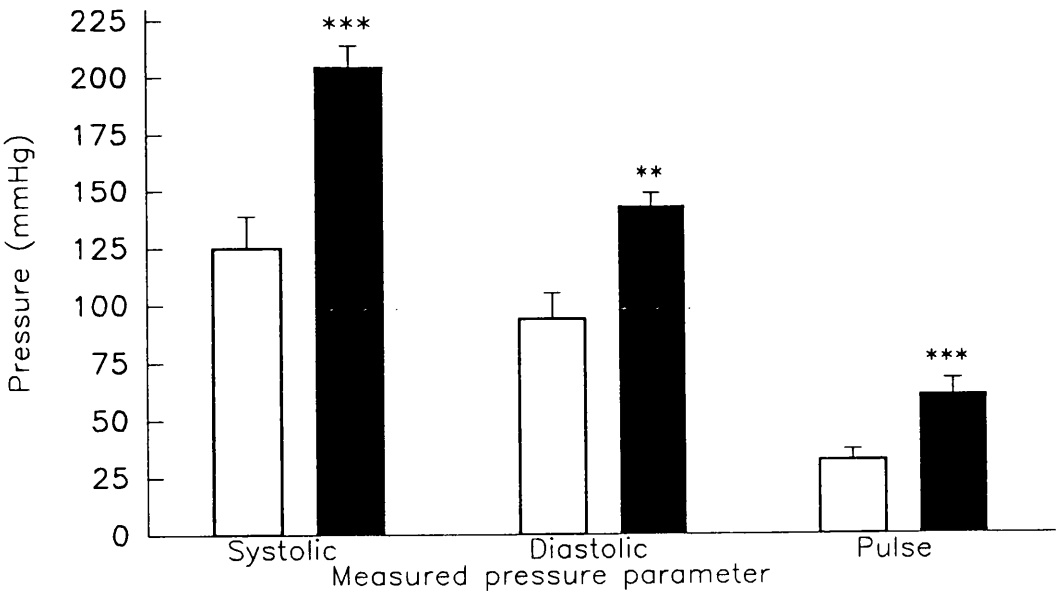

Figure R 1. Systolic, diastolic, and pulse pressures recorded in inactin-anaesthetised Wistar rats and SHRs. The data are from the rats used in the comparison of intravenous glucose tolerance and insulin action in Wistar rats and SHRs (figure R 3). Wistar rats (n=8) are represented by open bars and SHRs (n=6) by solid bars. ** P<0.01, *** P<0.001 compared with Wistar rats, by Student's unpaired t-test.

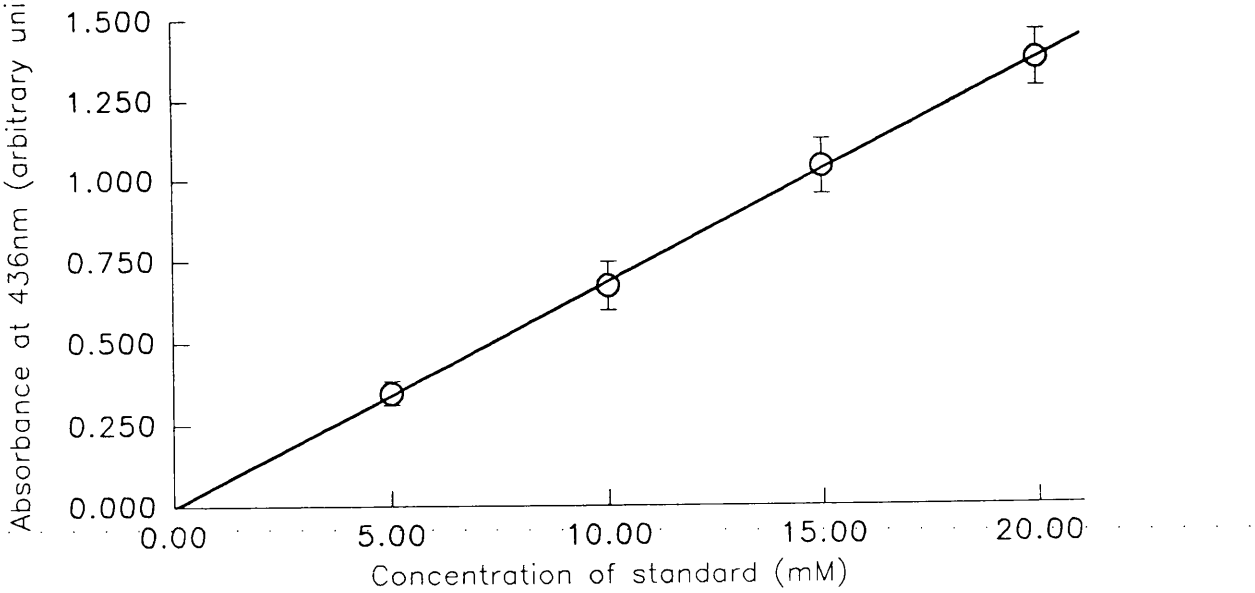

Figure R 2. Standard curve for glucose oxidase-based glucose assay. Graph shows mean absorbance ±95% confidence intervals and mean gradient (see text). The data represent six experiments each performed in triplicate.

Comparison of intravenous glucose tolerance and insulin action in Wistar rats and SHRs.

Plasma glucose concentration was significantly higher in SHRs than Wistar rats at t=0 (3.4±0.1 (n=8) vs. 4.7±0.4mM (n=6), $P<0.01$) and remained so except at t=30 minutes. Moreover, the increase in concentration upon injection of glucose was greater in SHRs than in Wistar rats (7.4±0.7 vs. 5.5±0.2mM, $P<0.01$) at t=2 minutes (figure R 3, A). However, the area under the curve above initial concentration was not significantly different in SHRs compared with Wistar rats (81.4±7.9 vs. 80.1 ±10.1mM.min respectively), and at the end of the IVGTT the concentration of plasma glucose was still significantly higher than basal values in Wistar rats whereas it had returned to normal in SHRs.

The insulin response during the IVGTT (figure R 3, B) was significantly greater ($P<0.01$) in the SHR group than in the Wistar rat group, although the rise in plasma insulin concentration at t=2 minutes was not significantly larger in SHRs than in Wistar rats (5.74 ±0.76 (n=6) vs. 4.51 ±0.24 (n=4), respectively).

The 10 minute infusion of insulin (3.6mU.Kg^{-1}.min^{-1}) to which the animals were subjected caused a significant drop in the plasma glucose concentration of both strains of rat (figure R 4). Interestingly, the response of SHRs appeared more marked than that in Wistar rats, although this did not reach statistical significance.

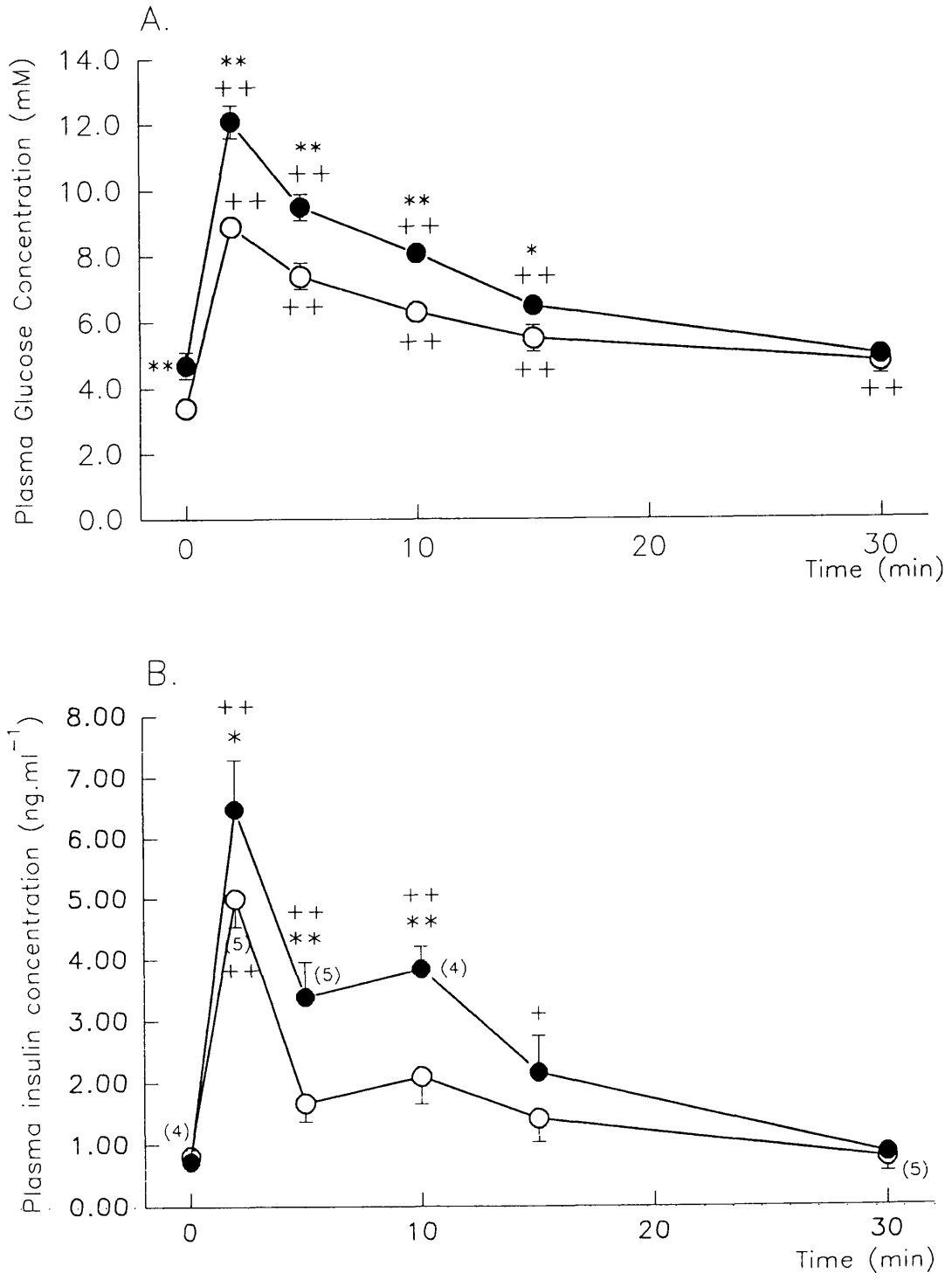

Figure R 3. Comparison of intravenous glucose tolerance in inactin-anaesthetised Wistar rats and SHRs (A), and the insulin response during IVGTT (B). 1.39mmol.Kg^{-1} glucose solution was administered intravenously at t=0. Symbols are: O - Wistar rats (n=8), or ● - SHRs (n=6) * P<0.05, ** P<0.01 compared with Wistar rats. + P<0.05, ++ P<0.01 compared with t=0 in the same strain

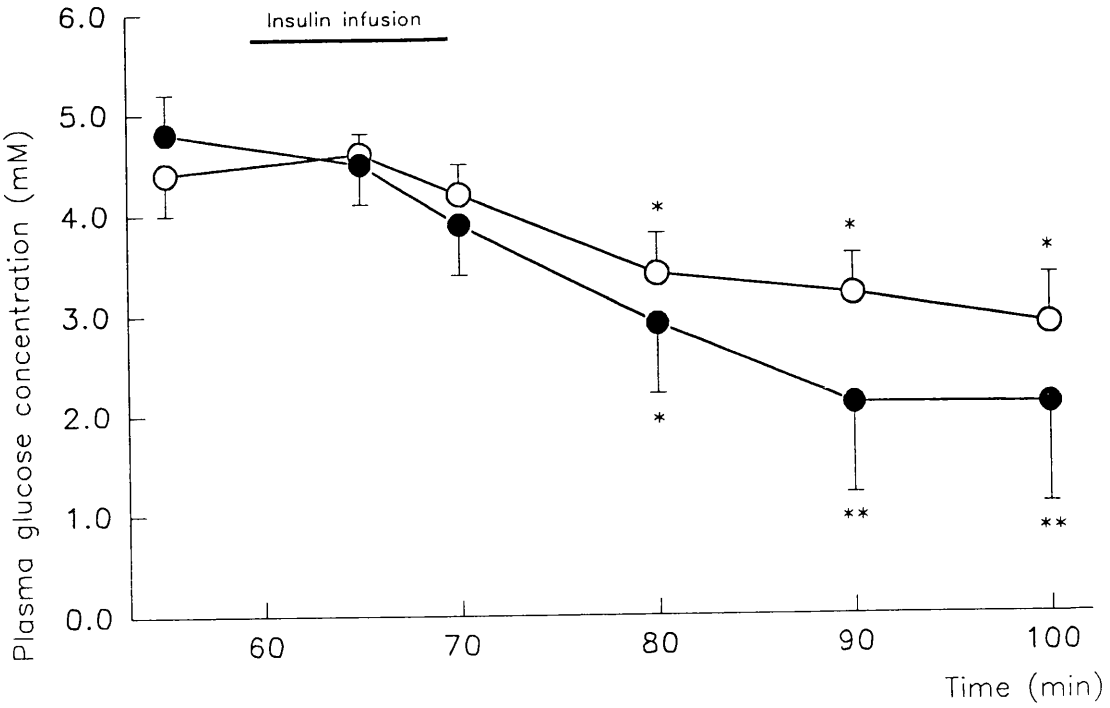

Figure R 4. Effect of intravenous insulin infusion (3.6mU.Kg^{-1} min^{-1}) on plasma glucose concentration in inactin-anaesthetised Wistar rats and SHRs. Symbols are. O - Wistar rats (n=7), or ● - SHRs (n=6). Bar indicates duration of infusion. * P<0.05, **P<0.01 compared with t=55 minutes in the same strain.

The effect of captopril, lisinopril, and enalaprilat on blood pressure and glucose tolerance in the SHR.

Captopril (2.3μmol.Kg^{-1}, iv) failed to cause a reduction in diastolic blood pressure, either compared with initial values in the test group (where it fell from 84 ±10 to 80 ±9mmHg, n=6) or compared with control animals (the fall in pressure after saline treatment was 16 ±5mmHg whereas it was 4 ±2mmHg after captopril treatment).

Enalaprilat (1.3μmol.Kg^{-1}, iv) caused a significantly greater reduction in systolic blood pressure than saline, falling by 21 ±5mmHg to 111 ±19mmHg (n=6) in controls compared with a decrease of 41 ±7mmHg to 97 ±15mmHg (n=6) in treated

animals. The reduction in diastolic blood pressure was not statistically significantly greater in enalaprilat-treated rats (21 ±4mmHg in controls vs. 37 ±7mmHg in enalaprilat-treated animals).

Lisinopril (275nmol.Kg^{-1}, iv) had no statistically significant effect on blood pressure parameters. In control animals diastolic blood pressure fell by 11 ±7mmHg to 58 ±9mmHg (n=6) after saline treatment. The reduction in this measurement in lisinopril-treated rats was by 22 ±13mmHg to 53 ±15mmHg (n=5). In spite of this, lisinopril at this dose had been previously shown to cause approximately a 100-fold shift to the right of the pressor dose:response curve in response to intravenously administered angiotensin I (figure R5).

Of the three ACE inhibitors, only captopril showed any effect on glucose tolerance (figures R 6 - R 8). Its action was to worsen glucose tolerance significantly ($P<0.05$) compared with the controls in which glucose tolerance did not change (figure R 6). The difference was demonstrated by analysis of variance, but post hoc Studentised range test revealed that no single pair of points differed significantly from each other.

The effect of chronic captopril treatment on glucose tolerance.

In the regime chosen for this experiment, captopril (230μmol.Kg^{-1}.day^{-1} for two weeks) reduced both systolic (from 176±7mmHg in controls (n=4) to 159±9mmHg in treated animals (n=7)) and diastolic (from 148±10 mmHg in the control group to 114 ±8mmHg in the test group) blood pressures, although only the difference in diastolic blood pressures was statistically significant ($P<0.01$). The two weeks of treatment with captopril did not affect glucose tolerance (figure R 9) with respect to time course of glucose elimination, peak glucose concentration, or area under the curve.

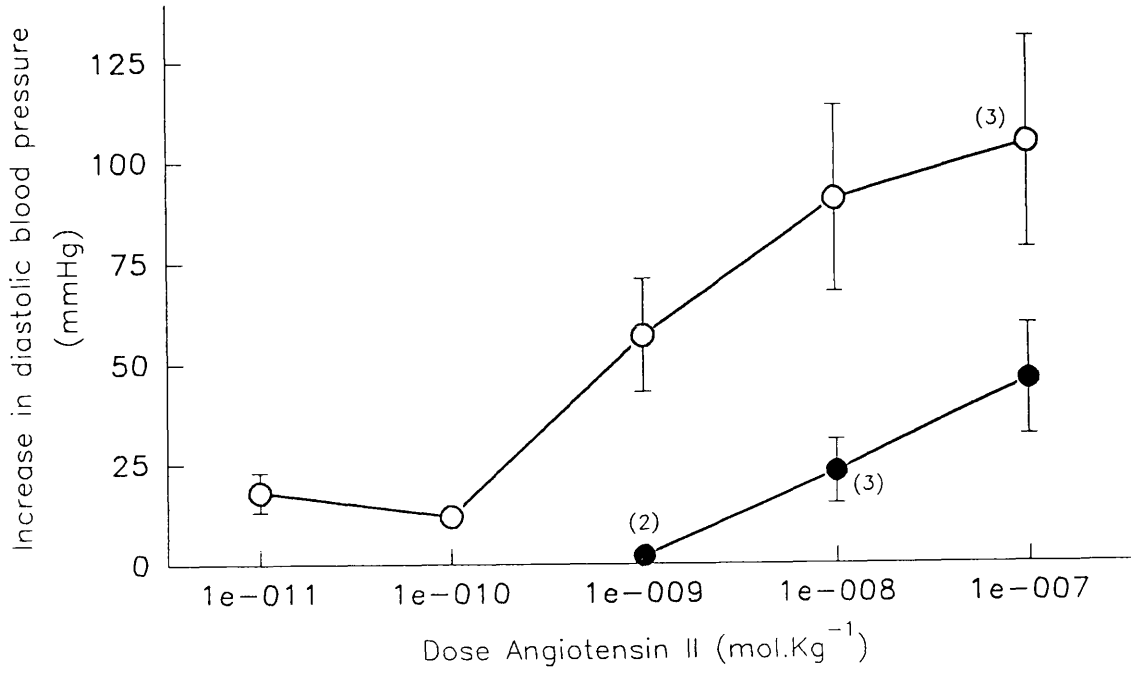

Figure R 5. Effect of lisinopril (275nmol.Kg^{-1}, iv) on pressor responses of diastolic blood pressure to angiotensin I (iv) in inactin-anaesthetised SHRs. Symbols are: O - control dose:response curve, ● - dose: response curve after treatment with lisinopril (n=4 except where indicated).

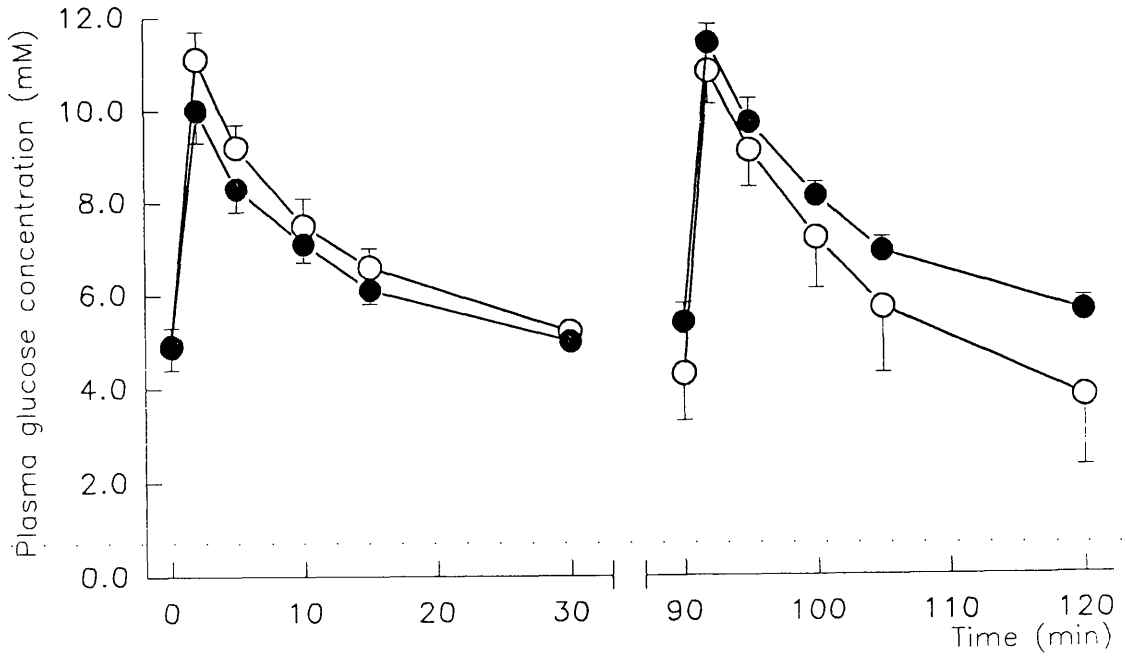

Figure R 6. Effect of captopril on glucose tolerance in inactin-anaesthetised SHRs. Captopril (2.3μmol.Kg^{-1}, iv) was administered at t=60 minutes. Symbols are: O - control (n=5), or ● - captopril (n=5). Captopril-treated animals have worse glucose tolerance than saline-treated controls ($P<0.05$, by ANOVAR).

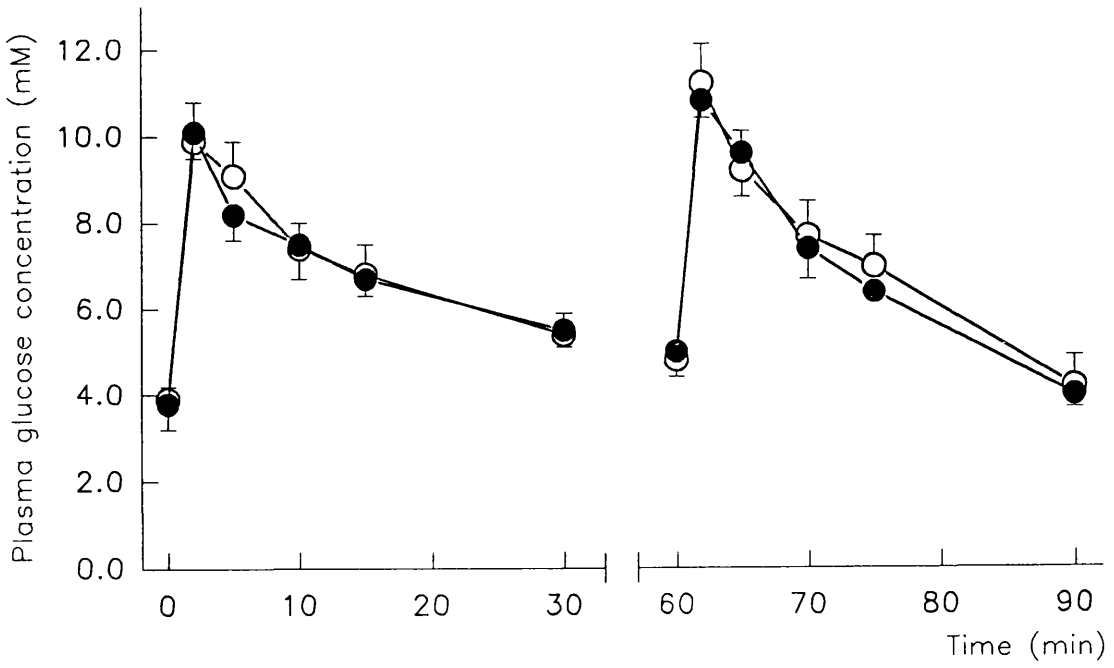

Figure R 7. Effect of enalaprilat on glucose tolerance in inactin-anaesthetised SHRs. Enalaprilat (1.3μmol.Kg^{-1}, iv) was administered at t=32 minutes. Symbols are: ○ - control (n=5), or ● - enalaprilat (n=7).

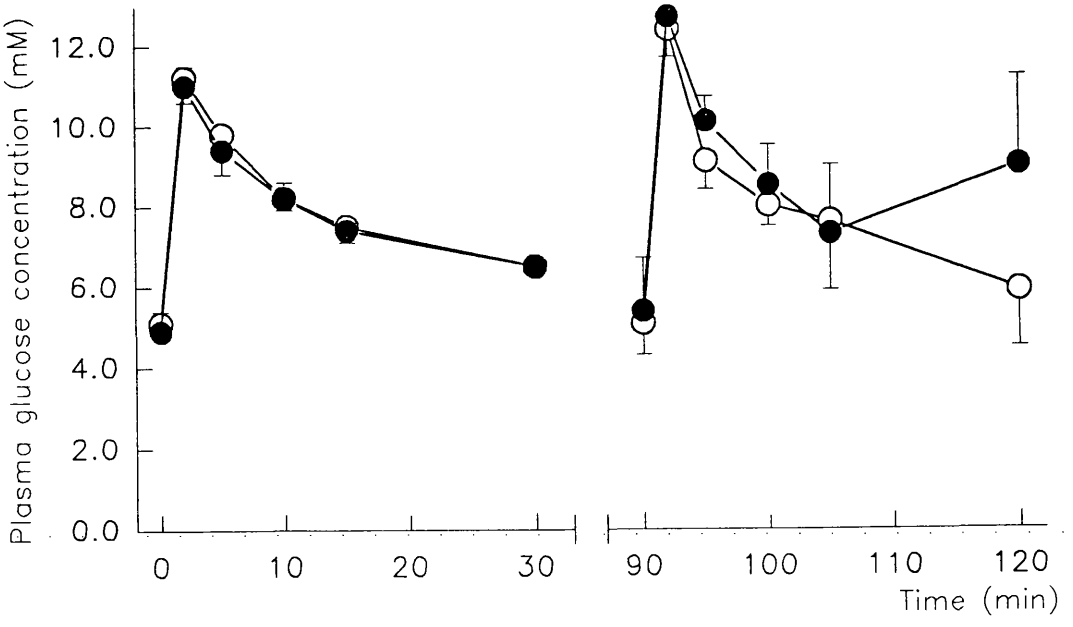

Figure R 8. Effect of lisinopril on glucose tolerance in inactin-anaesthetised SHRs. Lisinopril (275nmol.Kg^{-1}, iv) was administered at t=35 minutes. Symbols are: ○ - control (n=6), or ● - lisinopril (n=5).

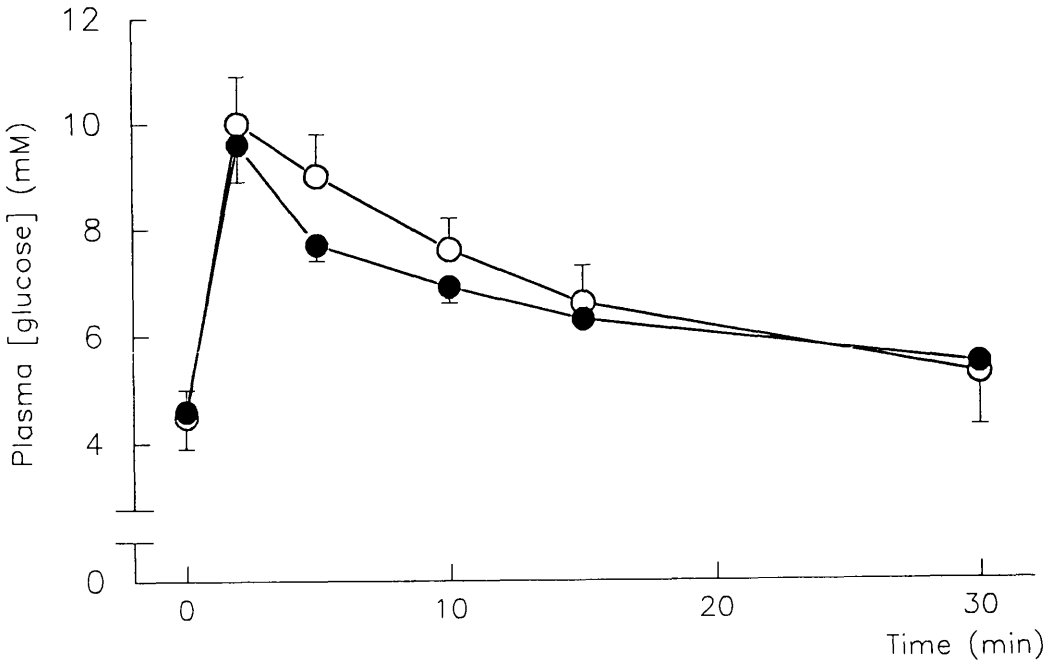

Figure R 9. Effect of chronic captopril treatment on glucose tolerance in inactin-anaesthetised SHRs. Captopril (115μmol.Kg^{-1}, sc) was administered at 9am and 4pm. Symbols are: O - control (n=4), or ● - captopril (n=7).

The effects of epicaptopril and L-cysteine on blood pressure and glucose tolerance.

Epicaptopril (2.3μmol.Kg^{-1}, iv) was without effect on blood pressure. Diastolic blood pressure was 37 ±4mmHg (n=5) both before and after saline treatment, and 39 ±1mmHg before epicaptopril treatment compared with 39 ±2mmHg (n=5) after it. L-cysteine (2.3μmol.Kg^{-1}, iv) was also without effect on blood pressure. While diastolic blood pressure was reduced from 113 ±4 to 86 ±7mmHg (n=5) in controls, the corresponding figures in L-cysteine-treated rats were 92 ±8 and 76 ±5mmHg (n=5) respectively. The difference in blood pressure reduction was not statistically significant.

Epicaptopril was used to determine whether the thiol group on captopril might

have any activity. A comparison of the post-treatment IVGTTs shows that the two curves are different ($P<0.01$ by ANOVAR), but no two points differ significantly (figure R 10). It should be noted that this experiment was carried out using hypnorm cocktail anaesthesia rather than inactin. This explains why the initial plasma glucose concentrations appear to be high, and may bring into doubt the validity of this result compared with the rest of the results.

L-cysteine, a compound with no known ACE inhibitory activity, is also a thiol-carrying compound. However, in contrast to the results obtained using epicaptopril, L-cysteine did not have any statistically significant effect upon glucose tolerance (figure R 11).

The effect of losartan on blood pressure and glucose tolerance.

Losartan is a recently developed non-peptide antagonist of angiotensin II type 1 receptors (Chiu *et al.*, 1990). It was assessed in the same way as the other drugs which interfere with the activity of the RAAS. The dose given (21.7μmol.Kg^{-1}, iv) had previously been shown to cause a 500-fold shift to the right of the AII pressor dose:response curve (figure R 12). In this experiment the dose of losartan given significantly worsened glucose tolerance ($P<0.01$ by ANOVAR), and the peak glucose concentration after treatment was significantly higher than in controls (figure R 13).

Losartan did not cause a significant reduction in any aspect of blood pressure. This was as a result of one animal responding to the drug with an increase in diastolic blood pressure of 25mmHg. The mean change for the other five animals was -59 ±13mmHg (range -102 to -25mmHg). If this rogue rat is removed, then the fall in diastolic blood pressure is statistically significant in losartan-treated animals (-59 ±13mmHg in treated rats (n=5) vs. -14 ±4mmHg in control rats (n=6), $P<0.01$).

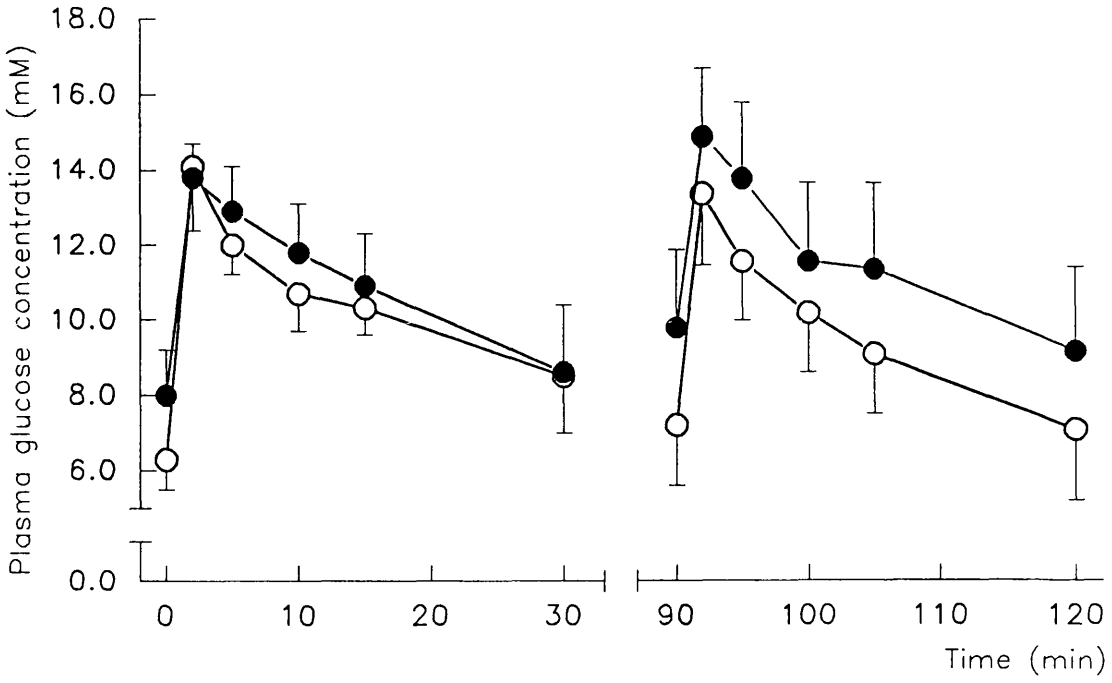

Figure R 10. Effect of epicaptopril on glucose tolerance in inactin-anaesthetised SHRs. Epicaptopril (2.3μmol.Kg^{-1}, iv) was administered at t=55minutes. Symbols are: O - control (n=5), or ● - epicaptopril (n=5). The post-treatment curves are statistically significantly different (P<0.01 by ANOVAR).

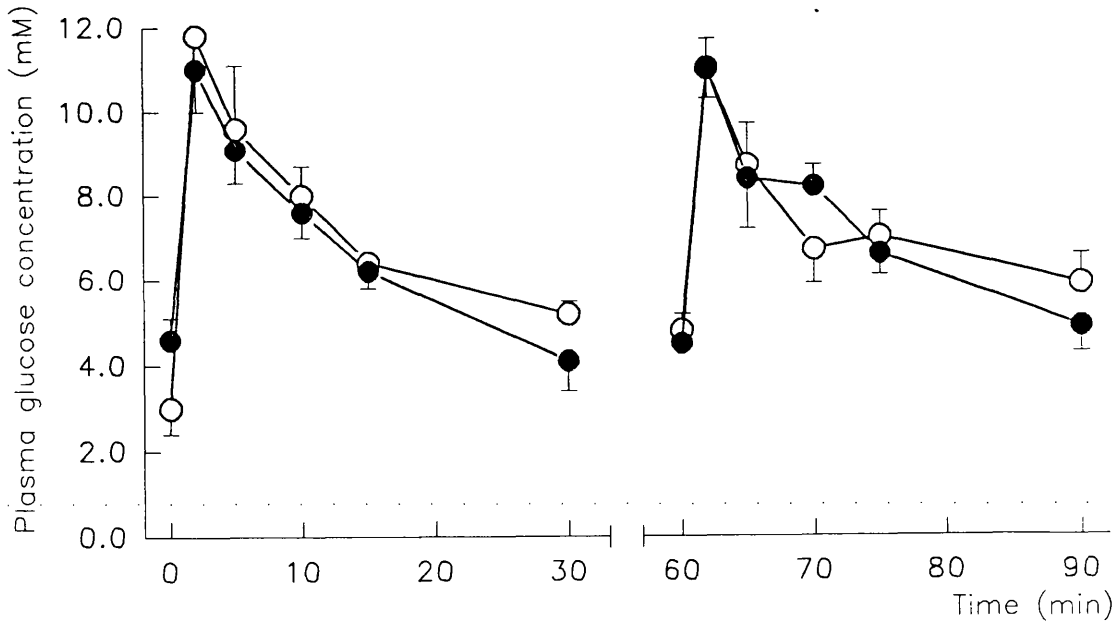

Figure R 11. Effect of L-cysteine on glucose tolerance in inactin-anaesthetised SHRs. L-cysteine (2.3μmol.Kg^{-1}, iv) was administered at t=32 minutes. Symbols are O - control (n=5), or ● - epicaptopril (n=5).

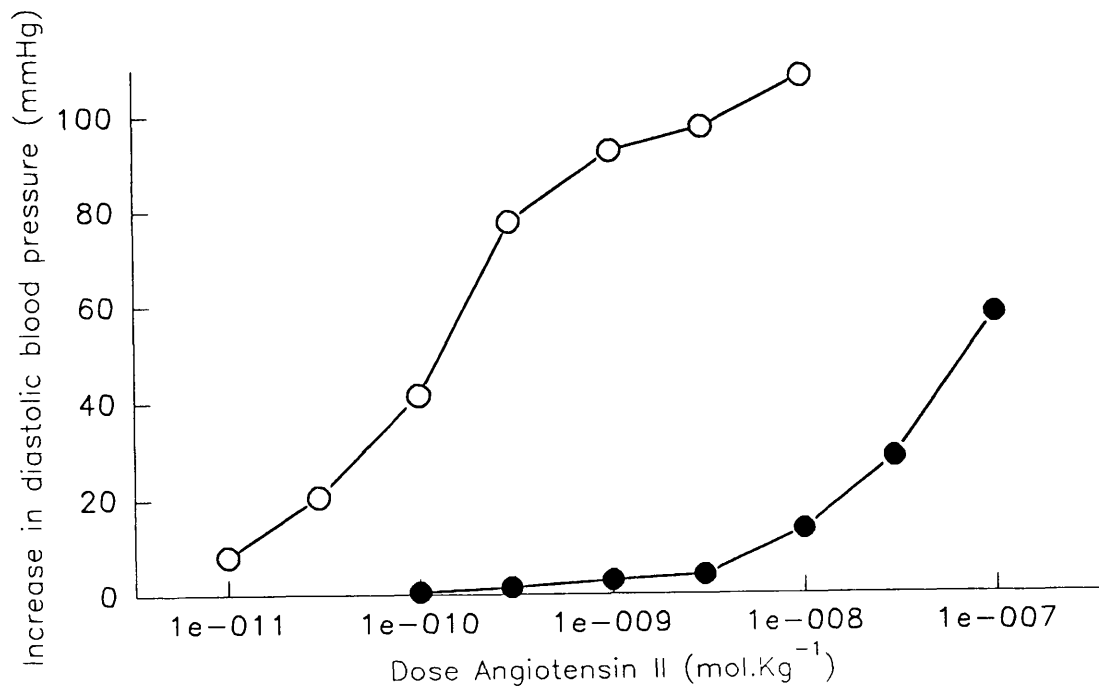

Figure R 12. Effect of losartan (21.7μmol.Kg^{-1}, iv) on pressor responses of diastolic blood pressure to angiotensin II (iv) in inactin-anaesthetised SHRs. Symbols are: O - control dose:response curve (n=2), ● - dose: response curve after treatment with losartan (n=2)

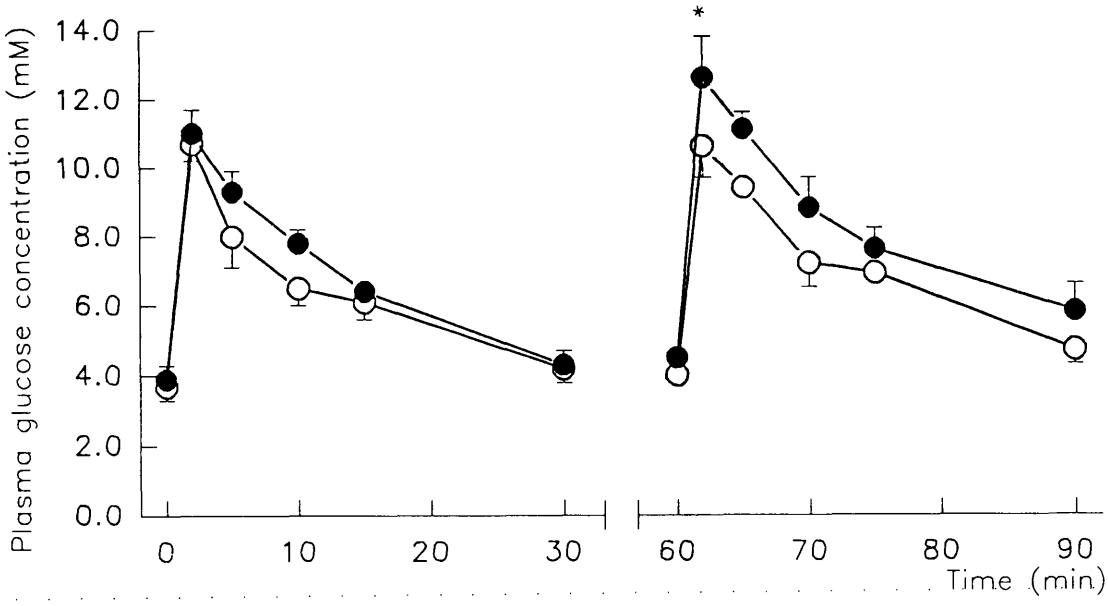

Figure R 13. Effect of losartan on glucose tolerance in inactin-anaesthetised SHRs. Losartan (21.7μmol.Kg^{-1}, iv) was administered at t=33 minutes. Symbols are: O - control (n=6), or ● - losartan (n=6). The post-treatment curves are statistically significantly different (P<0.01 by ANOVAR) * P<0.05, by ANOVAR and post hoc Studentised range test, compared with control at the same time point.

***In vitro* results: hemidiaphragms.**

Insulin concentration:effect curves.

Figure R 14 shows the effect of differing concentrations of insulin (10μU - 10mU (SHRs) or 100mU.ml^{-1} (Wistar rats)) on glucose uptake in both SHR and Wistar rat hemidiaphragms. Uptake was significantly stimulated at a concentration of 100μU. ml^{-1} in diaphragms from both strains of rat ($n=4$, $P<0.05$). There was no significant difference between the rat strains in the ability of insulin to stimulate uptake either, regarding total uptake after two hours, or ED_{50} (approximately 240μU.ml^{-1} in hemidiaphragms in either strain).

The effect of captopril on glucose uptake by Wistar rat and SHR hemidiaphragms.

Captopril was largely without effect on glucose uptake under either control or insulin-stimulated conditions.

In SHR hemidiaphragms the only effect of captopril was observed at a concentration of captopril of 10μM when hemidiaphragms were stimulated with 1mU.ml^{-1} insulin. In this case, glucose uptake was significantly reduced by captopril ($P<0.01$) (figure R 15, B). The other concentrations of the drug tested, at either high (1mU.ml^{-1}) or low (10μU.ml^{-1}) concentrations of insulin, were without effect (figure R15, A, C, D).

Likewise, captopril (10μM) had no effect on insulin-stimulated (10μU.ml^{-1} or 1mU.ml^{-1}) glucose uptake into Wistar rat hemidiaphragms (figure R 16 A, B).

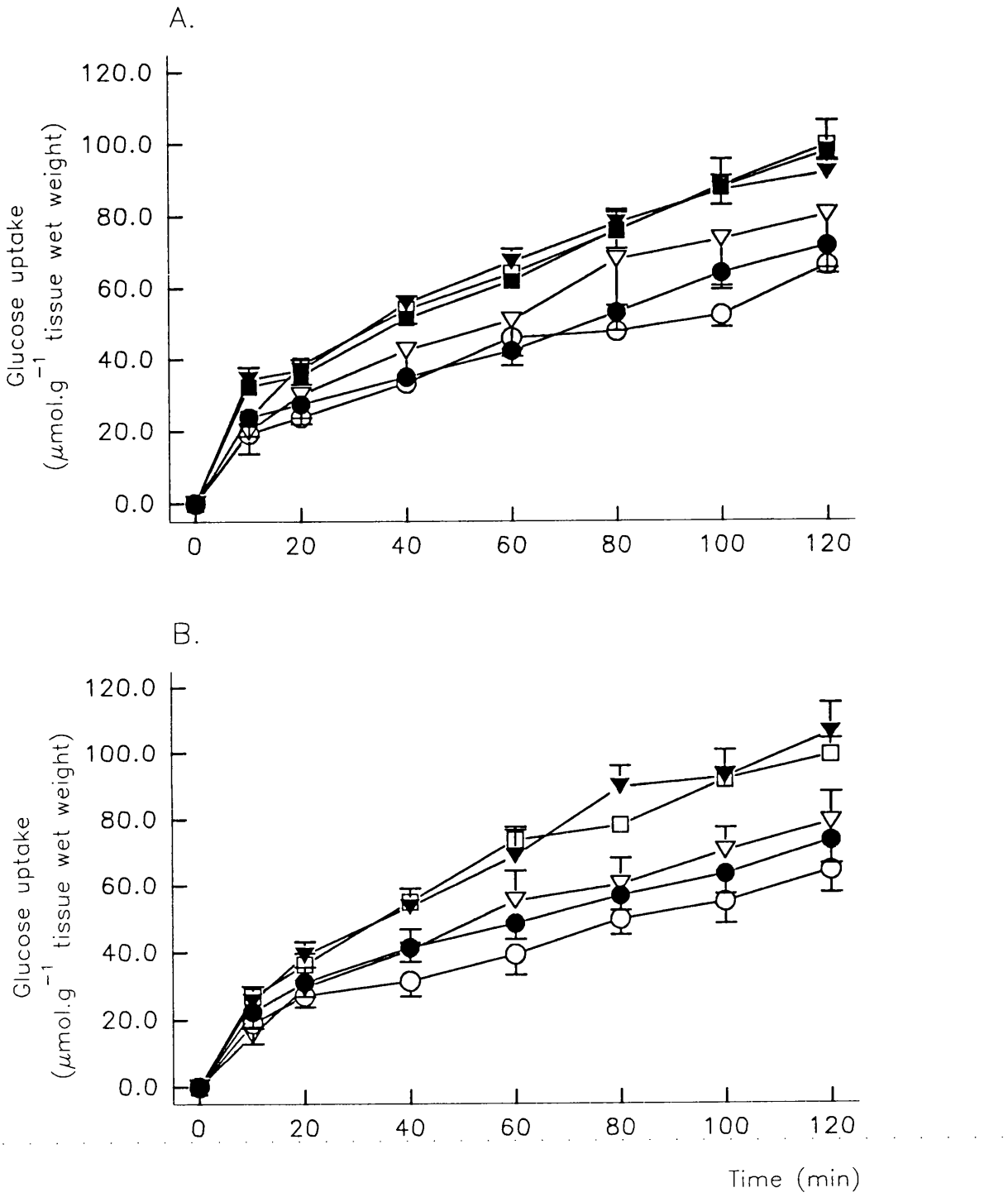

Figure R 14. Time course of insulin-stimulated glucose uptake in Wistar rat (panel A) and SHR (panel B) hemidiaphragms. Symbols are: ○ - no insulin (n=4); ● - 10μU.ml-1 insulin (n=4); ▽ - 100μU.ml-1 insulin (n=4), ▼ - 1mU.ml-1 insulin (Wistar rats n=4, SHRs n=6), □ - 10mU.ml-1 insulin (Wistar rats n=3, SHRs n=4), ■ - 100mU.ml-1 insulin (n=4, Wistar rats only) Significant stimulation of uptake occurred with 100μU.ml-1 insulin in both cases ($P<0.01$ by ANOVAR)

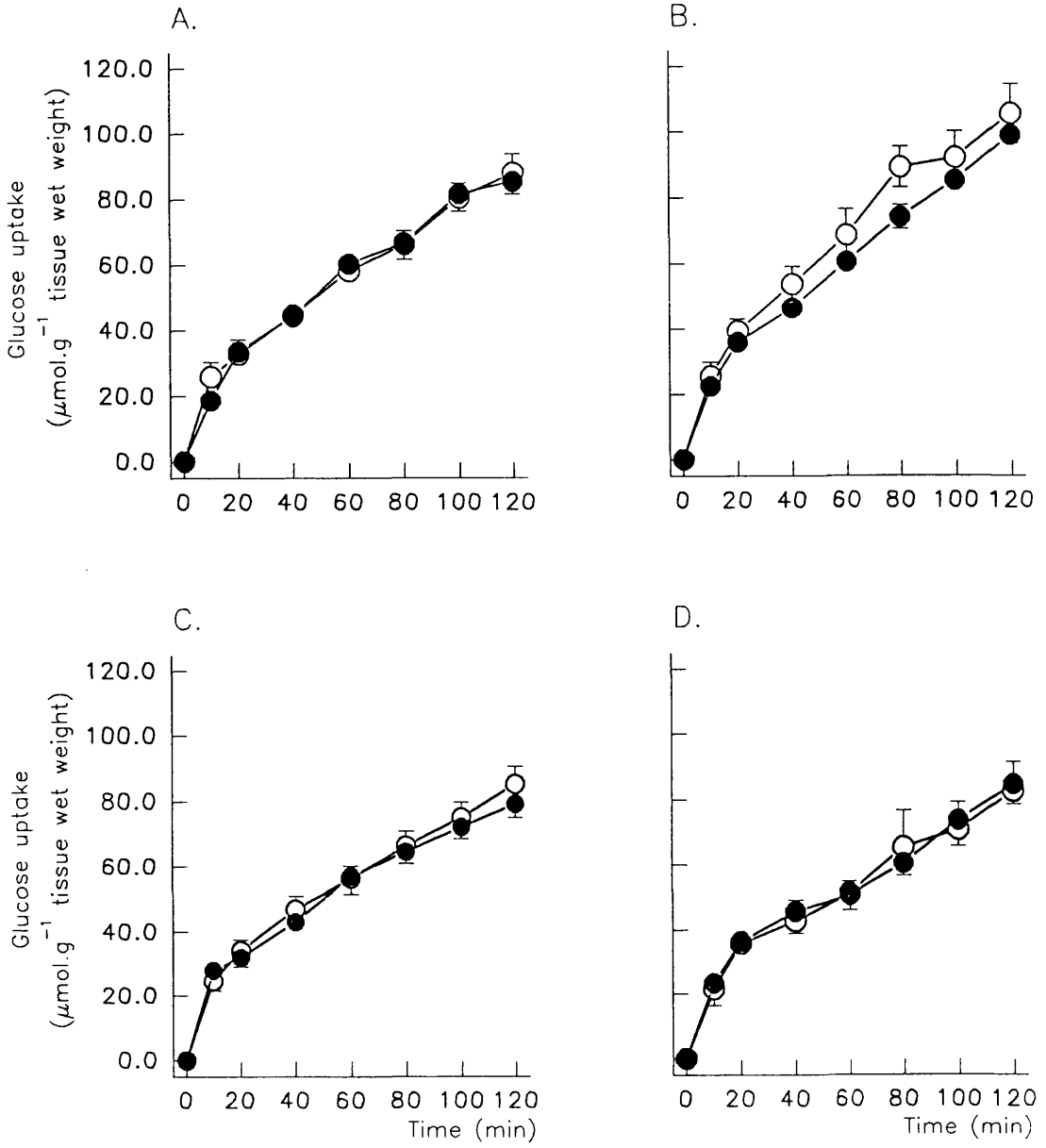

Figure R 15. Effect of captopril (1-30μM) on insulin-stimulated glucose uptake by SHR hemidiaphragms.

Graph	[Insulin]	[Captopril]
A	1mU.ml-1	1μM
B	1mU.ml-1	10μM
C	1mU.ml-1	30μM
D	10μU.ml-1	10μM

Symbols are: O - control (n=6), or ● - captopril (n=6). Glucose uptake was less in treated tissues than in controls in the experiment represented by graph B ($P<0.01$ by ANOVAR).

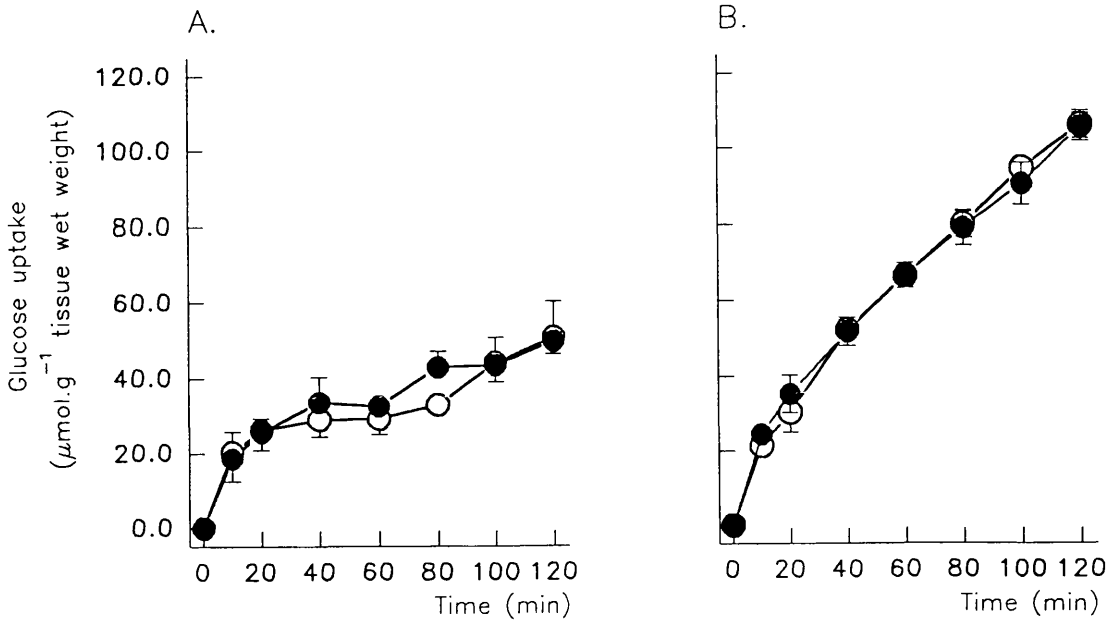

Figure R 16. Effect of captopril (10μM) on insulin-stimulated (10μU.ml-1 (A) or 1mUml-1 (B)) glucose uptake uptake in Wistar hemidiaphragms. Symbols are: O - control (n=6), or ● - captopril (n=6).

***In vivo* results.**

Investigations of associations between plasma glucose control and plasma potassium ion control.

These studies were carried out to determine whether the observations of Kim *et al.*, (1991), namely that aldosterone levels in SHRs are abnormally high, have any functional significance for control of plasma K^+ and glucose concentrations in the SHR.

The studies were undertaken in two parts. The first was the observation of plasma K^+ concentration during manipulation of glucose levels, and the second was the monitoring of glucose changes in response to attempts to manipulate K^+ concentration.

In inactin-anaesthetised rats the mean plasma K^+ concentrations were 3.08±0.16 (n=9) and 3.08±0.13mM (n=11) for Wistar rats and SHRs respectively.

Glucose administration (1.39mmol.Kg^{-1}, iv) did not have any statistically significant effect on plasma K^+ concentration (figure R 17). The ACE inhibitor lisinopril (275nmol.Kg^{-1}, iv) was unable to influence this lack of response to glucose administration (figure R 18). It must be noted, however, that when two IVGTTs were performed in the same rat (figure R 18), although no change in plasma K^+ concentration was observed during the IVGTTs, the basal plasma K^+ levels were significantly higher at the beginning of the second test than at the beginning of the first ($P<0.01$ in control group, $P<0.05$ in lisinopril group).

Chronic treatment of SHRs with an ACE inhibitor (captopril, 230μmol.Kg^{-1}.day^{-1} for 14 days) or saline appears to cause a response in plasma K^+ concentration different from that seen in naive animals. In both control (n=4) and treated animals (n=7) glucose tolerance tests had an effect on plasma K^+ concentration over time ($P<0.01$), this effect being a transient lowering of K^+ concentration. Captopril accentuated this lowering effect. After 10 minutes plasma K^+ concentration was not different from initial values in controls, whereas that in treated animals was significantly lower than in control animals (figure R 19).

The attempts to manipulate plasma K^+ concentration met with partial success. In animals given the Na^+/K^+ ATPase-stimulating drug aldosterone (1.39nmol.Kg^{-1}, iv), the plasma K^+ concentration gradually rose from a mean of 3.35mM to 5.1mM at t=140 minutes in the two strains of rat (figure R 20, A). This gradual upward trend in plasma K^+ concentration became a statistically significant increase at t=100 minutes and this level of significance remained or increased for the rest of the experiment. There was no significant difference in the response of the two strains to aldosterone

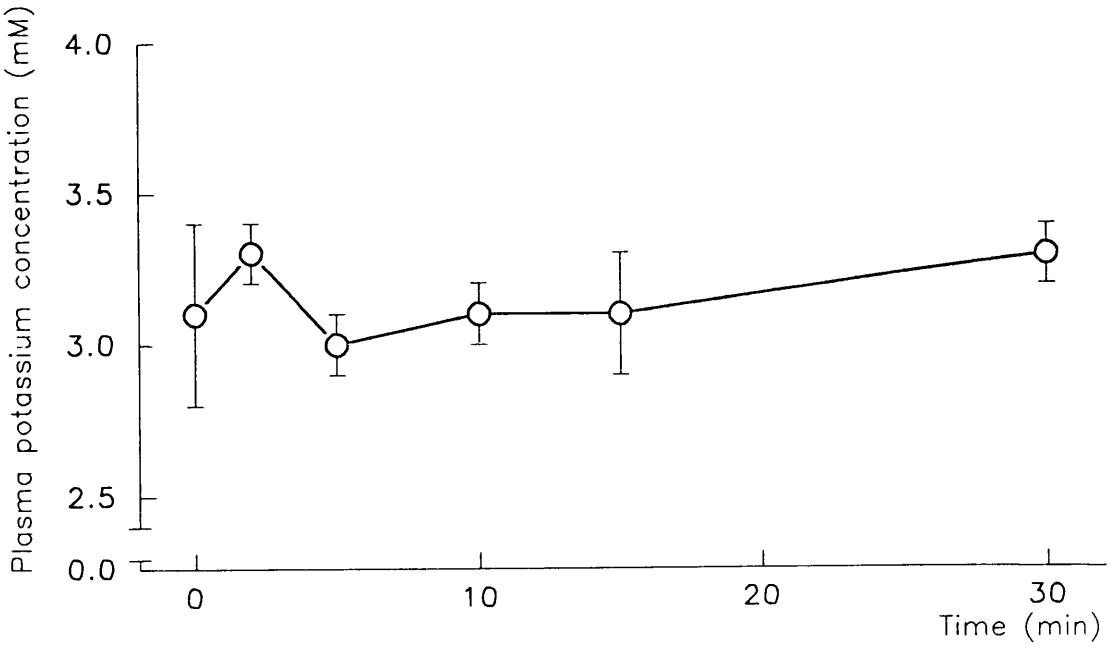

Figure R 17. Effect of glucose administration (1.39mol.Kg^{-1} iv, at t=0) on plasma K^+ concentration in inactin-anaesthetised SHRs (n=5)

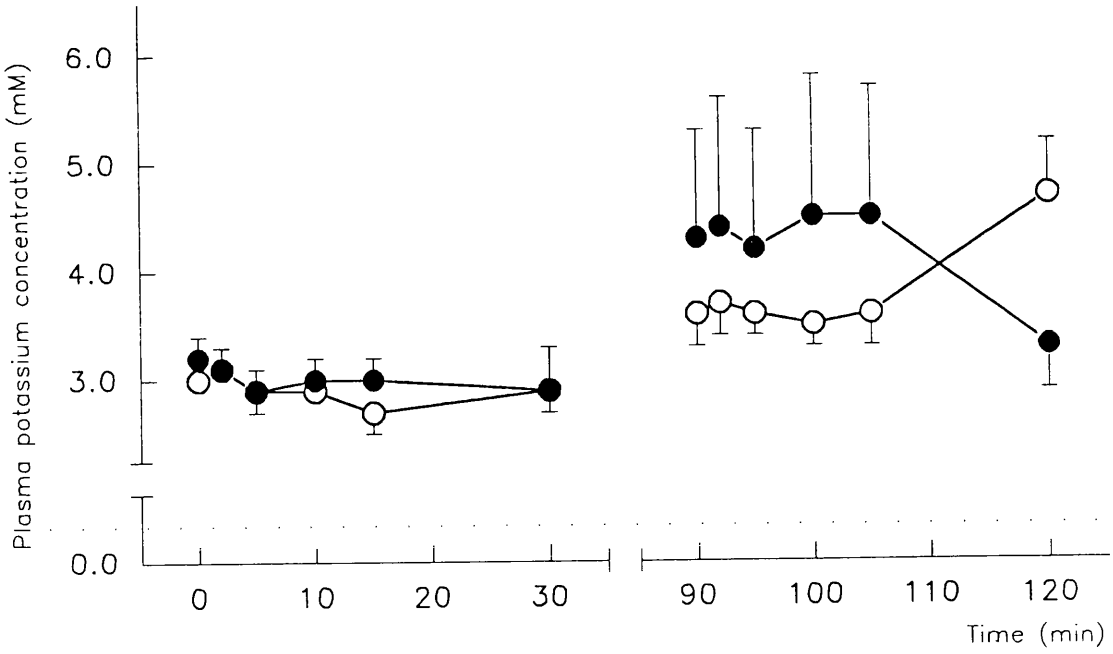

Figure R 18. Effect of lisinopril on plasma K^+ concentration during IVGTT in inactin-anaesthetised SHRs. Lisinopril (275nmol.Kg^{-1}, iv) was administered at t=33 minutes. Symbols are: O - control (n=6), or ● - lisinopril (n=5). * $P<0.05$, ** $P<0.01$ compared with t=0 in the same strain.

with respect to plasma K^+ concentration.

Plasma glucose concentration was significantly higher in the SHR group ($P<0.01$) than in the Wistar rat group over the course of the experiment. Aldosterone did not affect the plasma glucose concentration in SHRs, but did, after about an hour's lag, seem to reduce it in the Wistar group. This reduction did not reach statistical significance compared with initial plasma glucose concentration in the Wistar rats, but it did increase the frequency and level of significance of the difference between Wistar rats and SHRs (figure R 20, B).

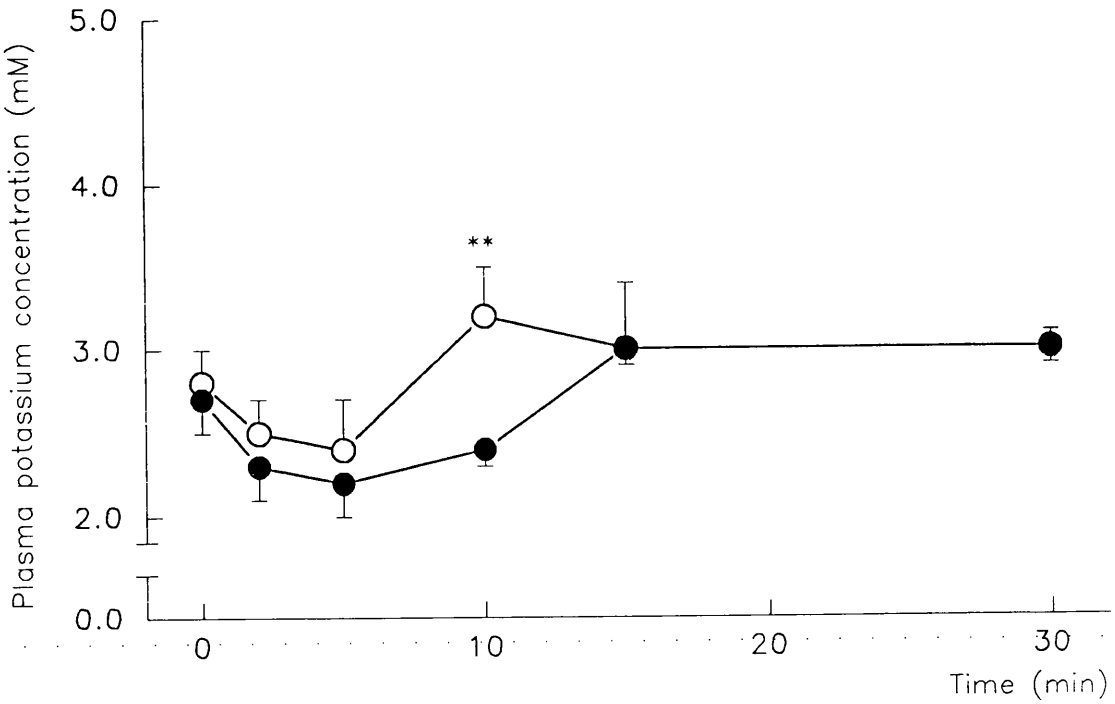

Figure R 19. Effect of chronic captopril treatment (230μmol.Kg^{-1}.day^{-1} for 14 days) on plasma K^+ concentration during IVGTT in inactin-anaesthetised SHRs. Symbols are: O - control (n=4), or ● - captopril (n=7). The curves are different, $P<0.01$ (by ANOVAR). ** $P<0.01$ by the post hoc Studentised range test.

The experiment was originally continued for another 40 minutes, but after about 2 hours 20 minutes the state of health of some of the animals was highly suspect. It should also be noted that, although the SHRs used in this experiment were of hypertensive blood stock (their parents were bought from Charles River Ltd.), their initial blood pressures were not statistically significantly higher than those of the Wistar rats (92 ±12mmHg diastolic in SHRs (n=4) compared with 93 ±14mmHg in Wistar rats (n=6)).

The opposite manipulation of plasma K^+ concentration, with the Na^+/K^+ ATPase antagonist canrenoate K^+, had a similar effect on plasma K^+ concentration as administration of aldosterone. In both strains K^+ concentration rose gradually throughout the experiment, this being from 2.9 ±0.2 to 3.7 ±0.3mM in Wistar rats, and 3.0 ±0.1 to 4.4 ±0.6mM in SHRs at t=180 minutes (figure R 21, A). The increase in plasma K^+ concentration, compared with t=0 in the same strain, became statistically significant at t=140 minutes in Wistar rats ($P<0.05$), and at t=120 minutes in SHRs ($P<0.05$). The final difference was statistically significant ($P<0.01$). The plasma K^+ concentration was statistically significantly higher in the SHR group ($P<0.01$ by ANOVAR).

ANOVAR indicated a significant difference between the two strains of rat ($P<0.01$) for plasma glucose concentration (figure R 21, B). ANOVAR also indicated an interaction of time with strain of rat, implying that the apparent transient increase in plasma glucose concentration in SHRs between t=20 and t=100 minutes is a real effect.

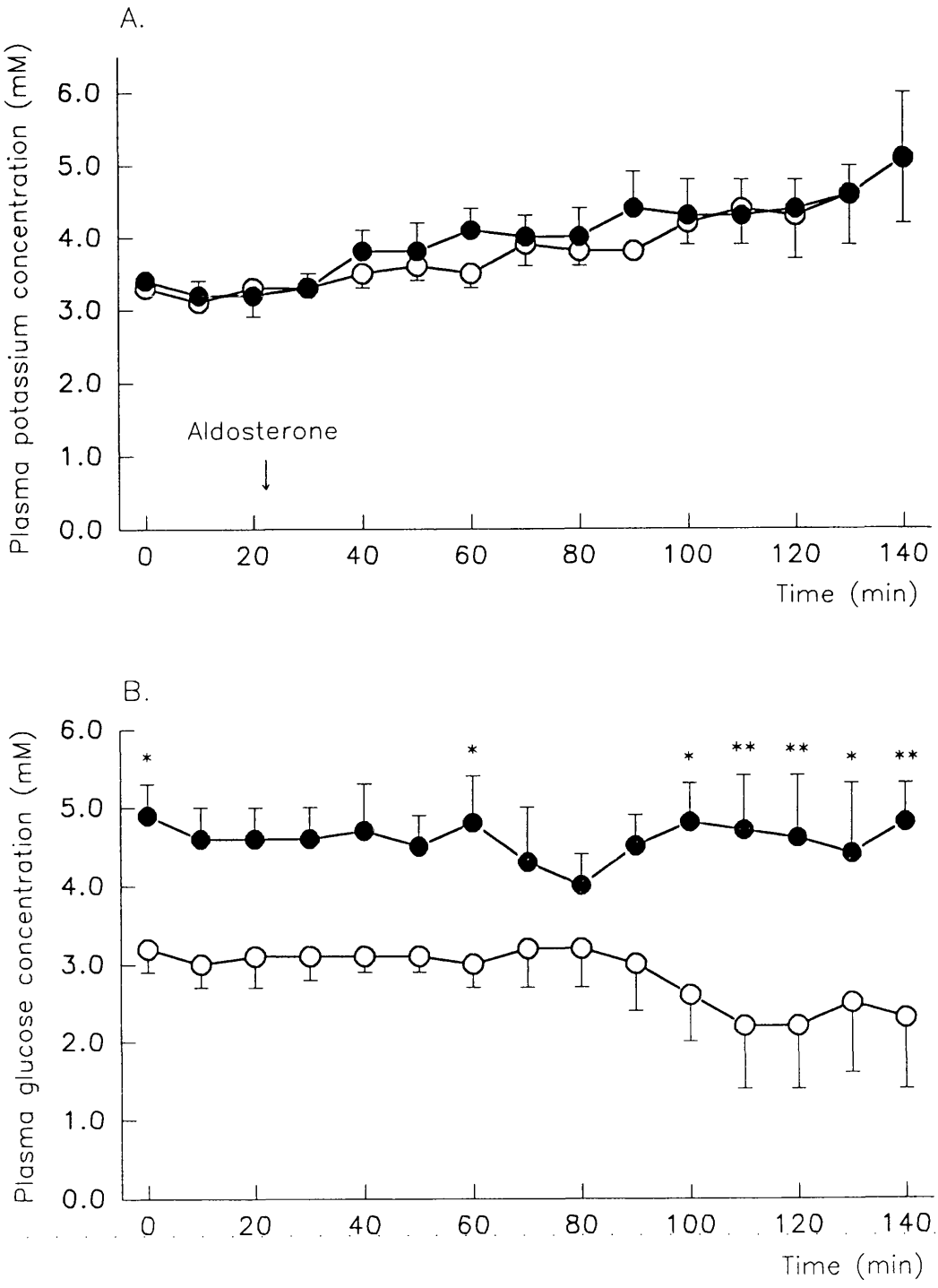

Figure R 20. Effect of aldosterone on plasma K^+ concentration (A), and plasma glucose concentration (B) in inactin-anaesthetised Wistar rats and SHRs. Aldosterone (1.39nmol.Kg^{-1}, iv) was administered at t=22 minutes. Symbols are: O - Wistar rats (n=4), or ● - SHRs (n=5) * $P<0.05$, ** $P<0.01$, by ANOVAR and post hoc Studentised range test, compared with Wistar rats

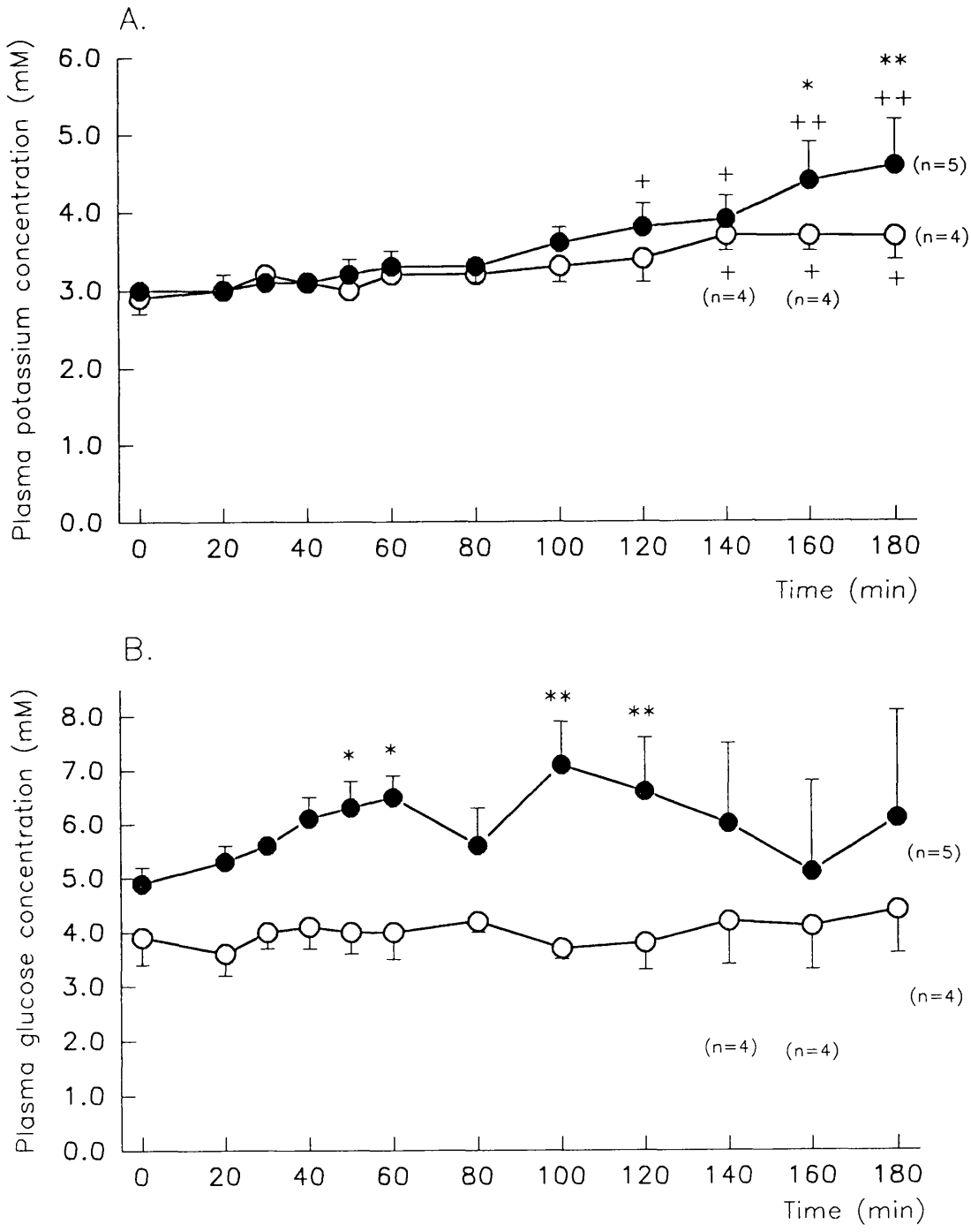

Figure R 21. Effect of canrenoate K^+ on plasma K^+ concentration (A), and plasma glucose concentration (B) in inactin-anaesthetised Wistar rats and SHRs. Canrenoate K^+ (75μmol.Kg^{-1}, iv) was administered at t=22 minutes. Symbols are: O - Wistar rats (n=6, except where indicated), or ● - SHRs (n=5, except where indicated). Plasma glucose concentration was different in the two strains ($P<0.01$, by ANOVAR). * $P<0.05$, ** $P<0.01$ by ANOVAR and post hoc Studentised range test, compared with Wistar rats + $P<0.05$, ++ $P<0.01$, by ANOVAR and post hoc Studentised range test, compared with t=0 in the same strain.

As in the previous experiment, the SHR group did not have a statistically higher initial diastolic blood pressure than the Wistar group (106 ±7mmHg (n=5) vs. 89 ±8mmHg (n=6), respectively). While blood pressure was significantly lower in both groups at t=181 minutes (the end of the experiment) than at t=19 minutes (immediately before the canrenoate K^+ administration) (87 ±7 (n=6) and 52 ±5 mmHg (n=5) in Wistar rats, respectively, and 90 ±7 (n=5) and 35 ±8mmHg (n=4) in SHRs, respectively) the falls in blood pressure in each strain were not statistically significant.

Discussion.

Discussion.

Summary.

In recent years the idea that insulin resistance may be a cause of, or at least be closely linked to, essential hypertension has become a popular topic for investigation. Much of the evidence to support this hypothesis has come from *in vivo* work with patients suffering from type 2 diabetes or obesity (e.g. Modan *et al.*, 1985) or from *in vitro* work with tissues from similar patients (Kashiwagi *et al.*, 1983). Some evidence has also come from studies of essential hypertensives (Ferrannini *et al.*, 1987). Likewise, animal work has been forthcoming which supports the human data both *in vivo* (Mondon and Reaven, 1988) and *in vitro* (Reaven *et al.*, 1989).

The work in this project stemmed from these findings, from the several reports that ACE inhibitors ameliorate insulin sensitivity in man (e.g. Pollare *et al.*, 1989), and from preliminary findings within the Pharmacology Group at the University of Bath showing that captopril also improved glucose tolerance in SHRs.

The approach, therefore, was to establish the SHR as a viable model of insulin resistance, to characterise its normal response in an IVGTT, and then to use it to test the efficacy of several ACE inhibitors in reducing glucose intolerance in the SHR. An attempt was then made to explain the actions of captopril. An *in vitro* assay was also set up to test the insulin responses of skeletal muscle under more rigidly defined conditions, and to test the effect of captopril on insulin-stimulated glucose uptake.

Having been unable to demonstrate substantial effects of the drugs tested, it was decided to look at the interplay between plasma glucose concentration and plasma K^+ concentration under various conditions since a number of papers, in particular that of Modan and colleagues (1985), have proposed that over-stimulation of Na^+/K^+ ATPase due to hyperinsulinaemia might be one route to essential hypertension. Two

drugs were therefore used to try to manipulate the plasma K^+ concentration and monitor plasma glucose concentration concurrently.

In vivo.

Blood Pressures.

As was stated previously, the blood pressure values depicted in figure R 1 were typical of the rats of each strain. The reason for the low blood pressures of the SHRs in the final two experiments (the assessments of aldosterone and canrenoate K^+ action) is unknown. The rats used in these experiments were the first offspring of a new colony of hypertensive stock which had just been bought in by the animal unit at the University of Bath. Their pedigree should therefore be assured, and there was no readily apparent reason for the low blood pressures recorded in these animals. The results of the last two experiments will therefore be discussed as if the SHR groups did have significantly higher blood pressures than their respective Wistar rat control groups, although it will be borne in mind that the results may not be as meaningful as they appear.

Anaesthetics.

The principal criteria upon which the choice of anaesthetic was based were the reliability of induction and maintenance of anaesthesia, which combine to determine the ease of use, and the effects of the anaesthetic on the system under investigation.

With these factors in mind both urethane and hypnorm cocktail were assessed. Although urethane was theoretically the easier to use (it should not need "topping up"), it failed to induce anaesthesia reliably, and, as can be seen in figure M 1, plasma

glucose concentration gradually tailed off during the experiment. Hypnorm cocktail, while also being unreliable in induction of anaesthesia, especially in SHRs, and requiring "topping up" throughout the experiment, did have the advantage of maintaining a relatively steady plasma glucose concentration. Consequently, hypnorm cocktail was chosen as the anaesthetic for the main studies. After a period of time, during which hypnorm cocktail appeared more and more to be losing efficacy, inactin became available. This proved to be a much better drug, on all counts, than hypnorm cocktail. It was not really known at the time how hypnorm cocktail might affect plasma glucose control, except that it did not appear to have a time dependent effect of its own (figure M 2). Hypnorm cocktail contains a component which is an α adrenoceptor antagonist (Kingsbury, PhD thesis, 1989), which tended to lower blood pressure, therefore making it very difficult to show that SHRs were actually hypertensive. Inactin, on the other hand, did not have this drawback. In a study by Hindlycke and Jansson (1992) on the suitability of various anaesthetics for glucose tolerance and pancreatic blood flow experiments, it was one of the two drugs recommended for such use because of its lack of effect on these two parameters. Consequently, inactin was used as the anaesthetic of choice from then on.

The reason for the difference between basal plasma glucose concentrations in hypnorm cocktail- and inactin-anaesthetised rats is unknown, but might relate to the depth of anaesthesia. An animal which is able intermittently to feel pain (as a result of partial recovery of consciousness) will probably respond to the stress by releasing hormones such as corticosteroids and catecholamines into its blood. The general effects of these are to prime the body for action, the classic "fight or flight" response, which include a major component of gluconeogenesis driven by glucocorticoid and adrenaline stimulation of peripheral tissues and growth hormone stimulation of the liver (Herd, 1984). This process could therefore account for the difference in plasma

glucose concentrations.

Evidence for insulin resistance in SHRs.

The result of the analysis of blood from freshly sacrificed rats suggests that plasma glucose concentration in SHRs is higher than in Wistar rats. When combined with the data comparing basal plasma glucose concentration in SHRs and Wistar rats over two hours (figure M 2), and the initial concentration in the experiment comparing glucose tolerance in the two strains (figure R 3, A), both of which show significant differences, it is clear that there is more glucose in the blood of SHRs under basal conditions. The difference between the analysis of blood from freshly killed rats and the plasma glucose levels obtained in anaesthetised rats is probably accounted for by the action of the anaesthetic which would depress plasma glucose concentration, and by the necessary handling of the rats immediately prior to killing them which would, to some degree, stress them.

Glucose tolerance in SHRs, however, is difficult to assess. Although administration of the glucose load caused a greater increase in plasma glucose concentration than in Wistar rats, the SHRs actually seemed *more* competent at removing the glucose than did the Wistar rats. Thus at the end of the glucose tolerance test (t=30 minutes) plasma glucose concentration was back to basal levels in SHRs, but still significantly higher than basal in Wistar rats. As a result, the area under the glucose extinction curve above the basal glucose value was similar in both types of rat. This may have been as a result of the insulin concentration in SHRs, which was significantly higher than in Wistar rats at t=2 ($P<0.05$), 5 and 10 minutes ($P<0.01$, figure R 3, B). The area under the insulin response curve was also greater in SHRs, but this was not statistically significant. It is therefore somewhat difficult to

draw firm conclusions about the insulin sensitivity of SHRs. The higher basal concentrations of plasma glucose in SHRs combined with normal basal plasma insulin could suggest insulin resistance, but could also result from one or more of several other mechanisms. The most important of these is the possibility that there is an excess of hormones which raise plasma glucose concentration. Glucagon is the most obvious candidate, and it is interesting that hyperglucagonaemia is present in type 2 diabetic patients (Lefèbvre, 1991). An overstimulation of glucagon-mediated processes (for example stimulation of glycogenolysis, gluconeogenesis and lipolysis, and inhibition of glycogen synthesis) could well account for the raised basal plasma glucose concentration seen in these studies. Adrenaline is another hormone which has glucagon-lke properties. In particular it is able to inhibit glycogen synthesis in skeletal muscle (Christopher *et al.*, 1992) and stimulate glucose production by the liver. Plasma catecholamine levles are generally increased in borderline essential hypertensives (Julius, 1991) and, like an excess of glucagon, this has the potential for explaining some or all of the raised basal plasma glucose concentration in the SHRs. Glucocorticoids are a third group with gluconeogenic properties. Other hormones, for example glucagon-like peptide 1 and cholecystokinin, are released from the gut during digestion and absorption of food, and these have the potential to raise plasma glucose indirectly by stimulating the secretion of glucagon (Dupre, 1991).

It cannot be said that the animals are glucose intolerant because the areas under the glucose extinction curves are similar. This seems to be as a result of hyperinsulinaemia during the glucose tolerance test compensating for the insulin resistance. The apparent extra sensitivity to insulin infusion in the SHRs confuses this scenario. However, assuming that the SHRs are insulin resistant, one might explain the phenomenon thus: since SHRs are frequently exposed to hyperinsulinaemia they may down-regulate their receptors more slowly than Wistar rats. The pancreas

normally releases insulin in small pulses which prevents insulin receptor down-regulation (Ward *et al.*, 1990). Therefore a prolonged infusion of insulin might be expected to have a greater effect, in terms of receptor down-regulation, in Wistar rats. If this is true then the results are more a product of the way in which the insulin was administered than of innate insulin sensitivity in the two strains of animal. Alternatively, one can hypothesise that because insulin has a longer half-life in SHRs than in normotensive rats (Mondon and Reaven, 1988), the plasma insulin concentration remained elevated for longer in the SHRs, and this would explain the apparently greater effect of the insulin infusion. This latter hypothesis seems the more credible, and therefore implies that the SHRs were not insulin resistant.

The results of the present studies support most of the recently published findings. Gaboury and colleagues (1991) demonstrated evidence that WKY rats are,in fact, more insulin resistant than SHRs during both IVGTTs and oral glucose tolerance tests. Likewise, both glucose disposal and glycogen synthesis were shown to be elevated in SHRs compared with WKY rats (Frontoni *et al.*, 1992). These reports imply that SHRs are not insulin resistant, a conclusion which is backed up by the present studies. The earlier data which seemed to support the idea that SHRs are insulin resistant (e.g. Yamori *et al.*, 1978; Mondon and Reaven, 1988) were gathered using less sophisticated techniques than the later evidence. The most recent report, however, which also used the insulin clamp technique, did find a very significant correlation of plasma insulin concentration with glucose disposal rate (Rao, 1993).

The SHR has long been regarded as a good model of hypertension, and probably the best available. Indeed, Trippodo and Frohlich (1981) stated that "several expert panels have reported that the SHR is an excellent model of experimental hypertension that could serve as a counterpart for clinical essential hypertension". The similarities between essential hypertension in man and hypertension in the SHR are

manifold. Both types of hypertension are influenced by two or three principle genetic loci, on top of which the environment also exerts an influence. In both man and the rat, blood pressure can be seen to be raised at an early age (Trippodo and Frohlich, 1981). Increased total peripheral resistance and cardiac output is a feature common to both species, as are hypertrophy of resistance vessels and the heart (Korner *et al.*, 1991) and the same vessels show a raised contractile response to a wide range of agonists (Mulvany *et al.*, 1978). Like the human situation (Trippodo and Frohlich, 1981), plasma catecholamines have been shown to be elevated in SHRs (Frontoni *et al.*, 1992). Renal function is also similar in the two species (Trippodo and Frohlich, 1981). For these and many other reasons, the SHR has been held in high regard as a model of hypertension for many years. However, in view of the present studies and those of other workers, it is probably not a good representation of the insulin resistance which is frequently found in human essential hypertensives.

Data obtained in experiments using patients with essential hypertension or type 2 diabetes tend to be more clear-cut. For example, there is usually a more defined (in terms of statistical significance) separation of glucose clearance curves in essential hypertensives and normotensives (Ferrannini *et al.*, 1987, Pollare *et al.*, 1989) compared with that between SHRs and Wistar rats.

Drug effects on blood pressure and glucose tolerance.

Captopril, enalaprilat and lisinopril.

The fact that captopril (2.3μmol.Kg^{-1}) failed to cause a significant reduction in diastolic blood pressure in SHRs was surprising, especially in view of the demonstration by Muller and colleagues (1990) that 280nmol.Kg^{-1} captopril (iv) caused an almost maximal increase in regional vascular conductance in conscious,

water-replete Brattleboro rats, and a ten-fold higher dose caused an almost maximal reduction in mean blood pressure in these same rats. 2.3μmol.Kg^{-1} captopril, assuming a blood volume of approximately 20ml in a 300g rat, would give a blood concentration of the drug of approximately 115μM, if it were administered intravenously. Many studies, besides that of Muller and co-workers (1990), have shown effective blockade of ACE, or secondary consequences of its blockade, at lower concentrations than this estimate. For example, captopril at 1μM and 10μM caused a significant increase in the spontaneous production of 6-keto-$PGF_{1\alpha}$ by Sprague-Dawley rat aortae *in vitro* (Hoffman *et al.*, 1990), and after an intravenous dose of 460nmol.Kg^{-1} captopril pressor responses to 1μg.Kg^{-1} AI (iv) were reduced to 32% of control (pre-captopril) values (Drummer and Kourtis, 1988). It remains unclear, therefore, why blood pressure was not reduced by the dose of captopril used in this study. One reason, under normal circumstances (normal being when captopril does reduce blood pressure), for the present finding is that the measurements were taken half an hour after captopril administration. The time course of blood pressure after captopril administration in the studies of Drummer and Kourtis (1988) shows that both systolic and diastolic blood pressure had nearly recovered to pre-captopril levels 24 minutes after the drug was given, having shown a transient reduction which peaked 30 seconds after injection. The rats used in the present experiments did not show this transient reduction in pressure at all. It may in the end be a futile exercise to attempt to explain why blood pressure did not fall. It has been noted that there is no correlation between the antihypertensive effect of ACE inhibitors and the ability of ACE inhibitors to inhibit the pressor effect of AI in rats (Sweet *et al.*, 1981). Therefore this result does not necessarily mean that ACE was not effectively inhibited by this dose of captopril. Indeed the previously quoted research (Drummer and Kourtis, 1988; Hoffman *et al.*, 1990; Muller *et al.*, 1990) strongly suggests that

2.3μmol.Kg^{-1} captopril is more than adequate to provide a very effective block of the enzyme. This is also borne out by the results obtained with lisinopril.

Lisinopril (275nmol.Kg^{-1}, iv) was shown to cause a 100-fold shift to the right of the AI pressor dose:response curve in SHRs (figure R 5). This activity is at the same order of concentration in the blood as that used by Hoffman and co-workers (1990) to cause a significant increase in the amount of 6-keto-$PGF_{1\alpha}$ spontaneously released by rat aorta *in vitro* (of the order of 10μM). However, like captopril, lisinopril failed to cause a significant reduction in blood pressure. This is further evidence in favour of the findings of Sweet and associates (1981), and strengthens the argument that a failure to reduce blood pressure probably has no meaning with respect to inhibition of ACE by the same drug.

In contrast with the foregoing results, enalaprilat (1.3μmol.Kg^{-1}, iv) did cause a significant reduction in systolic blood pressure, although the fall in diastolic blood pressure was not statistically significant. When a dose of 130nmol.Kg^{-1} (iv) was used in conscious, water-replete Brattleboro rats an almost maximal increase in regional blood flow and reduction in mean blood pressure was observed (Muller *et al.*, 1990). The ten-fold greater dose used here must, therefore, be at or very near to a maximally effective dose.

It is therefore apparent that acute administration of an ACE inhibitor has separate effects on blood pressure and ACE inhibition, with ACE inhibition occurring at doses which have no significant influence on blood pressure. Effective inhibition of ACE does not necessarily lead to a fall in blood pressure, and correspondingly, a failure of a drug to reduce blood pressure does not mean that ACE is uninhibited.

The results of the IVGTTs require careful consideration. Captopril significantly worsened glucose tolerance in SHRs (figure R 6), whereas enalaprilat and

lisinopril were without effect. The result with captopril was particularly surprising since several reports suggest that ACE inhibitors are able to improve glucose tolerance in man (Pollare *et al.*, 1989; Torlone *et al.*, 1991) and the most negative findings only suggest a lack of any activity of certain ACE inhibitors (captopril - Shionoiri *et al.*, 1987; ramipril - Küenberg *et al.*, 1990; lisinopril - Shionoiri *et al.*, 1990).

The varying data for captopril, enalaprilat and lisinopril work very well together in trying to explain the action of captopril. Since lisinopril inhibited ACE without affecting blood pressure, it seems that ACE inhibition *per se* is not responsible for worsening glucose tolerance. This is supported by the fact that captopril probably blocked ACE but also did not affect blood pressure. The lack of activity of enalaprilat on glucose tolerance, even though it reduced blood pressure (and presumably inhibited ACE), demonstrates that a reduction in blood pressure could not be the cause of captopril's activity either. This leaves two main possibilities for the mode of action of captopril. The first is that its action on glucose tolerance is not ACE-related and may be caused by the presence of the thiol group on the molecule. This will be discussed in the next section. The second possibility is that captopril's properties are related to an ability to inhibit a specific isomer of ACE which is not susceptible to enalaprilat or lisinopril. A return to this topic will be made later.

It is possibly unrealistic, however, to expect an acute dose of an ACE inhibitor to have much effect on glucose metabolism because the vast majority of published work has compared indices of glucose and insulin metabolism in chronically treated patients (Shionoiri *et al.*, 1987, 1990; Pollare *et al.*, 1989; Torlone *et al.*, 1991). Therefore captopril was given to a group of SHRs for two weeks. In this experiment diastolic blood pressure was significantly lower in the captopril-treated group after two weeks, but even so, glucose tolerance was unchanged.

The fact that blood pressure was reduced by chronic administration of captopril demonstrates that there was sufficient time for the treatment to affect a chronic condition, and implies that if captopril were to affect glucose tolerance it had adequate opportunity to do so. It cannot be ruled out, though, that the period of treatment was not long enough. One can imagine, for example, that changes in blood pressure and glucose tolerance might occur at different rates. Nevertheless, experiments investigating the chronic effects of ACE inhibitors on blood pressure have shown that four weeks' treatment ($3mg.Kg^{-1}.day^{-1}$ perindopril) in rats at a similar age to the ones in these experiments (6-8 weeks old) reduces blood pressure long term (Harrapp *et al.*, 1990). This also suggests that one ought to see an effect, if one is going to occur, at two weeks, even if it is not permanent at that stage.

These results challenge those published by other authors. Although some reports mention a lack of effect of ACE inhibitors (e.g. Shionoiri *et al.*, 1987, Küenburg *et al.*, 1990), others have shown an improvement in insulin resistance (Pollare *et al.*, 1989, Torlone *et al.*, 1991). The finding that, if anything, captopril worsens glucose tolerance in SHRs appears to be a new one, and it challenges the establishment. In the long term, however, a more clinically relevant situation, captopril had no effect, in accord with some of the published observations.

Epicaptopril and L-cysteine.

Although in a clinically important situation captopril does not seem to have any effect on glucose tolerance, it was decided to continue with this series of studies because it was possible that captopril might be having two opposing actions which result in no overall activity. In addition, an attempt needed to be made to explain why captopril worsened glucose tolerance in the acute phase. With this in mind, and results

from preliminary experiments which hinted at the involvement of captopril's thiol moiety, it was decided to investigate the possible role of this substituent in captopril's actions.

Epicaptopril was therefore chosen, being the 100-fold less active enantiomer of captopril, and given at the same dose as captopril to ensure an equivalent dose of thiol. Like captopril, epicaptopril significantly worsened glucose tolerance, indeed implying a role for the thiol group. It is not, however, totally without ACE inhibitory activity, and in order to avoid this possible confounding factor, L-cysteine was also tested at the same dose. In contrast to epicaptopril, L-cysteine did not affect glucose tolerance. This brings up the intriguing possibility that the active component of captopril (with respect to glucose tolerance) may not be the thiol group, but rather the epicaptopril which is also present (captopril is a racemic mixture of 50% active captopril and 50% epicaptopril). If epicaptopril were the active constituent, then it might be working in one of two ways. (Since L-cysteine did not affect glucose tolerance, the possibility of the involvement of the thiol group seems to have been ruled out.) These are that epicaptopril could have a specific activity unrelated to ACE inhibition or AII, or that it could be acting as an ACE inhibitor (even though it is 100-fold less potent than its active stereoisomer) at a site which is not susceptible to enalaprilat or lisinopril.

Both of these drugs (epicaptopril and L-cysteine) did not significantly affect any blood pressure parameter which leads to the conclusion that the thiol group on captopril in no way affects blood pressure. This, combined with the following section, further suggests that the thiol group on captopril does not have any activity relevant to the current investigations. It must be remembered, however, that the assessment of the efficacy of epicaptopril was undertaken using hypnorm cocktail anaesthesia. Since this as been shown to contain an element which is an α adrenoceptor antagonist (Kingsbury, PhD thesis, 1989) one cannot be certain that it will not affect glucose

tolerance. α agonists tend to inhibit the release of insulin. Therefore one would expect that an antagonist would have the opposite action. Assuming that this is the case, the predicted result of the experiment would be an improvement in glucose tolerance because the longer exposure of the animals to the anaesthetic by the start of the second IVGTT ought to induce a higher plasma insulin concentration during the second IVGTT. This implies that the epicaptopril effect on glucose tolerance might have been larger with a different anaesthetic. If, on the other hand, the argument is taken a step further, then the higher plasma insulin concentration may have caused insulin receptor down-regulation by the start of the second IVGTT and actually, therefore, worsened glucose tolerance. The fact that worsened glucose tolerance is the observed result hints that this extension of the hypothesis might be valid. However, it is probably much safer to accept the result at face value but, as a result of these doubts, accord them rather less weight when considering the results as a whole. Indeed it seems unlikely that epicaptopril is acting as an ACE inhibitor given the large difference in activity between it and active captopril, and considering the general lack of specificity of ACE inhibitors in general (Vago *et al.*, 1992). This leaves the possibility that this drug has a specific activity of its own, which seems less likely than it being a potent ACE inhibitor. By a process of elimination, then, it is probable that the result with epicaptopril is a spurious one and should be ignored.

Losartan.

The AII type 1 receptor antagonist losartan was assayed in similar fashion to the ACE inhibitors to provide further evidence of the role of AII in regulating glucose tolerance. The dose used had previously been tested against the AII pressor response to ensure it provided adequate blockade of AII receptors (figure R 12). Interestingly,

although previous results had suggested that any action of captopril was probably not due to ACE inhibition, losartan had a similar effect to captopril - i.e. it significantly worsened glucose tolerance (figure R 13). This suggests that AII actually promotes glucose tolerance in SHRs.

Perhaps the most likely explanation of the apparently conflicting data lies in different isoforms of ACE in different regions of the body. That these exist is a safe assumption. A study of [^{3}H]trandolaprilat binding by Brée and colleagues (1992) showed that at least two different forms of ACE exist in Wistar rats - one with two active sites, to be found in the brain and lung, and one with only one active site, found in the testis. A comprehensive study by Vago and associates (1992) compared binding and potency characteristics of thirteen ACE inhibitors in lung and heart samples from man and rats. It was notable that some ACE inhibitors did not show any selectivity for ACE from a particular sample (lung or heart), but others did. In the former group one finds captopril and quinapril, while in the latter one sees enalapril which preferentially bound to the lung isoform. Therefore one might expect certain ACE inhibitors to be more potent in certain regions of the body than others. Bearing this in mind, it can be hypothesised that captopril is able to inhibit an ACE at a site in the body which is not susceptible to enalaprilat and lisinopril. If losartan also has access to this system, that might explain the apparent anomaly. Thus captopril and losartan could inhibit the production or action, respectively, of AII, which would normally promote glucose uptake, at a local site, while lisinopril and enalaprilat are inactive. This conclusion also relies upon the worsening of glucose tolerance after epicaptopril treatment being a chance result.

In vitro.

Insulin concentration:effect curves.

The reasons for choosing the diaphragm as an assay tissue were largely a result of practicality, but also because it has been used in a similar fashion to the present studies by others (Kirby and Turner, 1975; Hothersall *et al.*, 1990). The muscle is easy to get to and dissect out without greatly damaging the muscle fibres (Hothersall *et al.*, 1990), it is thin and easily divided into two, thereby overcoming to a large extent the problems of diffusion and availability of control tissues, and it is a good example of red (slow oxidative) muscle. Red muscle has been shown by several studies to be the most insulin sensitive of the three types of skeletal muscle in the body (Kraegen *et al.*, 1985, James *et al.*, 1985). Therefore one would expect to see good insulin-stimulated glucose uptake in diaphragm, and if insulin resistance is an intrinsic property of muscle, then it ought to occur in red muscle since white (fast glycolytic) muscle probably does not have the capacity to reduce its uptake enough to account for the reduction in whole body glucose uptake which is seen in insulin resistance. Alternative tissues have been used by other investigators. For example, Stace and colleagues (1990) used soleus muscle of the rat. The disadvantage of using this and other large muscles is the problem of diffusion of substances into and out of the tissue and the damage done to the muscle in removing thin strips (to avoid diffusion problems) which is an inevitable consequence.

The insulin concentration:effect relationship in diaphragms from the two strains of rat was not significantly different. Both the EC_{50} and cumulative glucose uptake after two hours are similar in the two strains, suggesting, assuming that the Wistar rats are "normal", that there is no evidence from this experiment of insulin resistance in SHRs. The data presented in this experiment are in reasonable

accordance with published results, which state an ED_{50} *in vivo* of approximately $150\mu U.ml^{-1}$ in diaphragm of Wistar rats, (Kraegen *et al.*, 1985). One would expect to need a higher concentration for the same effect *in vitro* due to the problems of diffusion. Therefore an EC_{50} of approximately $240\mu U.ml^{-1}$ seems to indicate a viable assay set-up. Total stimulation of uptake is a little different, the maximum reported to be a ten-fold increase *in vivo* (Kraegen *et al.*, 1985) In the present study a two-fold increase was achieved. However, this is similar to the 50% increase of glucose uptake stimulated by $0.5mU.ml^{-1}$ insulin reported by Hothersall and colleagues (1990). Once again, in spite of the thinness of the muscle, this was due to diffusion problems, of glucose into the muscle in this instance.

The lack of any insulin resistance in isolated diaphragms brings to the fore an important point. Assuming that the results of the preliminary *in vivo* work do indicate insulin resistance in SHRs, it appears that insulin resistance is not purely an intrinsic property of skeletal muscle. Two explanations spring to mind. The first has been proposed in the literature (Lillioja *et al.*, 1987). These workers found that glucose intolerance correlated with both capillary density and fibre type in skeletal muscle in man. The less glucose-tolerant a patient was, the greater was the proportion of white (fast glycolytic) muscle in a biopsy of vastus lateralis, and the fewer the capillaries per unit of cross sectional area of muscle. Therefore the proportion of white muscle in SHRs may be higher than in Wistar rats, but the difference may not be great enough to show in such a small tissue as the rat diaphragm. Additionally, if the capillary density were lower in the SHR diaphragm this would be negated by the fact that in the *in vitro* situation all the components of the Krebs' solution diffuse in across the large flat surface of the tissue, thus putting muscle from the two strains of rat on an equal footing. The second explanation is more tenuous and has little published data to back it up directly. It is possible that some nervous or humoral factor makes the muscle

insulin resistant. What that factor might be is open to debate. A humoral substance is probably the favourite since studies like those of Bachmann and co-workers (1991) have shown the transmission of hypertension from one organism to another. Endogenous digitalis-like substance (EDLS) is a candidate (although it was ruled out by Bachmann and colleagues (1991) because their study showed no effect on Na^+/K^+ ATPase). This has been shown to be present in raised concentrations in SHRs (Hamlyn and Manunta, 1992), but it is not clear how it might affect glucose uptake. Amylin, as discussed in the introduction, is another candidate for such a factor. Since this has also been shown to be present in increased quantities in insulin resistant patients (Eriksson *et al.*, 1992) it also warrants further investigation.

The effect of captopril on glucose uptake by Wistar rat and SHR hemidiaphragms.

Whether the difference between the *in vivo* and *in vitro* situations is primarily caused by physical (due to capillary density or muscle composition) or humoral factors, captopril is unlikely to be an important modulator of glucose uptake.

It was only in one experiment that captopril had any influence on glucose uptake by the hemidiaphragm, this being when uptake was stimulated with $1mU.ml^{-1}$ insulin in the presence of 10μM captopril. As was demonstrated *in vivo*, rather than improving glucose uptake, captopril worsened it. This was a little surprising since this concentration of the drug was bracketed, and neither the higher nor the lower concentration was able to modify insulin's activity. If one assumes that this was not a spurious result, the most prudent explanation is that captopril has two actions in this tissue. The first action is to inhibit glucose uptake at a lower concentration than that which is needed for its second, uptake promoting activity. The only example of a dual

action of ACE inhibitors is the differential blocking of two different forms of ACE which have different affinities for drugs (Vago *et al.*, 1992). Perhaps a higher concentration (e.g. 100μM) of captopril would have resolved the problem by revealing an actual improvement in glucose uptake. Although this would only have been of academic interest (such concentrations are too high to be used therapeutically) it might have provided an insight into a target for a future generation of drugs.

One point which it is important to draw from these results is that while captopril may have some activity, depending on the concentration used, the SHR tissues are not insulin resistant in their own right. One must ask the question, therefore, whether captopril can actually treat the root cause of insulin resistance *in vivo*. Clearly the results from these studies suggest not, and where other researchers have found captopril to be effective in increasing insulin-stimulated glucose uptake (Pollare *et al.*, 1989), the current data imply that this is not an action upon the fundamental dysfunction in insulin resistance.

The fact that captopril had no effect on insulin-stimulated glucose uptake in all but one of the situations tested reinforces the *in vivo* data from chronically treated rats. Indeed, overall these results complement the *in vivo* data quite well by suggesting that captopril may worsen glucose uptake under some circumstances but that it is generally inactive.

In vivo.

Investigations of the interaction between plasma glucose and potassium concentrations.

The rationale for examining the possible links between plasma glucose and K^+ concentrations is threefold. Insulin is known to stimulate the Na^+/K^+ ATPase (Hundal

et al., 1992) and although it is not clear how alteration of Na^+/K^+ ATPase activity might affect glucose tolerance, it could have an effect on blood pressure. It is also known that aldosterone levels are substantially raised in SHRs (approximately four-fold) compared with Wistar rats, which has obvious implications for plasma K^+ control (Kim *et al.,* 1991). Finally, it is well documented that K^+ supplementation of the diet of both rats and man is able to reduce blood pressure (Cappuccio and MacGregor, 1991). It seems unlikely, therefore, that K^+ would not be involved in a complete explanation of the pathology of hypertension.

The first evidence from these studies, however, was not promising. The basal plasma K^+ concentrations were identical in SHRs and Wistar rats. This showed that the "set point" for K^+ concentration was similar in each strain.

The measurement of plasma K^+ concentration during testing of the effect of lisinopril on glucose tolerance revealed that this parameter increased after an IVGTT, even though it does not change during it. The reason for this is unknown. Moreover, one cannot draw the conclusion that the rise in K^+ concentration is different in lisinopril-treated animals compared with controls since the spread of values is so great in the treated group. It must be concluded, then, that acutely raising plasma concentrations of insulin and glucose had no immediate effect on K^+ dynamics. In addition, the rise in K^+ between the two IVGTTs is not explicable in conventional terms (the stimulation of Na^+/K^+ ATPase by insulin), since insulin would be expected to have the opposite effect from that which was seen.

While acute administration of glucose may have no effect on K^+ control, when animals were treated chronically with either captopril or saline, an effect appears to have been revealed. A transient lowering of plasma K^+ concentration occurred in response to glucose administration, which conforms to conventional explanations of

insulin action. Once again, why this should occur in animals chronically treated with saline, but not in previously untreated animals is not readily apparent. One must consider that the possibility that the process of injecting the animals twice a day for two weeks is in itself sufficient to bring about this change. Assuming this to be the case, the most likely explanation of the effect is that the regular release of stress hormones caused by the process of injection made the animals more sensitive to the Na^+/K^+ ATPase-stimulating abilities of insulin.

Treatment of SHRs with captopril for two weeks did produce the first evidence of these studies that the drug might be able to improve insulin sensitivity. In captopril-treated animals, the reduction in plasma K^+ lasted longer than in saline-treated subjects, thus implying that insulin was having a more prolonged effect than in controls. This could be explained in terms of a greater release of insulin from the pancreas, but to date all of the explanations in the literature have rationalised captopril's activity in terms of end organ efficacy (e.g. Pollare *et al.*, 1989). The lack of effect of chronic captopril treatment on glucose tolerance suggests that the link between K^+ and glucose control, if it exists, is not a strong one. However, the possibility of a link, however weak, merited further investigation, and the second part of this study was carried out.

The dose of aldosterone used was extracted from the literature. Kim and colleagues (1991) quoted a plasma value of 441pg.ml^{-1} in Wistar rats, and 490pg.ml^{-1} in SHRs, while others have reported a value of 11.3pg.ml^{-1} in Wistar rats (Ando *et al.*, 1991). The dose chosen, 1.39nmol.Kg^{-1}, assuming a blood volume of approximately 20ml, would therefore be expected to raise plasma concentration of the mineralocorticoid by between 15- and 70-fold in the first instance. This dose was able to increase plasma K^+ concentration in both strains of rat, although there was no difference between them. It may have been that the system through which aldosterone

exerts its effect on plasma K^+ was less sensitive than normal in the SHR, but that this was overcome by the large dose of aldosterone given. With more time, a dose response curve could have been constructed for each strain, and this question would have been resolved. Extrapolating from the results in the previous experiments, however, it seems highly likely that no major differences would be seen.

Aldosterone is normally thought of as a compound which promotes Na^+ absorption and K^+ excretion in the kidney (Zannad, 1991). The results obtained in this study (the increase of plasma K^+ concentration after aldosterone administration) do not reflect such an action. The change in K^+ levels is probably due to a reduction of cellular K^+ uptake by skeletal muscle, as seen *in vitro* by Adler (1970).

The administration of aldosterone provided the first evidence of a connection between plasma K^+ and glucose concentrations, and that there might be a differential response in the two strains of rat. For while plasma glucose concentration in SHRs seemed insensitive to aldosterone (Figure R 20, B) this was not the case for Wistar rats, where it decreased. There was a lag before the reduction occurred, which tempts one of two suggestions. Either aldosterone had a direct, but delayed, effect on glucose handling, or the change in plasma K^+ concentration, which its administration brought about, at some point reached a threshold which then affected glucose metabolism. It is not possible to say which of these two possibilities is more likely from the data presented here. The difference between the two strains can be explained in terms of different thresholds for aldosterone or K^+ action. It is not surprising that the SHR should be less sensitive to aldosterone than Wistar rats since the concentration of aldosterone increases quite dramatically over time in SHRs, whereas it stays relatively constant in Wistar rats (from 441pg.ml^{-1} at five weeks old to 317pg.ml^{-1} at 25 weeks old in Wistar rats, and from 490pg.ml^{-1} to 1351pg.ml^{-1} in SHRs (Kim *et al.*, 1991)). One would expect such high concentrations of aldosterone

in SHRs to occur as a result of resistance to its actions, or to create a resistance by down-regulation of receptors or by some other means.

The opposite manipulation, the inhibition of Na^+/K^+ ATPase as opposed to its stimulation, was attempted in order to cover the possibility that the basal concentration of aldosterone might have been stimulating Na^+/K^+ ATPase at a maximal rate in the SHRs, thereby explaining why exogenous aldosterone had no effect. Canrenoate K^+ had the same general effect on plasma K^+ concentration as aldosterone (i.e. plasma K^+ concentration gradually increased). This similarity of effect for oppositely acting drugs was surprising, and tends to suggest that the two drugs were acting at different sites in the body. Since canrenoate K^+ is very closely related to the diuretic spironolactone (it is the primary active metabolite of spironolactone) its main action is in the kidney. Inhibition of Na^+ resorption in the distal tubule of the nephron could, as was observed, gradually raise plasma K^+ concentration by preventing the concomitant kaliuresis. Any action it might have at extra-renal sites would also tend to inhibit the extraction of K^+ from the plasma and therefore promote a gradual accumulation of K^+ in the blood. Unlike aldosterone, however, canrenoate K^+ caused the plasma K^+ concentration to be greater in the SHR group than in the Wistar rat group. This implies that the SHR has a constitutively more active Na^+/K^+ ATPase than its Wistar rat counterpart, and the inhibition of this by canrenoate K^+ had a correspondingly larger effect on plasma K^+ concentration than in Wistar rats.

The plasma glucose concentrations were also different. The initial concentrations were as expected, with that in the SHRs being significantly higher than in the Wistar rats. In this experiment it was the Wistar rats which were unaffected by the treatment, and the SHRs which responded to canrenoate K^+ by increasing the plasma glucose concentration. The result of the analysis of variance indicated an

interaction between time and plasma glucose concentration, suggesting that the transient rise in plasma glucose concentration was a real effect. This is in contrast with the results of Clausen (1966) who found tha ouabain (which also inhibits Na^+/K^+ ATPase) stimulated production of glycogen in rat diaphragm *in vitro*, suggesting that *in vivo* this drug ought to increase glucose uptake. It is not known why quite such radically different findings should occur in *in vitro* and *in vivo* situations. However, the present finding complements the aldosterone results, and one can thus propose an explanation for the results which demonstrates that variations in plasma K^+ concentration do affect glucose metabolism. If one supposes that aldosterone, through its actions on Na^+/K^+ ATPase in skeletal muscle, increases glucose uptake then the data obtained in these studies suggest two things. The first is that in Wistar rats where aldosterone concentration is low, addition of further aldosterone will reduce plasma glucose concentration. In contrast, in SHRs, where aldosterone is high, aldosterone is already stimulating Na^+/K^+ ATPase as much as it may, and cannot have any additional effect. When canrenoate K^+ is administered to SHRs the existing promotion of glucose uptake is removed and plasma glucose concentration rises, while the same drug in the control animals has little effect because the stimulation of skeletal muscle Na^+/K^+ ATPase by the prevailing aldosterone concentration is negligible.

These results therefore provide indirect evidence that the control of plasma K^+ and glucose concentrations are interlinked, possibly through the action of aldosterone. This possibility is supported by a report of greatly raised plasma aldosterone concentrations in SHRs compared with Wistar rats (Kim *et al.*, 1991). The link may not be causal, in either direction, but could merely represent the coincidence of two actions of aldosterone. These experiments do not provide an explanation of insulin resistance but they are interesting observations upon which to build further investigations.

Conclusions.

Conclusions.

Taking this study in isolation, one is forced to conclude that the SHR does not show many signs of insulin resistance, and that ACE inhibitors do not have the potential to improve glucose tolerance. In the context of the available literature, the model is probably a not good representation of the human condition. The effect of captopril on insulin resistance has been reported as beneficial or negligible in the literature. The finding that in the acutely treated SHR this drug worsens glucose tolerance is therefore a new one. The activity does not appear to be a generic one, however, since the other ACE inhibitors tested did not have the same effect. It is not easy to draw conclusions about the mode of action of captopril. An action of the thiol group is an unlikely explanation of captopril's activity, which leaves a site-specific inhibition of ACE as the most probable cause of its effects on glucose tolerance.

Having failed to demonstrate insulin resistance in the isolated diaphragm of SHRs, the most pertinent deduction to be made is that the muscle fibres in this tissue are not themselves intrinsically insulin resistant. This is probably the reason for being unable to demonstrate substantial insulin resistance *in vivo*.

There does appear to be a link between plasma K^+ concentration and plasma glucose concentration. While the set point for plasma K^+ concentration is similar in the two strains of rat, the manipulation with aldosterone and canrenoate K^+ show that stimulation of Na^+/K^+ ATPase tends to reduce plasma glucose concentration, and that the constitutive activation of Na^+/K^+ ATPase in SHRs is much greater than that in Wistar rats. There are grounds, therefore, for examining this link more closely.

Future work.

For reasons of cost, the only measurements of plasma insulin concentration made *in vivo* were during the establishment of the intravenous glucose tolerance test model. Since plasma glucose concentration results from a balance between the insulin sensitivity of peripheral tissues and plasma insulin concentration, one needs to know both the first and last of these measurements before one can be sure of the second. Therefore to be certain of the effect of ACE inhibitors on glucose metabolism, one would ideally need to repeat the key *in vivo* experiments (those with captopril, epicaptopril, and losartan) incorporating a measurement of insulin at the same time.

The results showing that diaphragm from SHRs is not intrinsically resistant to insulin need further investigation. As was hypothesised in the discussion, this could mean that an external factor is responsible for creating insulin resistance. It would be wise, in the first instance, to find out whether this is representative of the majority of skeletal muscle by using other preparations and determining whether they show any resistance. Possible candidates are red (slow glycolytic) and white (fast glycolytic) gastrocnemius muscle, and soleus (mixed slow oxidative and slow glycolytic) muscle. Assuming that the results are similar to those from the diaphragm, one could then add some of the more common blood-borne anti-insulin factors (for example glucagon or adrenaline) to the incubation medium to determine whether the muscle is atypically sensitive to them. Digitalis and/or ouabain also merit investigation, especially since ouabain is thought by some to be EDLS (Blaustein and Hamlyn, 1991).

The final section of the studies described in this thesis relate to the interconnection of plasma glucose and K^+ control. These experiments appear to show that there is a connection. Therefore some investigation of what this might be is in order. The major drawback of the studies so far is that they employ only one dose

each of aldosterone and canrenoate K^+. A variety of doses of these drugs ought to be tested in a more extensive investigation, using a similar methodology to the one used here, to obtain information such as threshold dose, maximum possible effect, and ED_{50} of the drugs. It would also be very useful to measure the plasma concentrations of aldosterone before experimental intervention, and of aldosterone and canrenoate K^+ during the course of the experiment.

References.

References.

S. Adler. An extrarenal action of aldosterone on mammalian skeletal muscle. **Am. J. Physiol.**, 1970, *218*, 616-621.

K. Ando, Y. Sato, Y. Ito, E. Ogata and T. Fujita. Effect of salt loading on aldosterone response to long-term infusion of angiotensin II in rats. **J. Cardiovasc. Pharmacol.**, 1991, *17*, 386-389.

E.E. Änggård, R.M. Botting and J.R. Vane. Endothelins. **Blood Vessels**, 1990, *27*, 269-281.

The Australian Therapeutic Trial in Mild Hypertension. **Lancet**, 1980, *1*, 1261-1267.

J Bachmann, H. Schlüter, W. Storkebaum, H. Witzel, F. Wessels and W. Zidek. Effect of plasma from patients with essential hypertension on vascular resistance in the isolated perfused rat kidney. **Clin. Sci.**, 1991, *80*, 17-23.

J.F. Bak, N. Moller, O. Schmitz, A. Saaek and O. Pedersen. In vivo insulin action and muscle glycogen synthase activity in type 2 (non-insulin-dependent) diabetes mellitus: effects of diet treatment. **Diabetologia**, 1992, *35*, 777-784.

J.N. Barnes, P.J.T. Drew, S.S. Furniss, J.M.P. Holly, A.R. Knight, J.D. Skehan and F.J. Goodwin. Effect of angiotensin-converting enzyme inhibition on potassium-mediated aldosterone secretion in essential hypertension. **Clin. Sci.**, 1985, *68*, 625-630.

M. Baudouin-Legros, P. Guicheney and P. Meyer. Enhanced cell proliferation in essential hypertension. **J. Cardiovasc. Pharmacol.**, 1990, *16(suppl 1)*, S1-S3.

M.J. Bia and R.A. De Fronzo. Extrarenal potassium homeostasis. **Am. J. Physiol.**, 1981, *240*, F257-F268.

M.P. Blaustein and J.M. Hamlyn. Pathogenesis of essential hypertension. A link between dietary salt and high blood pressure. **Hypertension**, 1991, *18(suppl III)*, III184-III195.

E. Bonora, R.C. Bonadonna, S. Del Prato, G. Gulli, A. Solini, M. Matsuda and R.A. De Fronzo. In vivo glucose metabolism in obese and type II diabetic subjects with or without hypertension. **Diabetes**, 1993, *42*, 764-772.

A. Bornemann, T. Ploug and H. Schmalbruch. Subcellular localisation of GLUT4 in nonstimulated and insulin-stimulated soleus muscle of rat. **Diabetes**, 1992, *41(2)*, 215-

221.

M.W. Brands, D.A. Hildebrandt, H.L. Mizelle and J.E. Hall. Sustained hyperinsulinaemia increases arterial pressure in conscious rats. **Am. J. Physiol.**, 1991, *260*, R764-R768.

F. Brée, G. Hamon and J.P. Tillemant. Evidence for two binding sites on membrane-bound angiotensin-converting enzymes (ACE) for exogenous inhibitors except in testis. **Life Sci.**, 1992, *51*, 787-794.

T.A. Buchanan, G.F. Sipos, N. Madrilejo, C. Liu and V.M. Campese. Hypertension without peripheral insulin resistance in spontaneously hypertensive rats. **Am. J. Physiol.**, 1992, *262*, E14-E19.

R.D. Bukoski, P. De Wan and J. Bo. Mechanism of the enhanced epidermal growth factor-induced growth response of genetically hypertensive vascular myocytes. **Circ. Res.**, 1991, *69*, 757-764.

S.J. Bund, K.P. West and A.M. Heagerty. Effects of protection from pressure on resistance artery morphology and reactivity in spontaneously hypertensive and wistar-kyoto rats. **Circ. Res.**, 1991, *68(5)*, 1230-1240.

B.M.Th. Burgering, R.H. Medema, J.A. Maassen, M.L. van de Wetering, A.J. van der Eb, F. McCormick and J.L. Bos. Insulin stimulation of gene expression mediated by p21ras activation. **E.M.B.O. J.**, 1991, *10 (5)*, 1103-1109.

F.P. Cappuccio and G.A. MacGregor. Does potassium supplementation lower blood pressure? A meta-analysis of published trials. **J. Hypertens.**, 1991, *9*, 465-473.

J.-L. Carpentier. The cell biology of the insulin receptor. **Diabetologia**, 1989, *32*, 627-635.

A. Ceriello, A. Quatraro and D. Giugliano. Diabetes mellitus and hypertension: the possible role of hyperglycaemia through oxidative stress. **Diabetologia**, 1993, *36*, 265-266.

M.J. Christopher, M.W. Sleeman, F.P. Alford and J.D. Best. Contrasting action of short- and long-term adrenaline action on dog skeletal muscle glucose metabolism. **Diabetologia**, 1992, *35*, 399-405.

T. Clausen. The relationship between the transport of glucose and cations across cell membranes in isolated tissues. II Effects of K^+-free medium, ouabain and insulin

upon the fate of glucose in rat diaphragm. **Biochim. Biophys. Acta**, 1966, *120*, 361-368.

J.-P. Clozel, H. Kuhn and F. Hefti. Vascular protection with cilazapril in hypertension. **J. Cardiovasc. Pharmacol.**, 1992, *19(suppl 5)*, S28-S33.

J.-P. Clozel, M. Véniant, P. Hess and U. Sprecher. Effects of two angiotensin converting enzyme inhibitors and hydralazine on coronary circulation in hypertensive rats. **Hypertension**, 1991, *18(suppl II)*, II8-II14.

J.T.E. Cook, A.T. Hattersley, J.C. Levy, P. Patel, J.S. Wainscoat, T.D.R. Hockaday and R.C. Turner. Distribution of type II diabetes in nuclear families. **Diabetes**, 1992, *42*, 106-112.

G.J.S. Cooper, B. Leighton, G.D. Dimitriadis, M. Parry-Billings, J.M. Kowalchuk, K. Howland, J.B. Rothbard, A.C. Willis and K.B.M. Reid. Amylin found in amyloid deposits in human type 2 diabetes mellitus may be a hormone that regulates glycogen metabolism in skeletal muscle. **P.N.A.S.**, 1988, *85*, 7763-7766.

I. Cusin, J. Terrettaz, F. Rohner-Jeanrenaud, N. Zarjevski, F. Assimacopoulos-Jeannet and B. Jeanrenaud. Hyperinsulinaemia increases the amount of GLUT4 mRNA in white adipose tissue and decreases that of muscles: a clue for increased fat deposition and insulin resistance. **Endocrinology**, 1990, *127(6)*, 3246-3248.

P. Damsbo, A. Vaag, O. Hother-Nielsen and H. Beck-Nielsen. Reduced glycogen synthase activity in skeletal muscle from patients with and without type 2 (non-insulin-dependent) diabetes mellitus. **Diabetologia**, 1991, *34*, 239-245.

J.E. Davies, L.L. Ng, M. Ameen, P.D. Syme and J.K. Aronson. Evidence for altered Na^+/H^+ antiport activity in cultured skeletal muscle cells and vascular smooth muscle cells from the spontaneously hypertensive rat. **Clin. Sci.**, 1991, *80*, 509-516.

R.A. De Fronzo, J.D. Tobin and R. Andres. Glucose clamp technique: a method for quantifying insulin secretion and resistance. **Am. J. Physiol.**, 1979, *237(3)*, E214-E223.

R.A. De Fronzo, E. Ferrannini, Y. Sarto, P. Felig, and J. Wahren. Synergistic interaction between exercise and insulin and peripheral glucose uptake. **J. Clin. Invest.**, 1981, *68*, 1468-1474.

P. Dent, A. Lavoinne, S. Nakielny, F.B. Caudwell, P. Watt and P. Cohen. The molecular mechanism by which insulin stimulates glycogen synthesis in mammalian

skeletal muscle. **Nature**, 1990, *348*, 302-308.

O.H. Drummer and S. Kourtis. Bradykinin-potentiating activity of captorpil disulphide dimer (SQ 14,551). **Eur. J. Pharmacol.**, 1988, *153*, 11-17.

J. Dupre. Influences of the gut on the endocrine pancreas: an overview of established and potential physiological mechanisms. In: *The endocrine pancreas.* Edited by E. Samols, published by Raven Press Ltd., New York, 1991.

V.J. Dzau. Vascular renin-angiotensin system and vascular protection. **J. Cardiovasc. Pharmacol.**, 1993, *22 (suppl 5)*, S1-S9.

V.J. Dzau and G.H. Gibbons. Endothelium and growth factors in vascular remodelling of hypertension. **Hypertension**, 1991, *18(III)*, III115-III121.

J. Eckel, E. Gerlach-Eskuchen and H. Reinauer. G-protein-mediated regulation of the insulin-responsive glucose transporter in isolated cardiac myocytes. **Biochem. J.**, 1990, *272(3)*, 691-696.

C. Eglème, F. Cressier and J.M. Wood. Local formation of angiotensin II in the rat aorta: effect of endothelium. **Brit. J. Pharmacol.**, 1990, *100*, 237-240.

J. Eriksson, L. Koranyi, R. Bourey, C. Schalin-Jäntti, E. Widén, A.M. Permutt and L.C. Groop. Insulin resistance in type 2 (non-insulin dependent) diabetic patients and their relatives is not associated with a defect in the expression of the insulin-responsive glucose transporter (GLUT-4) gene in human skeletal muscle. **Diabetologia**, 1992a, *35*, 143-147.

J. Eriksson, M. Nakazato, M. Miyazato, K. Shiomi, S. Matsukura and L. Groop. Islet amyloid polypeptide plasma concentrations in individuals at increased risk of developing type 2 (non-insulin dependent) diabetes mellitus. **Diabetologia**, 1992b, *35*, 291-293.

J. Espinal. Insulin: chemistry, synthesis and secretion. In: *Understanding insulin action: principles and molecular mechanisms.* Published by Ellis Horwood Ltd., England, 1989.

R.D. Farhy, K.-L. Ho, O.A. Carretero and A.G. Scicli. Kinins mediate the antiproliferative effect of ramipril in rat carotid artery. **Biochem. Biophys. Res. Comm.**, 1992, *182(1)*, 283-288.

E. Ferrannini, G. Buzzigoli, R. Bonadonna, M.A. Giorico, M. Oleggini, L. Graziadei,

R. Pedrinelli, L. Brandi and S. Bevilacqua. Insulin resistance in essential hypertension. **New Eng. J. Med.**, 1987, *317(6)*, 350-357.

E. Ferrannini, A.Q. Galvan, D. Santoro and A. Natali. Potassium as a link between insulin and the renin-angiotensin-aldosterone system. **J. Hypertens.**, 1992, *10(Suppl 1)*, S5-S10.

E. Ferrannini, S.M. Haffner and M.P. Stern. Essential hypertension: an insulin resistant state. **J. Cardiovasc. Pharmacol.**, 1990, *15(suppl 5)*, S18-S25.

P. Ferrari and P. Weidmann. Insulin, insulin sensitivity and hypertension. **J. Hypertens.**, 1990, *8*, 491-500.

J.L. Frelson and J.F. Giudicelli. Compared myocardial and vascular effects of captopril and dihydralazine during hypertension development in spontaneously hypertensive rats. **Brit. J. Pharmacol.**, 1983, *80*, 533-543.

S. Frontoni, S.B. Choi, D. Banduch and L. Rossetti. In vivo insulin resistance induced by amylin primarily through inhibition of insulin-stimulated glycogen synthesis in skeletal muscle. **Diabetes**, 1991, *40*, 568-573.

S. Frontoni, L. Ohman, J.R. Haywood, R.A. DeFronzo and L. Rossetti. In vivo insulin action in genetic models of hypertension. **Am. J. Physiol.**, 1992, *262*, E191-E196.

K. Fujito, M. Yokomatsu, H. Numahata, N. Ishiguro and H. Koide. Acute effects of angiotensin-converting enzyme inhibitor on erythrocyte sodium ion transport in essential hypertension. **Life Sci.**, 1992, *51*, 2079-2087.

S.M. Furler, A.B. Jenkins, L.H. Storlien and E.W. Kraegen. In vivo location of the rate limiting step of hexose uptake in muscle and brain tissue of rats. **Am. J. Physiol.**, 1991, *261*, E337-E347.

C.L. Gaboury, N. Karanja, S.R. Holcomb, J. Torok and D.A. M^{c}Carron. Patterns of insulin secretion and responsiveness in Wistar Kyoto and spontaneously hypertensive rats. **Am. J. Hypertens.**, 1991, *4*, 661-666.

R.O.B. Gans. Insulin resistance. **New Eng. J. Med.**, 1992, *326(9)*, 646-647.

R.O.B. Gans, H.J.G. Bilo, J.J.P. Nauta, C. Popp-Snijders, R.J. Heine and A.J.M. Donker. The effect of angiotensin-I converting enzyme inhibition on insulin action in healthy volunteers. **Eur. J. Clin. Invest.**, 1991a, *21*, 527-533.

R.O.B. Gans, H.J.G. Bilo, W.W.A. v.Maarschalkerweerd, R.J. Heine, J.J.P. Nauta and

A.J.M. Donker. Exogenous insulin augments in healthy volunteers the cardiovascular reactivity to noradrenaline but not to angiotensin II. **J. Clin. Invest.**, 1991b, *88*, 512-518.

D. Ganten, S. Takahashi, K. Lindpaintner and J. Mullins. Genetic basis of hypertension. The renin angiotensin paradigm. **Hypertension**, 1991, *18(suppl III)*, III109-III114.

G.H. Gibbons. Angiotensin-converting enzyme inhibition and vascular structure in hypertension. **J. Cardiovasc. Pharmacol.**, 1991, *18(suppl 7)*, S19-S24.

L.C. Groop, R.C. Bonadonna, M. Shank, A.S. Petrides and R.A. De Fronzo. Role of free fatty acids and insulin in determining free fatty acid and lipid oxidation in man. **J. Clin. Invest.**, 1991, *87*, 83-89.

S.R. Hager, D. Pastovek, A.L. Jochen and D. Meier. Divergence between GLUT4 mRNA and protein abundance in skeletal muscle of insulin resistant rats. **Biochem. Biophys. Res. Comm.**, 1991, *181(1)*, 240-245.

T. Hamaguchi, H. Fukushima, M. Uehara, S. Wada, T. Shirotani, H. Kishikawa, K. Ichinose, K. Yamaguchi and M. Schichiri. Abnormal glucagon response to arginine and its normalisation in obese hyperinuslinaemic patients with glucose intolerance: importance of insulin action on pancreatic alpha cells. **Diabetologia**, 1991, *34*, 801-806.

J.M. Hamlyn and P. Manunta. Ouabain, digitalis-like factors and hypertension. **J. Hypertens.**, 1992, *10(suppl 7)*, S99-S111.

S.B. Harrapp, W.M. Van der Merwe, S.A. Griffin, F. Macpherson and A.F. Lever. Brief angiotensin converting enzyme inhibitor treatment in young spontaneously hypertensive rats reduces blood pressure long-term. **Hypertension**, 1990, *16*, 603-614.

H.U. Häring and H. Mehnert. Pathogenesis of type 2 (non-insulin-dependent) diabetes mellitus: candidates for a signal transmitter defect causing insulin resistance of the skeletal muscle. **Diabetologia**, 1993, *36*, 176-182.

R.J. Head. Hypernoradrenergic innervation: its relationship to functional and hyperplastic changes in the vasculature of the spontaneously hypertensive rat. **Blood Vessels**, 1989, *26*, 1-20.

J.A. Herd. Cardiovascular response to stress in man. **Ann. Rev. Physiol.**, 1984, *46*.

177-185.

P. Hilbert, K. Lindpainter, J.S. Beckmann, T. Serikawa, F. Soubrier, C. Dubay, P. Cartwright, B. De Gouyon, C. Julier, S. Takahashi, M. Vincent, D. Ganten, M. Georges and G.M. Lathrop. Chromsomal mapping of two genetic loci associated with blood-pressure regulation in hereditary hypertensive rats. **Nature**, 1991, *353*, 521-529.

M. Hindlycke and L. Jansson. Glucose tolerance and pancreatic islet blood flow in rat after intraperitoneal administration of different anaesthetic drugs. **Uppsala J. Med. Sci.**, 1992, *97*, 27-35.

D.E. Hinkle, W. Wiersma and S.G. Jurs. Chapter 16: Multiple comparison procedures. In *Applied statistics for the behavioural sciences.* Published by Houghton Mifflin Company, Boston, 1988.

G. Hoffmann, R. Pietsch, B.O. Göbel, B. Weisser, G. Bönner, H. Vetter and R. Düsing. Converting enzyme inhibition and vascular prostacyclin synthesis: effect of kinin receptor antagonism. **Eur. J. Pharmacol.**, 1990, *178*, 79-83.

J.S. Hothersall, R.P. Muirhead and S. Wimalawansa. The effect of amylin and calcitonin gene-related peptide on insulin-stimulated glucose transport in the diaphragm. **Biochem. Biophys. Res. Comm.**, 1990, *169*, 451-454.

S. Hulman, B. Falkner and Y.Q. Chen. Insulin resistance in the spontaneously hypertensive rat. **Metabolism**, 1991, *40*, 359-361.

H.S. Hundal, A. Marette, Y. Mitsumoto, T. Ramlal, R. Blostein and A. Klip. Insulin induces translocation of the a2 and b1 subunits of the Na^+/K^+-ATPase from intracellular compartments to the plasma membrane in mammalian skeletal muscle. **J. Biol. Chem.**, 1992, *267*, 5040-5043.

T. Ishizuka, D.R. Cooper, H. Hernandez, D. Buckley, M. Stendaert and R.V. Farese. Effects of insulin on diacylglycerol-protein kinase C signalling in rat diaphragm and soleus muscles and relationship to glucose transport. **Diabetes**, 1990, *39*, 181-190.

H.J. Jacob, K. Lindpainter, S.E. Lincoln, K. Kusumi, R.K. Bunker, Y-P. Mao, D. Ganten, V.J. Dzau and E.S. Lander. Genetic mapping of a gene causing hypertension in the stroke-prone spontaneously hypertensive rat. **Cell**, 1991, *67*, 213-224.

D.E. James, A.B. Jenkins and E.W. Kraegen. Heterogeneity of insulin action in individual muscles in vivo: euglycaemic clamp studies in rats. **Am. J. Physiol.**, 1985,

248, E567-E574.

R.J. Jarrett. In defence of insulin: a critique of syndrome X. **Lancet**, 1992, *340*, 469-471.

H.G. Joost and T.M. Weber. The regulation of glucose transport in insulin sensitive cells. **Diabetologia**, 1989, *32*, 831-838.

S. Julius. Autonomic nervous system dysregulation in human hypertension. **Am. J. Cardiol.**, 1991, *67*, 3B-7B.

R.C. Kahn and M.F. White. The insulin receptor and the molecular mechanism of insulin action. **J. Clin. Invest.**, 1988, *82*, 1151-1156.

A. Kashiwagi, M.A. Verso, J. Andrews, B. Vasquez, G.M. Reaven and J.E. Foley. In vitro insulin resistance of human adipocytes isolated from subjects with noninsulin-dependent diabetes mellitus. **J. Clin. Invest.**, 1983, *72*, 1246-1254.

F.W. Keeley, A. Elmoselhi and F.H.H. Leenen. Effects of antihypertensive drug classes on regression of connective tissue components in hypertension. **J. Cardiovasc. Pharmacol.**, 1991, *17(suppl 2)*, S64-S69.

M. Kellerer, B. Obermaier-Kusser, A. Pröfrock, E. Schleicher, E. Seffer, J. Mushack, B. Ermel and H.-U. Häring. Insulin activates GTP binding to a 40KDa protein in fat cells. **Biochem. J.**, 1991, *276*, 103-108.

Y. Kida, I. Raz, R. Maeda, B.L. Nyomba, K. Stone, C. Bogardus, J. Sommercorn and D.M. Mott. Defective insulin response of phosphorylase phosphatase in insulin-resistant humans. **J. Clin. Invest.**, 1992, *89*, 610-617.

S. Kim, M. Hosoi, K. Shimamoto, T. Takada and K. Yamamoto. Increased production of angiotensin II in the adrenal gland of stroke-prone spontaneously hypertensive rats with malignant hypertension. **Biochem. Biophys. Res. Comm.**, 1991, *178(1)*, 151-157.

M.P. Kingsbury. An investigation of the cardiovascular effects of the chronic administration of atenolol and nitrendipine given alone and in combination. PhD Thesis, 1989.

M.J. Kirby and P. Turner. Fenfluramine and norfenfluramine on glucose uptake into skeletal muscle. **Postgrad. Med. J.**, 1975, *51(suppl 1)*, 73-76.

A. Klip and A. Marette. Acute and chronic signals controlling glucose transport in

skeletal muscle. **J. Cell. Biochem.,** 1992, *48*, 51-60.

P.I. Korner, A. Bobik, G.L. Jennings, J.A. Angus and W.P. Anderson. Significance of cardiovascular hypertrophy in the development and maintenance of hypertension. **J. Cardiovasc. Pharmacol.**, 1991, *17(suppl 2)*, S25-S32.

E.W. Kraegen, D.E. James, A.B. Jenkins and D.J. Chisholm. Dose-response curves for in vivo insulin sensitivity in individual tissues in rats. **Am. J. Physiol.**, 1985, *248*, E353-E362.

E. Küenburg, M. Brunnbauer, B. Ludvik, G. Schernthaner and R. Prager. The effect of ramipril on glucose tolerance and insulin sensitivity in patients with hypertension. **Diabetologia**, 1990, *33(suppl 1)*, A145.

M. Laasko, S.V. Edelman, G. Brechtel and A.D. Baron. Impaired insulin-mediated skeletal muscle blood flow in patients with NIDDM. **Diabetes**, 1992, *41*, 1076-1083.

K. Landin, F. Lindgärde, B. Saltin and U. Smith. The skeletal muscle Na^+:K^+ ratio is not increased in hypertension: evidence for the importance of obesity and glucose intolerance. **J. Hypertens.**, 1991, *9*, 65-69.

J.C. Lawrence, Jr. Signal transduction and protein phosphorylation in the regulation of cellular metabolism by insulin. **Ann. Rev. Physiol.**, 1992, *54*, 177-193.

M.A.E. Lee, M. Paul, M. Böhm and D. Ganten. Effects of angiotensin-converting enzyme inhibitors on tissue renin-angiotensin systems. **Am. J. Cardiol.**, 1992, *70*, 12C-19C.

P.J. Lefèbvre. Diabetes: abnormal secretion of glucagon. In: *The endocrine pancreas.* Edited by E. Samols, published by Raven Press Ltd., New York, 1991.

B. Leighton and G.J.S. Cooper. Pancreatic amylin and calcitonin gene-related peptide cause resistance to insulin in skeletal muscle *in vitro*. **Nature**, 1988, *335*, 632-635.

B. Leighton, M. Parry-Billings, G. Dimitriadis, J. Bond, E.A. Newsholme, C. DaCosta and E.A. Foot. Physiological glucocorticoid levels regulate glutamine and insulin-mediated glucose metabolism in skeletal muscle of the rat: studies with RU 486 (mefipristone). **Biochem. J.**, 1991, *274*, 187-192.

A.F. Lever and S.B. Harrap. Essential hypertension: a disorder of growth with origins in childhood? **J. Hypertens.**, 1992, *10*, 101-120.

S. Lillioja, A.A. Young, C.L. Culter, J.L. Ivy, W.G.H. Abbott, J.K. Zawadzki, H. Yki-

Järvinen, L. Christin, T.W. Secomb and C. Bogardus. Skeletal muscle capillary density and fibre type are possible determinants of in vivo insulin resistance in man. **J. Clin. Invest.**, 1987, *80*, 415-424.

F.B. Lima, R.S. Thies and W.T. Garvey. Glucose and insulin regulate insulin sensitivity in primary cultured adipocytes without affecting insulin receptor kinase activity. **Endocrinology**, 1991, *128*, 2415-2426.

K. Lindpainter, S. Takahashi and D. Ganten. Structural alterations of the renin gene in stroke-prone spontaneously hypertensive rats: examination of genotype-phenotype correlations. **J.Hypertens.**, 1990, *8*, 763-773.

W. Linz and B.A. Schölkens. A specific B2-bradykinin receptor antagonist Hoe 140 abolishes the antihypertrophic effect of ramipril. **Brit. J. Pharmacol.**, 1992, *105*, 771-772.

H.O. Lithell, T. Pollare and C. Berne. Insulin sensitivity in newly detected hypertensive patients: influence of captopril and other antihypertensive agents on insulin sensitivity and related biological parameters. **J. Cardiovasc. Pharmacol.**, 1990, *15(suppl 5)*, S46-S52.

T. Matsukawa, E. Gotoh, E. Miyajima, Y. Yamada, H. Shionoiri, O. Tochikubo and M. Ishii. Angiotensin II inhibits baroreflex control of muscular sympathetic nerve activity and the heart rate in patients with essential hypertension. **J. Hypertens.**, 1988. *6(suppl 4)*, S501-S504.

M.C. McGuire, R.M. Fields, B.L. Nyomba, I. Raz, C. Bogardus, N.K. Tonks and J. Sommercorn. Abnormal regulation of protein tyrosine phosphatase in skeletal muscle of insulin resistant humans. **Diabetes**, 1991, *40*, 939-942.

J.A. Miller, E.L. Harris and N.J. Cassie. Mitogenesis in cultured vascular smooth muscle cells from two rat models of hypertension in response to fetal calf serum and angiotensin II. **J. Cardiovasc. Pharmacol.**, 1990, *16(suppl 7)*, S14-S16.

A. Mitrakou, M. Mokan, G. Bolli, T. Veneman, T. Jenssen, P. Cryer and J. Gerich. Evidence against the hypothesis that hyperinsulinaemia increases sympathetic nervous system activity in man. **Metabolism**, 1992, *41(2)*, 198-200.

K. Mizuno, K. Higashimori and T. Inagami. Direct evidence for the local generation of vascular angiotensin II and its prostaglandin-mediated release from isolated hind legs of the rat. **J. Hypertens.**, 1988, *6(suppl 4)*, S435-S437.

M. Modan, H. Halkin, S. Almog, A. Lusky, A. Eshkol, M. Shefi, A. Shitrit and Z. Fuchs. Hyperinsulinaemia. (Link between hypertension, obesity, and glucose intolerance). **J. Clin. Invest.**, 1985, *75*, 809-817.

D.E. Moller and J.S. Flier. Insulin resistance - mechanisms, syndromes, and implications. **New Eng. J. Med.**, 1991, *325(13)*, 938-948.

C.E. Mondon and G.M. Reaven. Evidence of abnormalities of insulin metabolism in rats with spontaneous hypertension. **Metabolism**, 1988, *37(4)*, 303-305.

E.R. Mortensen, J. Drachman and G. Guidotti. Guanosine nucleotides regulate hormone binding of insulin receptors. **Biochem. J.**, 1992, *281*, 735-743.

L. Mosthaf, B. Vogt, H.U. Häring and A. Ullrich. Altered expression of insulin receptor types A and B in the skeletal muscle of non-insulin-dependent diabetes mellitus patients. **P.N.A.S.**, 1991, *88*, 4728-4730.

A.F. Muller, S.M. Gardiner, A.M. Compton and T. Bennett. Regional haemodynamic effects of captopril, enalaprilat and lisinopril in conscious water-replete and water-deprived Brattleboro rats. **Clin. Sci.**, 1990, *79*, 393-401.

M.J. Mulvany, P.K. Hansen and C. Aalkjaer. Direct evidence that the greater contractility of resistance vessels in spontaneously hypertensive rats is associated with a narrowed lumen, a thickened media, and an increased number of smooth muscle cell layers. **Circulation Res.**, 1978, *43*, 854-864.

M. Mulvany. Structure and function of small arteries in hypertension. **J.Hypertens.**, 1990, *8(suppl 7)*, S225-S232.

M.J. Mulvany. Are vascular abnormalities a primary cause or secondary consequence of hypertension? **Hypertension**, 1991, *18(suppl I)*, I52-I57.

T. Nabika, Y. Nara, K. Ikeda, J. Endo and Y. Yamori. A new genetic locus cosegregating with blood pressure in F2 progeny obtained from stroke-prone spontaneously hypertensive rats and wistar-kyoto rats. **J. Hypertens.**, 1993, *11*, 13-18.

Y. Nara, T. Nabika, K. Ikeda, M. Sawamura, J. Endo and Y. Yamori. Blood pressure cosegregates with a microsatellite of angiotensin I converting enzyme (ACE) in F2 generation from a cross between original normotensive wistar-kyoto rat (WKY) and stroke-prone spontaneously hypertensive rat (SHRSP). **Biochem. Biophys. Res. Comm.**, 1991, *181(3)*, 941-946.

Y. Ohno, H. Suzuki, H. Yamakawa, M. Nakamura, K. Otsuka and T. Saruta. Impaired insulin sensitivity in young, lean normotensive offspring of essential hypertensives: possible role of disturbed calcium metabolism. **J. Hypertens.**, 1993, *11*, 421-426.

J.M. Olefsky. The insulin receptor (a multifunctional protein). **Diabetes**, 1990, *39*, 1009-1016.

F.J. Oliver, G. de la Rubia, E.P. Feener, M.-E. Lee, M.R. Loeken, T. Shiba, T. Quertermous and G.L. King. Stimulation of endothelin-1 gene expression by insulin in endothelial cells. **J. Biol. Chem.**, 1991, *266(34)*, 23251-23256.

M. Omatsu-Kanbe and H. Kitasato. Insulin stimulates the translocation of Na^+/K^+-dependent ATPase molecules from intracellular stores to the plasma membrane in frog skeletal muscle. **Biochem. J.**, 1990, *272*, 727-733.

Opie, L.H. The renin-angiotensin-aldosterone system and other proposed sites of action of angiotensin-converting enzyme inhibitors. In: *Angiotensin converting enzyme inhibitors. Scientific basis for clinical use*. 1992, pp. 1-17.

S.N. Orlov, T.J. Resink, J. Bernhardt and F.R. Bühler. Na^+-K^+ pump and Na^+-K^+ co-transport in cultured vascular smooth muscle cells from spontaneously hypertensive rats: baseline activity and regulation. **J. Hypertens.**, 1992, *10*, 733-740.

G. Paolisso, A. Scheen, F. D'Onofrio and P. Lefèbvre. Magnesium and glucose homeostasis. **Diabetologia**, 1990, *33*, 511-514.

R.M.J. Palmer, A.G. Ferrige and S.Moncada. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. **Nature**, 1987, *327*, 524-526.

O. Pedersen, C.R. Kahn, J.S. Flier and B.B. Kahn. High fat feeding causes insulin resistance and a marked decrease in the expression of glucose transporters (GLUT4) in fat cells of rats. **Endocrinology**, 1991, *129(2)*, 771-777.

J.E. Pessin and G.I. Bell. Mammalian facilitative glucose transporter family: structure and molecular regulation. **Ann. Rev. Physiol.**, 1992, *54*, 911-930.

M.A. Pfeifer amd V.L. Broadstone. Diabetes: abnormal secretion of insulin. In: *The endocrine pancreas*. Edited by E. Samols, published by Raven Press Ltd., New York, 1991.

T. Pollare, H. Lithell and C. Berne. A comparison of the effects of hydrochlorothiazide

and captopril on glucose and lipid metabolism in patients with hypertension. **New Eng. J. Med.**, 1989, *321(13)*, 868-873.

A.E. Pontiroli, M. Alberetto and G. Pozza. Patients with insulinoma show insulin resistance in the absence of arterial hypertension. **Diabetologia**, 1992, *35*, 294-295.

R.H. Rao. Insulin resistance in spontaneously hypertensive rats. Difference in interpretation based on insulin infusion rate or on plasma insulin in glucose clamp studies. **Diabetes**, 1993, *42*, 1364-1371.

G.M. Reaven. Role of insulin resistance in human disease. **Diabetes**, 1988, *37*, 1595-1607.

G.M. Reaven, H. Chang, B.B. Hoffman and S. Azhar. Resistance to insulin-stimulated glucose uptake in adipocytes isolated from spontaneously hypertensive rats. **Diabetes**, 1989, *38*, 1155-1160.

P.J. Roach. Control of glycogen synthase by hierarchical protein phosphorylation. **FASEB J.**, 1990, *4*, 2961-2968.

J. Saltis, A.D. Habberfield, J.J. Egan, C. Loudos, I.A. Simpson and S.W. Cushman. Role of protein kinase C in the regulation of glucose transport in the rat adipose cell. **J. Biol. Chem.**, 1991, *266(1)*, 261-267.

P. Sbraccia, P.A. Goodman, B.A. Maddux, K.Y. Wong, Y.-D.I. Chen, G.M. Reaven and I.D. Goldfine. Production of inhibitor of insulin -receptor tyrosine kinase in fibroblasts from patients with insulin resistance and NIDDM. **Diabetes**, 1991, *40*, 295-299.

S. Seino, M. Seino and G.I. Bell. Human insulin receptor gene. **Diabetes**, 1990, *39*, 129-133.

P.S. Sever. 1985 - the year of the hypertension trials: interpreting the results. **Trends Pharmacol. Sci.**, 1986, *6*, 134-139.

L.R. Shian and M.T. Lin. Insulin acts on the hypothalamic glucose-facilitated neurons to induce hyperglycaemia and hyperinsulinaemia in the rat. **Experientia**, 1991, *47*, 942-944.

H. Shionoiri, T. Miyakawa, I. Takasaki, Y. Ishikawa, S. Hiroto, Y. Kaneko and Y. Shindo. Glucose tolerance during chronic captopril therapy in patients with essential hypertension. **J. Cardiovasc. Pharmacol.**, 1987, *9*, 160-164.

H. Shionoiri, S. Ueda, E. Gotoh, T. Ito and T. Ogihara. Glucose and lipid metabolism during long-term lisinopril therapy in hypertensive patients. **J. Cardiovasc. Pharmacol.**, 1990, *16(6)*, 905-909.

S.E. Shoelson, S. Chatterjee, M. Chaudhuri and M.F. White. YMXM motifs of IRS-1 define substrate specificity of the insulin receptor kinase. **P.N.A.S.**, 1992, *89*, 2027-2031.

P. Skott, A. Vaag, N.E. Brunn, O. Hother-Nielsen, M.-A. Gall, H. Beck-Nielsen and H.-H. Parving. Effect of insulin on renal sodium handling in hyperinsulinaemic type 2 (non-insulin-dependent) diabetic patients with peripheral insulin resistance. **Diabetologia**, 1991, *34*, 275-281.

W.L.Smith. Prostaglandin biosynthesis and its compartmentation in vacular smoooth muscle and endothelial cells. **Ann. Rev. Physiol.**, 1986, *48*, 251-262.

R.L. Soffer, R. Reza and P.R.B. Caldwell. Angiotensin converting enzyme from rabbit pulmonary particles. **Proc. Nat. Acad. Sci.,** 1974, *71*, 1720-1724.

P.B. Stace, D.R. Marchington, A.L. Kerbey and P.J. Randle. Long term culture of rat soleus muscle in vitro. Its effects on glucose utilisation and insulin sensitivity. **FEBS Lett.**, 1990, *273*, 91-94.

X.J. Sun, P. Rothenberg, C.R. Kahn, J.M. Backer, E. Araki, P.A. Wilden, D.A. Cahill, B.J. Goldstein and M.F. White. Structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. **Nature**, 1991, *352*, 73-77.

C.K. Sung. Insulin receptor signalling through non-tyrosine kinase pathways: evidence from anti-receptor antibodies and insulin receptor mutants. **J. Cell. Biochem.**, 1992, *48*, 26-32.

S. Suzuki, K. Sugawara, Y. Satoh and T. Toyota. Insulin stimulates the generation of two putative insulin mediators, inositol-glycan and diacylglycerol in BC3H-1 myocytes. **J. Biol. Chem.**, 1991, *266*, 8115-8121.

C.S. Sweet, D.M. Gross, P.T. Arbegast, S.L.Gaul, P.M. Britt, C.T. Ludden, D. Weitz,.and C.A. Stone. Antihypertensive activity of N-[(S)-1-(ethxycarbonyl)-3-phenylpropryl]-L-Ala-L-Pro (MK421), an orally active converting enzyme inhibitor. **J. Pharmacol. Exp. Ther.**, 1981, *216*, 558-566.

P.D. Syme, L. Arnolda, Y. Green, J.K. Aronson, D.G. Grahame-Smith and G.K. Radda. Evidence for increased in vivo Na^+-H^+ antiporter activity and an altered

skeletal muscle contractile response in the spontaneously hypertensive rat. **J. Hypertens.**, 1990a, *8*, 1027-1036.

P.D. Syme, R.M. Dixon, J.K. Aronson, D.G. Grahame-Smith and G.K. Radda. Evidence for increased in vivo sodium-potassium pump activity and potassium efflux in skeletal muscle of spontaneously hypertensive rats. **J. Hypertens.**, 1990b, *8*, 1161-1166.

F. Takao, M.C. Laury, A. Ktorza, L. Picon and L. Pénicaud. Hyperinsulinaemia increases insulin action *in vivo* in white adipose tissue but not in muscles. **Biochem. J.**, 1990, *272*, 255-257.

S. Tartare, R. Ballotti and E.V. Obberghen. Interaction between heterologous receptor tyrosine kinases. Hormone-stimulated insulin receptors activate unoccupied IGF-1 receptors. **FEBS Lett.**, 1991, *295(1-3)*, 219-222.

E. Torlone, A.M. Rambotti, G. Pirriello, G. Botta, F. Santeusanio, P. Brunetti and G.B. Bolli. ACE-inhibition increases hepatic and extrahepatic sensitivity to insulin in patients with type 2 (non-insulin-dependent) diabetes mellitus and arterial hypertension. **Diabetologia**, 1991, *34*, 119-125.

G.J. Tortora and N.P Anagnostakos. Chapter 18 - the endocrine system. In *Principles of anatomy and physiology (fifth edition)*. 1987, pp 430 -432, edited by C.M. Wilson. R. Ginsberg and H.D. Gordon, published by Harper and Row, New York.

R.M. Touyz, F.J. Milne and S.G. Reinach. Platelet and erythrocyte Mg^{++}, Ca^{++}, Na^{+}, K^{+} and cell membrane adenosine triphosphatase activity in essential hypertension in blacks. **J. Hypertens.**, 1992, *10*, 571-578.

R.R. Townsend, R. Yamamoto, M. Nickols, D.J. DiPette and G.A. Nickols. Insulin enhances pressor responses to norepinephrine in rat mesenteric vasculature. **Hypertension**, 1992, *19(suppl II)*, II105-II110.

R.R. Traxinger and S. Marshall. Glucose regulation of insulin receptor affinity in primary cultured adipocytes. **J. Biol. Chem.**, 1990, *265(31)*, 18879-18883.

N.C. Trippodo and E.D. Frohlich. Similarities of genetic (spontaneous) hypertension. Man and rat. **Circulation Res.**, 1981, *48*, 309-319.

A. Vaag, P. Damsbo, O. Hother-Nielsen and H. Beck-Nielsen. Hyperglycaemia compensates for the defects in insulin-mediated glucose metabolism and in the activation of glycogen synthase in the skeletal muscle of patients with type 2 (non-

insulin dependent) diabetes mellitus. **Diabetologia**, 1992b, *35*, 80-88.

A. Vaag, J.E. Henrikson and H. Beck-Nielsen. Decreased insulin activation of glycogen synthase in skeletal muscles of young nonobese caucasian first-degree relatives of patients with non-insulin-dependent diabetes mellitus. **J. Clin. Invest.**, 1992a, *89*, 782-788.

T. Vago, M. Bevilacqua, F. Conci, G. Baldi, E. Ongini, E. Chebat, A. Monopoli and G. Norbiato. Angiotensin converting enzyme binding sites in human heart and lung: comparison with rat tissues. **Brit. J. Pharmacol.**, 1992, *107*, 821-825.

F. Vara and E. Rozengurt. Stimulation of Na^+/H^+ antiport activity by epidermal growth factor and insulin occurs without activation of protein kinase C. **Biochem. Biophys. Res. Comm.**, 1985, *130(2)*, 646-653.

M.C. Veroni, J. Proietto and R.G. Larkins. Evolution of insulin resistance in New Zealand obese mice. **Diabetes**, 1991, *40*, 1480-1487.

M.-W. Wang, P. Carlo, T.J. Rink and A.A. Young. Amylin is more potent and more effective than glucagon in raising plasma glucose concentration in fasted, anaesthetised rats. **Biochem. Biophys. Res. Comm.**, 1991, *181(3)*, 1288-1293.

G.M. Ward, J.M. Walters, P.M. Aitken, J.D. Best and F.P. Alford. Effects of prolonged pulsatile hyperinsulinaemia in humans. (Enhancement of insulin sensitivity). **Diabetes**, 1990, *39*, 501-507.

M. Weiland, F. Bahr, M. Höhne, A. Schürmann, D. Ziehm and H.G. Joost. The signalling potential of the receptors for insulin and insulin-like growth factor 1 (IGF-1) in 3T3 L1 adipocytes: comparison of glucose transport activity, induction of oncogene c-fos, glucose transporter mRNA, and DNA synthesis. **J. Cell. Physiol.**, 1991, *149*, 428-435.

G. Wiemar, B.A. Schölkens, R.H.A. Becker and R. Busse. Ramiprilat enhances endothelial autacoid formation by inhibiting breakdown of endothelium derived bradykinin. **Hypertension**, 1991, *18*, 558-563.

Y. Yamori, K. Ohta, M. Chtaka, Y. Nara, R. Horie and A. Ooshima. Glucose metabolism in spontaneously hypertensive rats. **Jap. Heart J.**, 1978, *19*, 559-560.

Y. Yarden and A. Ullrich. Growth factor receptor tyrosine kinases. **Annu. Rev. Biochem.**, 1988, *57*, 443-478.

K. Yin, Z.M. Chu and L.J. Beilin. Study of mechanisms of glucocorticoid hypertension in rats: endothelial related changes and their amelioration by dietary fish oils. **Brit. J. Pharmacol.**, 1992, *106*, 435-442.

C.C. Yip, C. Grunfeld and I.D. Golfine. Identification and characterisation of the ligand binding domain of insulin receptor by use of an antipeptide antiserum against amino acid sequence 241-251 of the α subunit. **Biochemistry**, 1990, *30*, 695-701.

F. Zannad. Vascular and cardiac actions of aldosterone and spironolactone. **Z. Kardiol.**, 1991, *80(suppl 7)*, 103-105.

B. Zhang, J.M. Tavaré, L. Ellis and R.A. Roth. The regulatory role of known tyrosine autophosphorylation sites of the insulin receptor kinase domain. **J. Biol. Chem.**, 1991, *266(2)*, 990-996.

J.R. Zierath, D. Galuska, Å. Engström, C. Betsholtz, P. Westermark and H. Wallberg-Henriksson. Human islet amyloid polypeptide at pharmacological levels inhibits insulin and phorbol ester-stimulated glucose transport in *in vitro* incubated human muscle strips. **Diabetologia**, 1992, *35*, 26-31.

Appendix.

Comparison of glucose uptake by diaphragm of normotensive and spontaneously hypertensive rats.

D.S.T. Crabbe, P.H. Redfern, B. Woodward, & K.I.Williams, School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY. United Kingdom.

In recent years it has been proposed that insulin resistance may be a cause of hypertension (Reaven, 1988). Soleus muscle of patients with type 2 diabetes mellitus, another syndrome of insulin resistance, has been shown to be less responsive to insulin than normal muscle (Dohm *et al.*, 1988), and Reaven and colleagues (1989) have demonstrated insulin resistance in adipocytes from spontaneously hypertensive rats (SHR). We have shown that SHR are glucose intolerant when compared with Wistar rats and that this intolerance is ameliorated by captopril treatment (Crabbe *et al.*, 1991). These factors have led us to compare glucose uptake in an *in vitro* skeletal muscle preparation of SHR and Wistar rats.

We have adapted the method of Kirby and Turner (1975) for measuring glucose uptake by hemidiaphragms from male Wistar rats or SHR (both University of Bath strain) weighing 200-250g. Diaphragms were excised, washed in ice cold, glucose-free Krebs-Henseleit solution (KHS), and incubated in 3ml of glucose-containing (15mM) KHS in a shaking (150 strokes.min^{-1}), heated (37°C) water bath for two hours. Each flask already contained appropriate incubation additions to the KHS (10μU.ml^{-1}-100mU.ml^{-1} human insulin or vehicle) and was gassed with moist 95%O_2/5%CO_2. 100μl samples were taken at t=10min, t=20min, and every 20min thereafter for subsequent glucose estimation using a colourimetric glucose oxidase assay. A second set of experiments was performed to determine the effect of captopril (1-30μM) on insulin-stimulated (1mU.ml^{-1}) glucose uptake.

Insulin-stimulated glucose uptake by hemidiaphragms between 10 and 120 minutes was concentration-related and linear. There was no significant difference between the rates of uptake in the two strains. For example at 100μU.ml^{-1} insulin the rate of uptake was 0.55$\pm$0.09μmol.min^{-1}g^{-1} tissue wet weight in Wistar rats and 0.54$\pm$0.04μmol.min^{-1}g^{-1} in SHR (mean $\pm$sem, n=4). Likewise, the total insulin-stimulated glucose uptake after two hours was similar (80.76$\pm$15.06μmol.min^{-1}g^{-1} in Wistar rats and 79.04$\pm$9.02μmol.min^{-1}g^{-1} in SHR, at 100μU.ml^{-1} insulin, n=4). Captopril (1-30μM) did not alter insulin-induced glucose uptake in either strain of rat.

The results show that isolated diaphragm of SHR is not insulin resistant under these conditions. Since it has been demonstrated that this same strain of rat is glucose intolerant (Crabbe *et al.*, 1991) it seems that a systemic input is necessary to render the animals insulin resistant. Perhaps, therefore, reduced insulin sensitivity in SHR is not purely a defect of insulin signalling, but is dependent on other factors. These factors remain obscure, but might include glucagon and other glucose regulating hormones.

DSTC is a SERC-funded scholar.

Crabbe, D.S.T., Redfern, P.H., Woodward, B., & Williams,K.I. (1991) *Br. J. Pharmac.* **104**, 127P.
Dohm, G.L., Tapscott, E.B., Pories, W.J., Dabbs, D.J., Flickinger, E.G., *et al.* (1988) *J. Clin. Invest.* **82**, 486-494.
Kirby, M.J., & Turner, P. (1975) *Postgrad. Med. J.* **51**(Suppl. 1), 73-76.
Reaven, G.M. (1988) *Diabetes* **37**, 1595-1607.
Reaven, G.M., Chang, H., Hoffman, B.B., & Azhar, S. (1989) *Diabetes* **38**, 1155-1160.