DISCUSSION

Comparison among different rat models of diabetes mellitus with or without hypertension

STZ-induced hypertension is a well-established form of experimental hypertension using the downhill effect in streptozotocin in diabetic rats by Kawashima (1978). Other workers have reported that STZ not only induces diabetes but also hyperglycemia in vivo (Cropani et al. 1978, Handa et al. 1989). In this paper, only STZ-induced rats with hypertension and a rise in blood pressure in early rats. Measurement of blood pressure in normal rats (Kawashima et al. 1980) and animals (or rats) and guinea pigs (Ferris et al. 1981) at various ages (in the present study) showed that STZ-induced diabetic rats showed hyperglycemia, hypertension, and hypertension in other experiments where the combination of hypertension and diabetes has been described. This suggests that the mechanism of hypertension in STZ-diabetic rats may be different from that in diabetic rats without hypertension. While the mechanism of sympathetic nervous system (SNS) activity in rats with type I diabetes mellitus is controversial (References in 1987) and patients with hypertension tend to develop a progressive increase in blood pressure and blood pressure in insulin-dependent diabetes mellitus (IDDM) rats (Cropani et al. 1978, Handa et al. 1989). All new drugs developed for STZ-bolus rats may be beneficial in reducing sympathetic nervous activity, even though their effect on blood vessels is hazy under the current conditions (References in 1987). However, it is not clear whether the action of ACE inhibitors is effective in reducing the renovascular system (RAS) activity when increased in diabetes mellitus (STZ-diabetic rats) (References in 1987). However, it is not clear whether the action of ACE inhibitors is effective in reducing the renovascular system (RAS) activity when increased in diabetes mellitus (STZ-diabetic rats) (References in 1987).
(1) Comparison among different rat models of diabetes-mellitus with or without hypertension

STZ-induced hypertension is a well established form of experimental hypertension since its demonstration in Sprague-Dawley rats by Kawashima (1978). Other workers also reported that STZ not only induces diabetes but also hypertension in rats (Hayashi et al 1983, Funakawa et al 1983). In the present study STZ-diabetes was associated with a rise in blood pressure in Wistar rats. Measurement of blood pressure, directly (Shah et al 1995) and indirectly (Sevak and Goyal 1996) in our laboratory has revealed that STZ-diabetic Wistar rats develop hypertension. Alterations of the prostaglandin and/or the kallikrein-kinin systems, impaired renal prostaglandin E_2 synthesis (Hayashi et al 1993), and altered hypothalamic pressor responses (Sasaki and Bunag 1983) have been suggested to be involved in the pathogenesis of hypertension in STZ-diabetes in rats. Further, Bunag et al (1982a) have suggested that although diabetic rats are predisposed to hypertension, mechanisms such as hypothalamic depression may be activated to restrict further elevation in blood pressure. Rasch (1977) suggested that renal disease may be one of the mechanisms of hypertension in STZ-diabetic rats. Findings from our laboratory support this hypothesis. High serum creatinine levels coupled with several histological changes of renal tissue like ruptured nephron, decreased compactness and loss of cell shape, clustering and picknosis of nuclei as a result of damaged walls of convoluted tubule and glomeruli, indicate renal disease in STZ-diabetic rats (Shah et al 1995). Elevation of blood pressure in STZ-diabetic rats could also be due to increased ACE activity in serum and other tissues and a decrease in plasma renin activity (PRA) (Eman et al 1993). Increase in serum ACE activity could be either a consequence of damaged endothelial cells (Porta et al 1987) or increase in tissue renin-angiotensin system (RAS) activity which may lead to spillover of ACE into circulation (Carbonnel et al 1987). All these changes, following STZ treatment, may contribute to disorders of cardiovascular regulation including blood pressure elevation via their direct effects on body sodium handling and/or through direct vasoconstrictor effects (Tomlinson et al 1990). However, there are other reports that do not support the existence of hypertension in STZ-diabetic rats (Kohler et al 1980, Pfaffman 1980). Yamamoto (1988) reported that STZ-diabetes in Wistar-Kyoto rats reduced the systolic, mean and diastolic blood pressure when it was measured directly in a conscious state with an arterial catheter. Somani et al (1979) reported that blood pressure, when measured directly, in
Wistar-Kyoto rats rises progressively with increasing dose of STZ, whereas, STZ induces a dose- dependent decrease in blood pressure in spontaneously hypertensive (SH) rats. Rodgers et al (1985) measured blood pressure indirectly and reported that STZ induces a depressor effect in SH rats and has no effect in Wistar-Kyoto rats. Further, Kusaka et al (1987) reported similar findings and suggested that measuring blood pressure by the indirect tail-cuff method may result in higher blood pressure values due to emaciation of the rat tail in diabetic rats that may result in structural changes that require extra pressure above the maximum to occlude the tail artery. This controversy led us to select a rat model of simultaneously occurring diabetes mellitus and hypertension where diabetic animals were also made hypertensive by subcutaneous administration of DOCA. This model probably mimics the clinical situation when patients develop hypertension after the onset of diabetes mellitus.

Hebden et al (1990) have reported that the DOCA-hypertensive STZ diabetic rat may be a useful model to study the effects of different pharmacologic agents on atherosclerosis and hyperlipidemia. We found that DOCA-hypertensive STZ diabetic rats developed hypertension that was significantly less severe than that induced by STZ diabetes or DOCA saline treatment alone. This suggests that DOCA probably produces a sort of counteraction to STZ-induced hypertension. Dai and McNeill (1992) also reported a similar counteraction by DOCA to STZ diabetes-induced myocardial dysfunction in rats. Further, DOCA was also found to alter glucose-homeostasis in STZ-diabetic rats. Therefore, we chose the nondiabetic DOCA-hypertensive rat to study the effects of antihypertensives. DOCA saline treatment was found to produce elevation of blood pressure in 10 days from initiation of the treatment schedule. The mechanism by which this treatment increases blood pressure is not fully understood. There are quite a few factors believed to be involved in the pathogenesis of DOCA salt hypertension, however, it is not clear as to what are the most important factors in the pathogenesis of mineralocorticoid-induced hypertension. Initiation of hypertension may involve sodium retention and a subsequent volume expansion, however, it is not likely that sodium retention causes hypertension by means of plasma expansion and increased extracellular fluid volume alone (Schenck and McNeill 1992). Ferrario et al (1987) suggested that increased sodium levels may alter hormonal pressor baroreflexes which can then contribute to the initiation and/or maintenance of hypertension. Several studies have indicated that the involvement and altered activity of the brain renin-angiotensin system is

The SH rats have a genetic predisposition towards hypertension. The pathogenesis of hypertension in this rat model is not well understood. Although, it is not easy to define the control state, this is a useful model of hypertension as it is thought to closely resemble human essential hypertension. Further, this model has been shown to be insulin resistant and hyperinsulinemic (Hulman et al 1991, Mondon and Reaven 1988) and thus seemed to be appropriate for studying the effects of antihypertensive agents on insulin and glucose metabolism. Buchanan et al (1992a) and Frontoni et al (1992) have demonstrated that SH rats are hyperinsulinemic and proposed that increased insulin levels may contribute to the elevation of blood pressure. Reaven and Chang (1991) have shown that hyperinsulinemia precedes the development of hypertension in SH rats. High insulin levels may produce hypertension by one or a combination of the following mechanisms:-

(a) Insulin-induced natriuresis (Endre et al 1994, Kageyama et al 1994)

(b) Activation of sympathetic activity by insulin (Rowe et al 1981)

(c) Trophic effects of insulin on vascular smooth muscle (Banskota et al 1989, King et al 1985)

(d) Insulin-mediated increase in intracellular sodium levels (Boon et al 1985, Canessa et al 1984, Postnov and Orlov 1985) which could sensitize the arteriolar smooth muscle cells to the pressor effects of catecholamines and angiotensin II

(e) Resistance to insulin-mediated attenuation of vascular smooth muscle calcium influx through both receptor and voltage-operated calcium channels (Standley et al 1993) which would cause an increase in intracellular calcium levels and a consequent enhancement of vascular tone and blood pressure.
Injection of STZ to SH rats was found to further elevate blood pressure. Similar findings have been reported earlier (Sevak and Goyal 1996). STZ-treated SH rats were found to develop severe apoplexy and showed a high mortality. This could be due to severe stress caused by simultaneous presence of diabetes and high blood pressure.

Rats treated with STZ on day 5 of life were found to develop hypertension at 16 weeks of age. These rats were also found to be hyperinsulinemic. The mechanism of development of hypertension in the neonatal STZ-diabetic rats is not clear. Hypertension observed in the hyperinsulinemic neonatal STZ-diabetic rats probably develops through one or a combination of the above mentioned effects of insulin.

Intravenous administration of STZ to adult Wistar, DOCA-hypertensive and SH rats produced loss of body weight. Hofteizer and Carpenter (1973) also reported loss of body weight in STZ-treated rats. They suggested that loss of body weight could be due to dehydration and the catabolism of fats and proteins seen during diabetes mellitus. Treatment of Wistar rats with DOCA produced significant reduction in body weight initially up to 1 week. However, after 1 week the animals gained weight and their body weight was comparable to the control Wistar rats. This was probably due to acclimatization of animals. STZ administration to 5 day old Wistar rat pups produced retardation in weight gain initially, however, neonatal STZ-diabetic rats gained weight which was comparable to Wistar rats at 16 weeks of age. SH rats had lower body weight when compared to control Wistar rats. Genetic hypertension in these rats probably resulted in retardation in weight gain.

STZ-diabetic rats exhibit polyphagia, polydipsia and glycosuria (>2%) which are considered as cardinal signs of diabetes mellitus (Sevak and Goyal 1996, Shah et al 1995, Rodrigues et al 1986). In our studies also we found these signs in Wistar, DOCA-hypertensive and SH rats. The food and water intake of neonatal STZ-diabetic, DOCA-hypertensive and SH rats was comparable to control Wistar rats. This further affirms that polyphagia, polydipsia and glycosuria are the cardinal signs of STZ-induced diabetes-mellitus.

Bradycardia has been frequently observed in STZ-diabetic rats (Rodrigues et al 1986, Joshi et al 1996, Sevak and Goyal 1996). The development of STZ-induced
bradycardia has been attributed to a down regulation of myocardial beta-adrenoceptors and an increase in circulation and heart levels of catecholamines (Savaress and Berkowitz 1979). In our laboratory we found that STZ diabetes produced hypothyroidism (Sevak and Goyal 1996). Goyal et al (1987) have reported similar findings. Hypothyroidism induced by STZ may be another factor responsible for changes in myocardial adrenoceptors (Goyal et al 1987). Goyal et al (1987) reported that bradycardia in STZ-diabetic rats was a consequence of hypothyroidism in these rats. They demonstrated that administration of triodo-1-thyronine prevented bradycardia in hypothyroid STZ-diabetic rats. Data from several laboratories indicate that both, hypothyroidism and diabetes mellitus cause a decrease in beta-adrenoceptor activity and an increase in alpha-adrenoceptor activity (Kunos et al 1974, Ciaraldi and Marinetti 1977, Simpson et al 1981, Heyliger et al 1982, Goyal et al 1987). Further, quantitative changes in the calcium channels in STZ-diabetic rats may be responsible for hypothyroid bradycardia as changes in electrical properties of cardiac tissues can produce depression of chronotropic functions. Altered myocardial calcium metabolism and reduced uptake of calcium by the sarcoplasmic reticulum ATPase activity has been reported in STZ-diabetic rats (Nordin and Gilat 1990, Legayle and Beiglemann 1988, Ganguly et al 1983). Moreover, diminished calcium uptake, calcium ATPase (Heyliger et al 1987, Makino et al 1987), sodium calcium exchange, sodium potassium ATPase activity (Pierce and Dhalla 1983) and sodium hydrogen exchange (Pierce and Dhalla 1990) of sarcolemmal membranes from STZ treated rats have also been reported. In contrast to STZ-diabetic rats, heart rate in neonatal STZ-diabetic, DOCA-hypertensive and SH rats was not significantly different from control Wistar rats.

Insulin sensitivity was assessed in vivo by oral glucose tolerance test (OGTT) and by insulin tolerance test (ITT). OGTT is a closed-loop method that is simple and indirectly measures the action of endogenous insulin in response to a glucose stimulus. This method does not allow the separate evaluation of beta islet cell or peripheral dysfunction since the interrelationship between beta islet cells and peripheral insulin sensitive tissue still exists. AUC glucose (mg/dl . min) indicates insulin-stimulated glucose disposal whereas AUC insulin (U/ml . min) indicates insulin response to glucose stimulus. In the present study these parameters were used to estimate insulin sensitivity. Several workers argue that mere AUC glucose and AUC insulin do not give any idea about insulin sensitivity and that only the euglycemic-hyperinsulinemic method should be performed to
assess insulin sensitivity. Although it is true that the euglycemic-hyperinsulinemic method of assessment of insulin sensitivity is the gold standard method, AUC glucose and AUC insulin are simple and can still be considered as reliable means of assessing insulin response to external oral glucose stimulus and endogenously released insulin-mediated glucose disposal. They can be useful in deciding if there is an improvement or a decrease in insulin sensitivity when AUC glucose and AUC insulin of the control animals are compared with those of the chronically treated animals. ITT, on the other hand provides direct measurements of the metabolic responses to exogenous insulin. The peripheral insulin resistance as measured by ITT is a net result of defect in insulin action at the receptor or post receptor levels at different sites such as the liver and the target tissues like skeletal muscle and adipocytes. Peripheral insulin resistance is ideally assessed in vivo by the euglycemic-hyperinsulinemic clamp technique (Bergman et al 1985). However, the insulin concentrations required during clamp technique are usually much higher than physiologic concentrations of insulin. Moreover, 3-4 ml of blood is needed to perform the test. Compared to euglycemic method, ITT is a simple procedure and gives equally good assessment (Prato et al 1986). We therefore chose to use ITT to assess peripheral insulin resistance.

STZ-treatment produced hyperglycemia and hypoinsulinemia in Wistar and DOCA-hypertensive rats. In SH rats STZ-treatment produced a significant decrease in elevated insulin levels. STZ, by its selective beta-cell toxic effect produces fragmentation of pancreatic beta-cell DNA due to a general alkylating effect thereby stimulating poly (ADP-ribose) synthase and NAD depletion. Depletion of NAD has cytotoxic effect on islet beta cell. Pancreatic beta cell death causes insulin deficiency that eventually causes hyperglycemia in STZ treated rats. Hyperglycemia in STZ treated DOCA-hypertensive rats was milder. This suggests that DOCA may have some action on glucose metabolism.

Neonatal administration of STZ has been reported to produce destruction of beta-cells resulting in hypoinsulinemia and severe hyperglycemia in these rat pups (Weir and Clore 1981). However, as the rats grow there appears to be partial regeneration of pancreatic beta-cells along with development of characteristics of NIDDM such as hyperinsulinemia and mild hyperglycemia. Bonora et al (1983) have reported hyperinsulinism with low hepatic extraction and hypersecretion of beta cells in mild glucose intolerance and in obese subjects. We found normal beta cell secretion (as indicated by
insulin time-concentration curves and AUC insulin after a glucose load) in neonatal STZ-diabetic rats which suggests that the peripheral hyperinsulinemia in neonatal STZ-diabetic rats is probably not due to increased beta cell secretion. In these rats, the metabolic clearance rate of insulin might have been altered. Most of the insulin degradation has been demonstrated to follow hormone receptor binding (Gliemann and Sonne 1978). Reduced binding of insulin to its receptor is reported in mild glucose intolerance (Olefsky 1981). Therefore, the hyperinsulinemia in neonatal STZ-diabetic rats could be due to decreased hepatic extraction of insulin and/or decreased number of insulin receptors, resulting in decreased insulin binding and lowered insulin degradation. Therefore, the high insulin concentration found in neonatal STZ-diabetic rats need not be pancreatic in origin. It could also be due to metabolic alterations at extra pancreatic levels. The presence of hyperinsulinemia, higher AUC glucose and low KITT values in neonatal STZ-diabetic rats indicate insulin resistance in these rats. The specific mechanisms underlying in insulin resistant states are heterogeneous and may include a decrease in insulin sensitivity (receptor defect), or a decrease in responsiveness to insulin (post receptor defect), or a combination of both processes (Kahn 1978). Studies show that post receptor defects are more important than defects at the site of receptors (Crettaz and Jeanrenand 1980).

Further, insulin resistance may be peripheral or hepatic. Thourburn et al (1995) reported that active glycogen synthase in skeletal muscle was reduced in New Zealand obese (NZO) mice when compared to New Zealand lean (NZL) mice. Ortmeyer (1996) reported that NIDDM is accompanied by low glycogen content in the muscle of overtly diabetic Rhesus monkey. Therefore, it appears that development of hyperinsulinemia is also related to alteration in insulin action on muscle glycogen synthase, glycogen phosphorylase and whole body glucose disposal rates. The glycogen content in liver and skeletal muscle in neonatal STZ-diabetic rats was comparable to Wistar control rats suggesting that there is probably no defect in peripheral uptake of glucose and neither is there any increase in hepatic glucose output. Further, the activity of liver phosphorylase enzyme, which is an insulin-dependent enzyme responsible for glycogenolysis was decreased in neonatal STZ-diabetic rats suggesting that the inactivation of phosphorylase is not resistant to insulin action. However, until further studies are done the mechanism for insulin resistance in neonatal STZ-diabetic rats remains unclear.
SH rats were found to be hyperinsulinemic. Hyperinsulinemia in SH rats has been reported by other laboratories (Bhanot et al 1994). Hyperinsulinemia in SH rats may be secondary to insulin resistance (Hulman et al 1991, Mondon and Reaven 1988) and/or due to decreased insulin clearance in SH rats (Mondon et al 1989). We found lower insulin sensitivity index for SH rats when compared to Wistar controls suggesting that SH rats are probably insulin resistant. Reaven et al (1989 a) have reported a lower insulin stimulated glucose uptake in SH rat adipocytes and this decrease was observed despite normal insulin receptor number, affinity and tyrosine kinase activity suggesting that the defect in insulin action resided distal to the insulin receptor (Reaven et al 1989a). However, interesting results were revealed by OGTT in SH rats. AUC insulin was unaltered, whereas, AUC glucose was significantly higher in SH rats when compared to Wistar controls. The time-concentration curves showed that insulin release and insulin-mediated glucose disposal was impaired in SH rats when compared to Wistar controls. These findings further confirm insulin resistance in SH rats. It was interesting to note that there was a significant increase in skeletal muscle glycogen in SH rats. This would suggest better uptake of glucose peripherally. The mechanism for this increase despite a decrease in insulin sensitivity index in SH rats, remains unclear. It is, however, possible that the skeletal muscle glucose-uptake is not the site of insulin resistance in SH rats. Liver or kidney may be the other sites of insulin resistance in these rats.

DOCA has been reported to decrease plasma insulin levels in male Sprague-Dawley rats (Dai and McNeil 1992). However, in the present study DOCA treatment was not found to alter serum insulin levels in female Wistar rats. In DOCA-hypertensive rats the AUC insulin was lower, although the difference was not significant and the AUC glucose was comparable to Wistar controls. Interestingly, although the KTT value of these rats was comparable to Wistar control rats, the time-concentration curves revealed enhanced glucose disposal in DOCA-hypertensive rats when compared to Wistar controls suggesting that DOCA may have an action on glucose metabolism, either directly inhibiting the assimilation or production and/or increasing the utilization of glucose or indirectly enhancing the metabolic effects of insulin. Similar findings have been reported by Dai and McNeil (1992). DOCA treatment was also found to produce a decrease in liver glycogen and increase in skeletal muscle glycogen again suggesting that DOCA probably has some effect on glucose metabolism in Wistar rats.
STZ diabetes produced dyslipidemia in Wistar, DOCA-hypertensive and SH rats. It has been well documented that diabetes mellitus is associated with changes in lipid metabolism. Rats treated with STZ have increased plasma levels of triglycerides, total cholesterol, free fatty acids and phospholipids (Rodrigues et al. 1986). In STZ diabetes insulin deficiency may be responsible for dyslipidemia. Insulin has an inhibitory action on HMG CoA reductase, a key rate limiting enzyme in the metabolism of cholesterol rich LDL particles. Hypoinsulinemia would therefore, be responsible for the elevation of cholesterol levels. The mechanisms responsible for the development of hypertriglyceridemia in uncontrolled diabetes in humans (and possibly in insulin deficient STZ-diabetic rats) are due to number of metabolic abnormalities that occur sequentially. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue, resulting in increased secretion of VLDL-triglyceride from liver (Balasse et al. 1972). With longer insulin deficiency, liver converts free fatty acids into ketone bodies, and VLDL-triglyceride secretion diminishes (Basso and Havel 1970). At the same time, lipoprotein lipase activity falls (Nikkila et al. 1977) resulting in impaired clearance of VLDL and chylomicrons from plasma (Bagdade et al. 1968).

Neonatal STZ-diabetic and SH rats had serum total cholesterol comparable to control Wistar rats. Serum triglycerides were normal in neonatal STZ-diabetic rats, however, they were significantly low in SH rats. This indicated that these models of diabetes and/or hypertension do not develop dyslipidemia. In neonatal STZ-diabetic rats it appears that though there is resistance to the glucoregulatory action of insulin, the insulin action in maintaining lipid homeostasis via its action on lipoprotein lipase is probably not impaired. Interestingly, DOCA-hypertensive rats were found to have low serum total cholesterol and triglycerides when compared to Wistar control rats. Dai and McNeill (1992) and Hebden et al. (1990) have reported that DOCA hypertension is associated with an increase in plasma cholesterol and triglycerides. Although, these workers have suggested that DOCA increases insulin sensitivity they have not specified as to why then there is a disturbance in lipid profile in the DOCA-hypertensive rat. If there was an improvement in insulin sensitivity with DOCA then the lipid levels in DOCA-saline treated rats should have been normal since insulin plays a vital role in lipid metabolism. In the present study DOCA hypertension was associated with significantly lower levels of serum total cholesterol levels and triglycerides when compared to control Wistar rats. These findings suggest that DOCA probably enhances insulin action on lipid metabolism.
STZ treatment results in changes in various hepatic enzyme systems. Many fundamental alterations in hepatic function related to changes in metabolism also occur following STZ treatment. Since liver is a very important organ involved in carbohydrate metabolism, uncontrolled hyperglycemia could lead to adverse effects on the liver. In patients with maturity onset diabetes fatty infiltration of hepatocytes occurs. This may be due to hyperlipidemia. Elevated activity levels of alkaline phosphatase, serum glutamate oxaloacetate translocase (GOT) and glutamate pyruvate translocase (GPT) have been reported in patients with NIDDM (Falchuk and Tray 1985). High levels of serum GPT indicate deterioration of liver function with degeneration of liver tissue. Serum levels of GPT give an idea about the liver function. In the present study serum GPT levels in STZ treated Wistar, DOCA-hypertensive and SH rats were found to be elevated. Relative hypoxia is an important factor in the development of tissue change in diabetes (Ditzel and Standl 1975). Serum GPT levels in neonatal STZ-diabetic and DOCA-hypertensive rats were comparable to control Wistar rats whereas, those in SH rats were higher when compared to control Wistar rats. Long standing hypertension in the genetically hypertensive SH rats probably causes microvascular changes in liver leading to liver damage.

Rats treated with STZ also develop changes in renal function including altered renal haemodynamics and structural changes which can be attributed to the development of diabetes and are relevant when considering cardiovascular control (Tomlinson et al 1990). STZ has no significant nephrotoxic potential and its direct effect on the kidney need not be considered when using the drug in order to study the effect of diabetes on renal function and structure (Evan and Mong 1984). Rise in serum creatinine levels has also been reported in patients with diabetes mellitus (Thomson et al 1989). STZ treatment in Wistar, DOCA-hypertensive and SH rats resulted in elevation of serum creatinine levels. Neonatal STZ-diabetic, DOCA-hypertensive and SH rats had normal serum creatinine levels when compared to control Wistar rats.

In the light of the above discussion it may be concluded that STZ treatment produces all the cardinal signs of diabetes-mellitus, namely, loss of body weight, polyphagia, polydipsia, hyperglycemia, hyperlipidemia, renal and hepatic damage. Bradycardia and hypothyroidism were also induced by STZ diabetes. Hypertension was
seen in all the rat models used namely, the STZ-diabetic rat, the neonatal STZ-diabetic rat, the SH rat and the DOCA-hypertensive rat. Although, all the rat models manifested a disruption in the ‘insulin-glucose’ balance to varying degrees, there were differences in the mechanism of induction of such an imbalance. STZ-diabetic rats were hypoinsulinemic, whereas, NIIDM and SH rats were hyperinsulinemic and insulin resistant. The STZ-diabetic, neonatal STZ-diabetic and SH rats may be considered as good animal models for studying the effects of chronic treatment with antihypertensives on metabolic status as they appear to be models for at least some mechanisms leading to hypertension and metabolic disturbances in humans. On the other hand, the DOCA-hypertensive rat may not be a suitable model for studying the effects of antihypertensives on insulin sensitivity as DOCA itself appears to influence insulin and glucose homeostasis.

(2) Effect of different antihypertensives on cardiovascular and metabolic parameters in the rat models of diabetes and/or hypertension

Effects of different antihypertensives on the general and cardiovascular parameters in the rat models of diabetes and/or hypertension

Treatment with nifedipine, amlodipine, ramipril or spirapril prevented STZ-induced loss of body weight, polyphagia and polydipsia in STZ-diabetic Wistar rats. Polyphagia and polydipsia may be a consequence of STZ-induced impairment in glucose utilization, hyperglycemia and disrupted lipid profile. Nifedipine, amlodipine, ramipril and spirapril were found to lower the elevated glucose levels and also correct the metabolic imbalance to a certain extent. This may in turn have led to a decrease in food and water intake in STZ-diabetic rats.

Treatment of 5-day old rat pups with STZ produced retardation in gain in body weight, however, the body weight of 16 weeks old neonatal STZ-diabetic rats was comparable to age-matched Wistar control rats suggesting that neonatal STZ administration does not produce any change in body weight in later life. SH rats as such, showed a retarded gain in body weight. Treatment with all the antihypertensives used did not alter body weight in neonatal STZ-diabetic, SH and DOCA-hypertensive rats.
Food and water intake of neonatal STZ-diabetic, SH and DOCA-hypertensive animals was comparable to control Wistar rats. Chronic treatment with the antihypertensives did not produce any change in food or water intake in neonatal STZ-diabetic, SH and DOCA-hypertensive rats.

STZ-diabetic rats were found to have significantly lower heart rate when compared to control Wistar rats. Bradycardia in STZ-diabetic rats has also been reported by other workers (Rodrigues et al 1988). Mechanisms responsible for bradycardia in STZ-diabetic rats may be the downregulation of myocardial beta-adrenoceptors, the increase in heart levels of catecholamines (Savaress and Berkowitz 1979) and/or hypothyroidism (Goyal et al 1987, Sevak and Goyal 1996). Treatment with nifedipine, amlodipine and spirapril prevented bradycardia in STZ-diabetic rats. Chronic treatment with these agents prevented not only STZ-induced bradycardia, but also hypothyroidism as indicated by the significantly low levels of serum thyrotropin (TSH). ACE inhibitor lisinopril has also been reported to prevent STZ-induced bradycardia and also produce an increase in the serum tri-iodothyronine (T3) levels in STZ-diabetic rats, suggesting that hypothyroidism may be one of the major causes of bradycardia in STZ-diabetic rats (Sevak and Goyal 1996). In the present investigation, treatment with ramipril was found neither to prevent bradycardia nor hypothyroidism in STZ-diabetic rats. This again suggests that hypothyroidism may be one of the causes of STZ-induced bradycardia. Heart rate of neonatal STZ-diabetic and SH rats was comparable to control Wistar rats. Heart rate in Wistar rats was not altered by DOCA-saline schedule. Chronic treatment with the antihypertensives used in the present study did not alter heart rate in neonatal STZ-diabetic, SH and DOCA-hypertensive rats. Short term treatment with nifedipine produces tachycardia (Lyons et al 1994). In the present study, however, chronic treatment with nifedipine did not produce any such effect in STZ-diabetic, neonatal STZ-diabetic or SH rats.

Blood pressure in STZ-diabetic, neonatal STZ-diabetic, SH and DOCA-hypertensive rat rats was elevated. Chronic treatment with the antihypertensives used in the present study were found to prevent hypertension in STZ-diabetic, neonatal STZ-diabetic, SH and DOCA-hypertensive rats. Ramipril was found to lower elevated blood pressure in DOCA-hypertensive rats, however, the decrease in blood pressure was not significant. The mechanism of antihypertensive action of calcium antagonists involves an increase in the free intracellular level of calcium which is required for the contraction of
cardiac and vascular smooth muscle cells (Lyons et al 1994). When these cells are stimulated, voltage sensitive channels in the cell membrane are opened, allowing a very small quantity of the large concentration of extracellular calcium to enter the cell (Lyons et al 1994). The resultant small increase in free intracellular calcium initiates a release of larger amounts of stored calcium within the sarcoplasmic reticulum. The combined higher levels of free intracellular calcium combine with calmodulin to activate the enzyme myosin kinase. This binds actin to myosin and leads to contraction of the cell (Lyons et al 1994).

In addition to this contractile effect, free calcium is needed for the formation and conduction of the impulse in the sinoatrial and atrioventricular nodes of the heart. Calcium antagonists block the voltage sensitive calcium channel and reduce the entry of calcium into the cells. Since cardiac and smooth muscle cells are much more dependent than skeletal muscle cells on this extracellular calcium to initiate their contraction, the contraction of smooth muscle cells and not skeletal muscle cells is thereby inhibited (Lyons et al 1994). The decrease in the level of free intracellular calcium leads to a decrease in the force of contraction and the tone of vascular smooth muscle, thereby reducing peripheral resistance and lowering blood pressure. Calcium antagonists predominantly affect the voltage dependent calcium channels rather than the receptor operated calcium channels. What adds to the antihypertensive effect of calcium antagonists is their diuretic and mild natriuretic action which has been attributed to their selective vasodilatory action on the renal afferent arterioles.

In the present study amlodipine was found to attenuate hyperinsulinemia in SH and neonatal STZ-diabetic rats. Since high circulating levels of insulin may contribute to rise in blood pressure in one or more ways, as mentioned in earlier sections, attenuation of hyperinsulinemia may contribute, in part, to the antihypertensive effect of amlodipine in SH and neonatal STZ-diabetic rats.

Use of ACE inhibitors in diabetics is particularly advantageous since they are either neutral or may actually improve glycemic control and lipid profile (Kendall et al 1988). The mechanism of action of ACE inhibitors relates to the renin angiotensin system which plays a central role in cardiovascular homeostasis and in the aetiology of hypertension (Kostis 1988). ACE inhibitors inhibit the conversion of angiotensin I (Ang I) to the powerful vasoconstrictor angiotensin II (Ang II) both in the circulation and in the vascular and other tissues. They reduce aldosterone secretion to induce a natriuresis.
They specifically enhance renal vasodilation resulting in natriuresis and inhibit the degradation of vasodilator, bradykinin. Further actions include inhibition of Ang II mediated adrenergic vasoconstriction. Several studies suggest that ACE inhibitors exert their effect primarily by inhibiting local angiotensin II production (Unger et al. 1984, Unger et al. 1985, Agürela et al. 1981, Campbell and Haebner 1986, Naftilan et al. 1991).

**Effect of different antihypertensives on serum lipids and hepatic and renal function in the rat models of diabetes and/or hypertension**

The abnormalities in lipid profile which are often present in diabetic hypertensive patients accelerate atherosclerosis (Messerli and Grossman 1996). Total cholesterol, triglyceride and low density lipoprotein (LDL) levels are elevated, high density lipoprotein (HDL) levels are decreased, there is augmented oxidation of LDL cholesterol and accelerated formation of glycated LDL (Messerli and Grossman 1996). All these factors enhance foam cell formation which in turn leads to atherosclerosis. Therefore, it would be beneficial to use antihypertensives that have beneficial or neutral effect on lipid profiles or do not, at least, worsen it. Rats treated with STZ had high serum levels of total cholesterol and triglycerides. Similar findings have been reported by other workers (Rodrigues et al. 1988). Insulin plays a role in both production and removal of triglyceride-rich proteins and therefore, insulin deficiency may lead to defective removal of triglyceride-rich lipoproteins (New et al. 1963). Insulin administration has been shown to restore normal lipid profile in insulin deficient humans (Nikkila et al. 1977). Neonatal STZ-diabetic and SH rats had serum total cholesterol levels comparable to Wistar control rats. Although serum triglyceride levels in neonatal STZ-diabetic rats were comparable to Wistar control rats, in SH rats they were significantly lower. Houston et al. (1990) demonstrated that nifedipine produces significant increase in HDL, HDL-2 and apolipoprotein A-I and A-II levels. Nifedipine (Shah et al. 1995) and nitrendipine (Joshi et al. 1996) have been shown to prevent hyperlipidemia in STZ-diabetic rats. Klauser et al. (1991) reported that isradipine had neutral effects on serum lipids. In the present study treatment with nifedipine and amloidipine was found to lower total cholesterol levels in STZ-diabetic rats. In neonatal STZ-diabetic or SH rats amloidipine and nifedipine produced either a decrease or no effect on serum total cholesterol and triglycerides. This is in agreement with several studies that indicate beneficial or neutral effect of calcium channel blockers on lipid levels (Gotto 1990, Houston et al. 1990, Shah et al. 1995, Joshi et al. 1996).
Treatment with ACE inhibitor captopril (50 mg) for 3 months has been reported to correct hypercholesterolemia and dyslipidemia in hypertensive patients (Catalano et al 1992). Enalapril (20 mg, for 10 weeks) was found to have neutral effects on serum total and lipoprotein lipid fractions and also on apolipoprotein A-I and B in diabetic hypertensive patients (Ferrier et al 1992). However, Libertti and Catalano (1993) demonstrated that enalapril significantly reduced the total cholesterol, triglyceride and LDL-cholesterol levels in hypertensive patients. Lisinopril (Sevak and Goyal 1996) and perindopril (Reddy 1996) have also been shown to prevent hypercholesterolemia in STZ-diabetic rats. Hasslacher (1996) demonstrated that lisinopril treatment (2.5, 5, 10 and 20 mg) for 3 months in NIDDM patients significantly decreases total cholesterol, triglycerides and LDL-cholesterol, at the same time, the HDL cholesterol levels are increased. Thurg et al (1995) demonstrated that lisinopril is neutral to serum lipoproteins in essential hypertensive patients. In the present study ramipril and spirapril were found to significantly improve dyslipidemia in STZ-diabetic rats. Ramipril treatment was not found to alter total total cholesterol and triglyceride levels in neonatal STZ-diabetic rats. In SH rats ramipril was found to decrease the serum triglycerides. Treatment with spirapril was not found to alter serum triglycerides in neonatal STZ-diabetic or SH rats. Although, various studies (Weidmann et al 1993, Weidmann et al 1988, Berne et al 1991, de Courten et al 1993, Trost and Weidmann 1988b) have indicated that ACE inhibitors are metabolically neutral, treatment with spirapril in the present study was found to produce an elevation in serum total cholesterol concentration in neonatal STZ-diabetic rats. This may indicate that long term treatment with spirapril probably disrupts the lipid metabolism. However, the mechanism for this remains obscure. DOCA-hypertensive rats were found to have significantly lower levels of serum total cholesterol and triglycerides when compared to control Wistar rats. Treatment with nifedipine, amlodipine, ramipril or spirapril was not found to alter serum levels of total cholesterol and triglycerides in DOCA-hypertensive rats.

Many fundamental alterations in hepatic function related to changes in metabolism occur following STZ treatment (Sevak and Goyal 1996, Joshi et al 1996, Reddy 1996). Elevated levels of alkaline phosphatase, serum GOT and serum GPT have been reported in STZ-diabetic rats (Sevak et al 1996, Joshi et al 1996, Reddy 1996). Falchuk and Tray (1985) reported similar findings in NIDDM patients. In the present study STZ-diabetes
was associated with high serum levels of GPT. Serum levels of GPT were not altered in neonatal STZ-diabetic rats whereas, they were significantly elevated in SH rats. Long standing hypertension in SH rats may be responsible for liver damage in these rats. Sevak and Goyal (1996) have shown that ACE inhibitor lisinopril has beneficial effect on liver function in STZ-diabetic rats. Similar findings were reported with calcium antagonist nitrendipine (Joshi et al 1996). In the present study treatment with amlodipine, ramipril or spirapril lowered the elevated serum GPT concentrations in STZ-diabetic rats suggesting that these agents may have a protective effect on liver function in these rats. Nifedipine was found to significantly lower serum GPT levels in SH rats. All the antihypertensive agents used reduce peripheral vascular resistance and improve tissue blood flow. This effect may be responsible for the protection of liver damage in STZ-diabetic rats.

STZ-diabetic rats were found to have high levels of serum creatinine. Hyperglycemia leads to elevated glucose levels in mesangial cells and this activates protein kinase C (Kreisberg et al 1994a) which in turn increase synthesis of fibronectin, laminin and type (IV) collagen (Kreisberg et al 1994b). Thus, there occurs an imbalance in matrix proteins leading to development of mesangial hypertrophy and eventually nephropathy. Increase in blood pressure occur early in diabetic renal disease (Ritz et al 1989, Mogensen and Christensen 1985). Hyperfiltration and microproteinuria, resulting from glomerular leakage, are hallmarks of diabetic nephropathy (Corry and Tuck 1986). On the other hand, the early phase of hypertensive renal disease is characterised by a normal glomerular filtration rate (GFR), a decrease in renal blood flow (RBF), and elevated filtration fraction. Untreated hypertension is therefore, associated with declining renal function (National High Blood Pressure Education Program 1991). The combined presence of hypertension and diabetes will therefore affect both RBF, accelerating the decline in renal function. Rigorous control of blood pressure in diabetic hypertensive patients may slow down progression of renal disease. Further, several studies show an inverse correlation between creatinine clearance and arterial pressure in diabetic patients (Ritz et al 1989, Mogensen and Christensen 1985). SH and neonatal STZ-diabetic rats had serum creatinine levels comparable to Wistar control rats. Since impaired renal function is considered an independent predictor of cardiovascular morbidity and mortality (Liungman 1990, Yudkin et al 1988), antihypertensives that improve or, at least, do not worsen renal function may produce improvement of cardiovascular and/or renal prognosis in diabetic hypertensive patients. Calcium antagonists such as verapamil or diltiazem are
effective in microalbuminuria (Weidmann 1996), whereas, nifedipine (in its short-acting form) is reported to be ineffective (Mimran et al 1989). Another study showed that calcium antagonists are beneficial in microalbuminuria (Baba et al 1989). Nicardipine has been reported to decrease renal vascular resistance and thereby improve renal function (Hamedouche et al 1990). Bauer et al (1985) reported that short or long-term administration of calcium antagonists did not produce any consistent changes in GFR or effective renal blood flow. However, in patients with hypertension with or without concomitant glomerulonephritis, increases in GFR and RBF were observed when treated with nifedipine or diltiazem (Reams et al 1988, Sunderrajan et al 1986). In contrast, Bellini et al (1984) showed that a single oral dose of nifedipine significantly reduces GFR in hypertensive patients on normal or low sodium diet. Several studies demonstrate an acute natriuretic effect of calcium antagonists without any changes in renal function, although, the magnitude of the effect appears to be variable with different agents (Bauer et al 1985, Kubo et al 1986). However, there has been one report of acute reversible deterioration in renal function after the use of nifedipine in four patients with chronic renal insufficiency (Diamond et al 1984). In our laboratory calcium antagonist nitrendipine has been shown to lower elevated serum creatinine levels in STZ-diabetic rats (Joshi et al 1996). In the present study chronic treatment with nifedipine and amlodipine did not alter serum creatinine levels in neonatal STZ-diabetic, SH and DOCA-hypertensive rats.

ACE inhibitors are renoprotective. ACE inhibitors have been reported to significantly decrease glomerular pressure, which results in a marked reduction of renal sclerosis and renal deterioration (Brenner 1985, Azar et al 1977). Wee and Epstein (1993) carried out a meta-analysis and reported that short-term (< 12 months' duration) treatment with ACE inhibitors either does not modify or slightly increases GFR and does not alter RBF. Renal protein excretion was reduced in 20 of the 23 studies. Bianchi et al (1991, 1992) and Bigazzi et al (1993) demonstrated that enalapril significantly decreased urine protein excretion. Several studies have shown that enalapril (Lebowitz et al 1994), captopril (Viberti et al 1994) and other ACE inhibitors (Ravid et al 1993) markedly reduce the progression from incipient to overt diabetic nephropathy. Further, Weidmann et al (1995) reported that in patients with already established overt diabetic nephropathy, ACE inhibitors also tend to preserve GFR. Lisinopril was found to produce a fall in serum creatinine levels in 46 % of NIDDM hypertensive patients with elevated pretreatment values, but there was no change in serum levels of creatinine in patients with normal
pretreatment values (Hasslacher 1996). In our laboratory ACE inhibitors lisinopril (Sevak and Goyal 1996) and perindopril (Reddy 1996) have been shown to lower elevated serum levels of creatinine in STZ-diabetic rats. Weidmann (1996) also reported that ACE inhibitors are at present the first choice for treatment of microalbuminuria or overt proteinuria in normotensive or mildly hypertensive diabetic patients. In the present study ACE inhibitor ramipril was not found to alter serum creatinine levels in STZ-diabetic rats. ACE inhibitors ramipril and spirapril were not found to alter serum creatinine levels in neonatal STZ-diabetic, SH and DOCA-hypertensive rats. The effects of the calcium antagonists and ACE inhibitors on the serum levels of GPT and creatinine suggest that chronic treatment with these agents may not have toxic effects on the renal or hepatic tissue. Sevak and Goyal (1996) have reported that DOCA hypertension does not alter serum GPT and creatinine levels. Similar findings were observed in the present study suggesting that DOCA-saline schedule does not have toxic effects on the liver and kidney tissue. Treatment with the antihypertensives revealed that the liver and kidney functions are not affected by chronic treatment with these agents in DOCA-hypertensive rats.

**Effect of different antihypertensives on insulin and glucose in the rat models of diabetes and/or hypertension**

Studies with calcium antagonists, albeit, in isolated perfused pancreas and islet cell plasma membranes have shown a dose related decrease in insulin output with nicardipine, a dihydropyridine calcium antagonist (Marre and Fressinaud 1990). In this context, calcium antagonists may theoretically alter insulin release and consequently affect glucose tolerance in NIDDM patients and individuals with borderline glucose intolerance. Morris et al (1993) demonstrated that a 2-week treatment with lacidipine had no influence on insulin sensitivity evaluated by the glucose clamp technique in healthy, male volunteers. Pollare et al (1999b) demonstrated, in their randomized, double blind, parallel comparison study, that diltiazem (mean dose of 329 mg/Kg) does not produce any change in insulin mediated glucose uptake. Several other clinical trials do not show any clinically important effect on glucose tolerance when calcium antagonists are used in therapeutic doses (Collins et al 1987, Faguer de Moustier and Paoli 1990, Hedner et al 1987, Kihara 1991, Marre and Fressinaud 1990, Tebtorio et al 1989, Pasanisi et al 1989). This was confirmed by a continuous long term study in elderly NIDDM patients on antihypertensive monotherapy with nitrendipine that, antidiabetic regimen and body weight
remaining constant, no change in carbohydrate homeostasis was found for upto 5 years (Trost and Weidmann 1988a). Shah et al (1995) reported that despite decreasing serum insulin levels, chronic treatment with nifedipine did not impair glucose homeostasis in Wistar rats. Satia (1995) reported that a reduction in the dose of oral hypoglycemic agent was necessary in diabetic-hypertensives treated with nifedipine. In the present study nifedipine was found to further decrease serum insulin levels in STZ-diabetic rats. Impairment of insulin secretion by nifedipine has been reported in non-diabetic individuals (Charles et al 1981) and in patients with impaired glucose tolerance (Gugliano et al 1980). On the other hand, treatment with amlodipine did not produce further decrease in insulin levels in STZ-diabetic rats. However, both nifedipine and amlodipine were found to significantly lower the elevated serum glucose levels in STZ-diabetic rats. The observation that despite lower levels of insulin, glucose levels were decreased suggests that both nifedipine and amlodipine probably improve insulin sensitivity in STZ-diabetic rats.

As mentioned earlier SH rats were hyperinsulinemic with normal glucose levels when compared to Wistar controls. On the other hand, although, neonatal STZ-diabetic rats were hyperinsulinemic they showed mild hyperglycemia. Chronic treatment with nifedipine and amlodipine was found to significantly attenuate hyperinsulinemia in SH rats. Despite lowering of insulin levels, the serum glucose levels were not altered. This suggests that these agents probably improve insulin sensitivity in SH rats. The finding that both these agents produced an increase in $K_{ITT}$ further substantiate the hypothesis that nifedipine and amlodipine produce improvement in insulin sensitivity. The results of OGTT further strengthen this contention. Nifedipine and amlodipine were found to significantly decrease the AUC glucose with a concomitant decrease in AUC insulin in neonatal STZ-diabetic rats. The nifedipine treated neonatal STZ-diabetic and SH rats showed significantly greater concentrations of muscle glycogen indicating that nifedipine probably improves insulin sensitivity by enhancing peripheral uptake of glucose in these rats. Chronic treatment with nifedipine in SH rats was not found to alter AUC insulin or AUC glucose. Amlodipine treatment was found to produce a significant decrease in AUC insulin without any change in AUC glucose in SH rats. There was a significant decrease in insulin released after a glucose challenge in neonatal STZ-diabetic and SH rats treated with amlodipine. This was still associated with effective lowering of glucose levels in neonatal STZ-diabetic and SH rats. This further supports the contention that amlodipine improves insulin sensitivity in rats.
Nifedipine treatment did not alter the plasma glucose or insulin levels in DOCA-hypertensive rats. The insulin sensitivity index in nifedipine treated rats was also comparable to Wistar controls. The AUC glucose in nifedipine treated DOCA-hypertensive rats was significantly higher than Wistar controls. The time-concentration curves also revealed slower glucose disposal despite greater insulin release in the nifedipine treated DOCA-hypertensive rats. This suggests that nifedipine treatment probably impairs glucose disposal after an oral glucose challenge in DOCA-hypertensive rats. It was interesting to note that treatment with amlodipine produced a decrease in $K_{ITT}$ indicating insulin resistance in amlodipine treated DOCA-hypertensive rats. A rise in insulin levels and a decrease in glucose levels was also produced by amlodipine treatment. The fall in serum glucose levels was accompanied by an increase in liver glycogen content. It is possible that insulin resistance leads to a compensatory rise in insulin secretion and a concomitant fall in glucose levels in amlodipine treated DOCA-hypertensive rats. It is probable that the high insulin levels increase the hepatic uptake of glucose in amlodipine treated DOCA-hypertensive rats. Although the $K_{ITT}$ for amlodipine treated DOCA-hypertensive rats was significantly lower than DOCA-hypertensive control rats, the time-concentration curves during OGTT revealed enhanced glucose disposal despite less insulin release in amlodipine treated DOCA-hypertensive rats. The AUC insulin in amlodipine treated DOCA-hypertensive rats was lower, although the difference was not significant. The effects of amlodipine and nifedipine in DOCA-hypertensive rat model are different than those observed in all other models used. However, since DOCA, by itself affects glucoregulatory mechanisms, further experiments are required to be done to modify the statement that amlodipine produces an alteration in insulin sensitivity in rats.

ACE inhibitors are also reported to improve insulin sensitivity (Donnelly 1992, Berntorp et al 1992, Baba et al 1993, Berne et al 1991) and produce hypoglycemia. It has been reported that ACE inhibitors like captopril (Donnelly 1992, Berntorp et al 1992), enalapril (Baba et al 1993) and lisinopril (Paolisso et al 1992, Sevak and Goyal 1996) improve insulin sensitivity, which causes enhanced insulin mediated glucose disposal causing reduction in blood glucose level. Captopril or enalapril have been shown to increase insulin sensitivity in NIDDM patients (Jauch et al 1987, Catalano al 1992, Pollare et al 1999a, Santaro et al 1992, Torlone et al 1991) with a subsequent reduction in fasting blood sugar levels (Arauz-Pachecho et al 1990, Ferrari et al 1991, Prince et al 1988).
However, in contrast, several other studies have shown that ACE inhibitors do not cause any significant alteration in serum insulin or glucose tolerance in hypertensive or normotensive subjects (Alleman et al 1992, Segheiri et al 1992, Chen et al 1991). The mechanism by which ACE inhibitors improve insulin sensitivity is not known. The possible explanation could be the association between insulin mediated glucose disposal and vasodilatory effect of these drugs. They may improve insulin sensitivity by enhancing blood flow to skeletal muscle (Baba and Ishikawa 1992). Several reports have indicated that ACE inhibitors are the major cause of hypoglycemia in patients with diabetes mellitus (Rett et al 1988a, Ferriere et al 1985, Donnelly 1992, Uehara 1994, Berne 1991). The hypoglycemic effect of ACE inhibitors has been attributed to increased insulin sensitivity, probably due to enhanced insulin-mediated peripheral glucose disposal from muscular tissue (Uehara 1994, Berne 1991). In the present study, chronic treatment with spirapril but not ramipril significantly lowered elevated glucose levels without altering insulin levels in STZ-diabetic rats. This suggests that spirapril improves the glucoregulatory action of insulin. Spirapril was also found to decrease serum insulin on day 15 without altering the glucose levels in SH rats. However, the insulin levels were found to be increased later and the insulin levels on the final day of treatment were comparable to the initial high values. Ramipril, on the other hand, was found to decrease both insulin and glucose levels in SH rats. Similar findings were observed with ramipril and spirapril in the hyperinsulinemic, hyperglycemic neonatal STZ-diabetic rats. These findings suggest that probably ramipril and spirapril improve insulin sensitivity in rats. This is further supported by results of OGTT. Ramipril was found to significantly decrease AUC insulin in neonatal STZ-diabetic and SH rats which was associated with a significant decrease in AUC glucose in SH rats and no change in neonatal STZ-diabetic rats. Further, the time-concentration curves for glucose and insulin after a glucose load in ramipril treated neonatal STZ-diabetic and SH rats showed significantly lower levels of glucose and insulin suggesting that ramipril decreases insulin release after an oral glucose challenge. Effective lowering of glucose levels despite low insulin levels is probably due to improvement in insulin sensitivity. The muscle glycogen in ramipril treated neonatal STZ-diabetic rats was significantly greater than neonatal STZ-diabetic control rats suggesting that probably ramipril improves insulin sensitivity by increasing the peripheral uptake of glucose. ACE inhibitors ramipril and spirapril were found to significantly lower serum glucose levels in DOCA-hypertensive rats. Lowering of serum glucose levels by ACE inhibitors was accompanied with higher serum insulin levels in DOCA-hypertensive rats. It is probable that ACE inhibitors produce
hypoglycemia by increasing insulin levels in DOCA-hypertensive rats. It is probable that these agents decrease insulin degradation or decrease hepatic extraction of insulin. However, the mechanism for increased insulin levels by ACE inhibitor treatment in DOCA-hypertensive rats is not clear. Ramipril was found to significantly decrease AUC glucose with a concomitant, although, insignificant decrease in AUC insulin. Spirapril treatment was not found to alter AUC glucose or AUC insulin in DOCA-hypertensive rats. However, the time-concentration curves during CGTT revealed enhanced glucose disposal in DOCA-hypertensive rats treated with these ACE inhibitors. Enhanced glucose disposal after a glucose challenge indicates that these agents probably enhance the glucoregulatory action of insulin by either increasing insulin sensitivity or causing insulin receptor upregulation. As DOCA itself enhances insulin mediated glucose disposal it would again be difficult to assess the true impact of ACE inhibitors on insulin action in DOCA-hypertensive rats.

In conclusion our data suggest that calcium antagonists nifedipine and amlodipine and ACE inhibitors ramipril and spirapril produce a number of beneficial effects on glucose homeostasis in diabetic and/or hypertensive rats. The data also suggests that chronic treatment with these agents may not affect the glucose-insulin balance adversely in the hypoinsulinemic, hyperglycemic STZ-diabetic rats or the hyperinsulinemic, insulin resistant SH and neonatal STZ-diabetic rats. Further, these agents do not appear to have deleterious effects on the serum lipids (except for spirapril) and renal and hepatic function. Although, direct extrapolation of animal data to human patients may be oversimplistic and incorrect, the present data may have some clinical relevance as it suggests that the use of these antihypertensive agents may be preferable in diabetic-hypertensive patients. Further, it may be concluded that chronic treatment with antihypertensives may affect insulin levels and glucose homeostasis in DOCA-hypertensive rats. However, the DOCA-salt hypertensive rat may not be a suitable model for assessing the impact of antihypertensive therapy on glucose homeostasis and insulin sensitivity as DOCA itself appears to alter insulin action.