CHAPTER - 2

EFFECTS OF VAGOTOMY AND CISPLATIN TREATMENT ON GENERAL CARBOHYDRATE METABOLISM IN RAT KIDNEY

Dysfunction of the autonomic nervous system (ANS), manifested by a variety of cardiovascular, genitourinary, alimentary abnormalities is a frequent complication of type-I and type-II diabetes (Schmidt and Scharp, 1982). Glucose intolerance can develop as a result of abnormalities in the metabolic variables, and the severity of glucose intolerance will vary as a function of the relationship between the degree of insulin resistance and diminished insulin secretory capacity (Reaven, 1980). Three groups of investigators found that electrical stimulation of the efferent vagi enhanced plasma insulin concentrations (Frohman et al.; Daniel and Henderson; Kaneto et al. 1967). Bergman and Miller (1973) demonstrated that rise in insulin was due to direct effect of the nerves on hormone secretion. Lee and Miller (1984) opined that hepatic vagus plays a regulatory role in insulin secretion. Changes in neuronal activity caused by ventromedial hypothalamuic (VMH) lesions was observed by Kita et al. (1982). Effects of VMH lesions have been prevented by vagotomy or denervation of pancreas (Inoue et al., 1977). On the other hand stimulation of the vagi enhances insulin secretion in rats, cats and pigs (Holst et al., 1981). If vagal stimulus increases insulin secretion, vagotomy might be expected to lower the plasma concentrations of insulin in certain physiological circumstances (Miller, 1981). Selective truncal vagotomy resulted in a decreased insulin response to oral glucose (Russel et al., 1974). Vagotomy is known to produce hyperglycemia (Pilo and Verma, 1984).

Hyperglycaemia and autonomic dysfunction goes hand in hand. Autonomic neuropathy in diabetic rats have been observed by Yagihashi and Sima (1985). The structural abnormalities observed by them were characterized by axonal dystrophic changes and loss of synaptic contacts. Neurological disorders and neuropathies were observed by Van Lis and Jennikens (1977). Thickening of the glomerular and renal tubular basement membrane in diabetic neuropathy was observed by Johnson et al. (1981). Axonal neuropathy has also been observed by Xu et al. (1989): Marin et al. (1979), Von Hoff et al. (1979) after pyridoxine and cisplatin (CDDP) administration. Peripheral neuropathy as a toxic effect of cisplatin was reported by Kedar et al. (1978). Association between cisplatin administration and peripheral neuropathy was observed by Mollman (1990). Polyneuropathy has been observed by Lambert and Berry (1985) and Van der Zee (1989) after cisplatin administration.

CDDP though being an anticancer agent does have deleterious effects. The mechanism by which the drug becomes effective is through its binding to DNA (Rosenberg, 1981). CDDP is known to cause hyperglycaemia (Schaeppi et al., 1972; Goldstein et al., 1982). Streptozotocin (STZ) and Alloxan which act as inducers of diabetes are cytotoxic in nature and have some what similar mechanisms. These agents are capable of breaking DNA strands in pancreatic islets (Okamoto), [illegible]. The proposed mechanism of these drugs are thought to act via a common pathway that induce DNA strand breaks, thereby inhibiting B cell function.

Calcium chloride ($CaCl_2$) was found to be somewhat effective in rectifying the deleterious effects caused by these antitumor agents (Aggarwal, 1980). $CaCl_2$ was found to reduce the renal toxicity specifically. Since several similarities between CDDP treatment and autonomic dysfunction are reported, the present study on comparative effects on vagal denervation and CDDP administration was undertaken.

Materials and Methods

Healthy male albino rats of the Charles Foster strain weighing about 250-300 gms were used for the study. They were acclimated to the laboratory conditions about 3 weeks prior to the experiment and were grouped into 8 sets each consisting of 6 animals.

Set 1 : Rats were subjected to subdiaphragmatic vagotomy under mild anaesthesia (See Chapter 1).

Set 2 : Sham operated rats served as controls for animals of Set 1.

Set 3 : Vagotomized rats were administered with 1.3% calcium chloride (1 ml i.p.) twice a day (morning and evening) for 48 hours.

Set 4 : Sham operated rats with similar dose of $CaCl_2$ were taken as control for Set 3.

Set 5 : Animals were subjected to cisplatin treatment with a dosage of 7 mg/kg body weight in 0.9% saline.

Set 6 : Controls received 0.9% saline only.

Set 7 : Cisplatin treated rats were subjected to $CaCl_2$ treatment as explained in Set 3.

Set 8 : Controls for Set 7 received only $CaCl_2$ along with a saline treatment.

All the VgX and CDDP treated rats were provided with water and were sacrificed by exsanguination under mild anaesthesia at 48 and 72 hours respectively. Kidneys from both sides were extirpated, weighed and processed for further investigation. Biochemical analysis was carried out for enzymes such as glycogen synthetase, G-6-Pase, phosphorylase, aldolase, LDH, SDH and pyruvate carboxylase (PC). Estimation for glycogen content of the kidney was also carried out. Methods followed for various analysis are given in Chapter 1.

Results

Vagotomy (VgX) :

Vagotomized rat kidney showed an increase in almost all the biochemical and enzymatic parameters studied. Phosphorylase, aldolase, LDH, SDH and pyruvate carboxylase all showed increased activities in the rat kidney following vagotomy. Glycogen synthetase showed an increased activity but there was no corresponding increase in the glycogen content. G-6-Pase was the only enzyme that did not show any deviation in its activity in the kidney following vagal denervation.

TABLE 1 : EFFECT OF VAGOTOMY AND CISPLATIN TREATMENT ALONE AND IN COMBINATION WITH $CaCl_2$ IN RAT KIDNEY ON GLUCOSE METABOLISM

	GLYCOGEN	GLYSYNTH	G-6-PASE	PHOSPHORY-LASE	ALDOLASE	LDH	SDH	PC
VgS	0.014± 0.002	0.028± 0.007	0.240± 0.014	89.37± 5.00	0.155± 0.020	85.97± 2.734	18.394± 1.838	0.643± 0.004
VgX	0.017± 0.002	0.044± 0.012	0.230± 0.015	96.10± 5.37	0.244± 0.029	92.40± 1.532	27.4936± 2.876	0.493± 0.002
Con	0.024± 0.004	0.061± 0.028	0.169± 0.009	151.35± 10.21	0.150± 0.010	93.57± 0.895	25.150± 2.427	0.493± 0.002
CDDP	0.024± 0.005	0.041± 0.003	0.222± 0.008	169.81± 3.38	0.239± 0.013	104.77± 1.247	23.449± 0.905	0.478± 0.002
VgS+Ca	0.019± 0.0009	0.015± 0.0007	0.315± 0.018	58.21± 4.05	0.134± 0.020	57.04± 2.110	25.535± 4.669	0.587± 0.001
VgX+Ca	0.018± 0.002	0.110± 0.001	0.121± 0.004	62.43± 1.58	0.308± 0.003	55.90± 0.878	33.807± 1.585	0.652± 0.001
Con+Ca	0.026± 0.003	0.120± 0.009	0.171± 0.009	93.7± 5.008	0.257± 0.002	67.34± 2.417	18.506± 0.877	0.545± 0.006
CDDP+Ca	0.017± 0.001	0.241± 0.012	0.417± 0.012	115.75± 0.161	0.496± 0.011	80.33± 3.288	19.684± 1.501	0.558± 0.003

$P \leq 0.02$, $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$

TABLE 2 : EFFECT OF VAGOTOMY AND CISPLATIN TREATMENT ALONE AND IN COMBINATION WITH $CaCl_2$ IN RAT KIDNEY ON GLUCOSE METABOLISM IN TERMS OF % CHANGES

	GLYCOGEN	GLY-SYNTH	G-6-PASE	PHOSPHORY-LASE	ALDOLASE	LDH	SDH	PC
VgS (VgX)	21.4 ↑	57.14 ↑	4.1 ↓	7.5 ↑	57.4 ↑	7.4 ↑	49.4 ↑	1.8 ↑
Con (CDDP)	NS	32.7 ↓	31.3 ↑	12.1 ↑	59.3 ↑	12 ↑	5.9 ↓	3.5 ↓
VgS+Ca (VgX+Ca)	5.26↓	63.3 ↑	61.6 ↓	7.2 ↑	13 ↑	1.99 ↓	32.3 ↑	11.07 ↑
Con+Ca (CDDP+Ca)	34.6 ↓	100.8 ↑	144 ↑	24 ↑	93 ↑	19.3 ↑	6.3 ↑	2.38 ↑

% is corrected to nearest whole number, expressed as increase [↑], decrease [↓] in value of the group in parenthesis compared to its adjoining group.

EXPLANATION TO FIGURES

Effect of Vagotomy and Cisplatin treatment alone or in combination with calcium with respect to:

Fig (1) : Glycogen content and activity of glycogen synthetase in the kidney.

Fig (2) : Activities of glucose-6-phosphatase and phosphorylase in the kidney.

Fig (3) : Activities of LDH and aldolase in the kidney.

Fig (4) : Activities of SDH and pyruvate carboxylase in the kidney.

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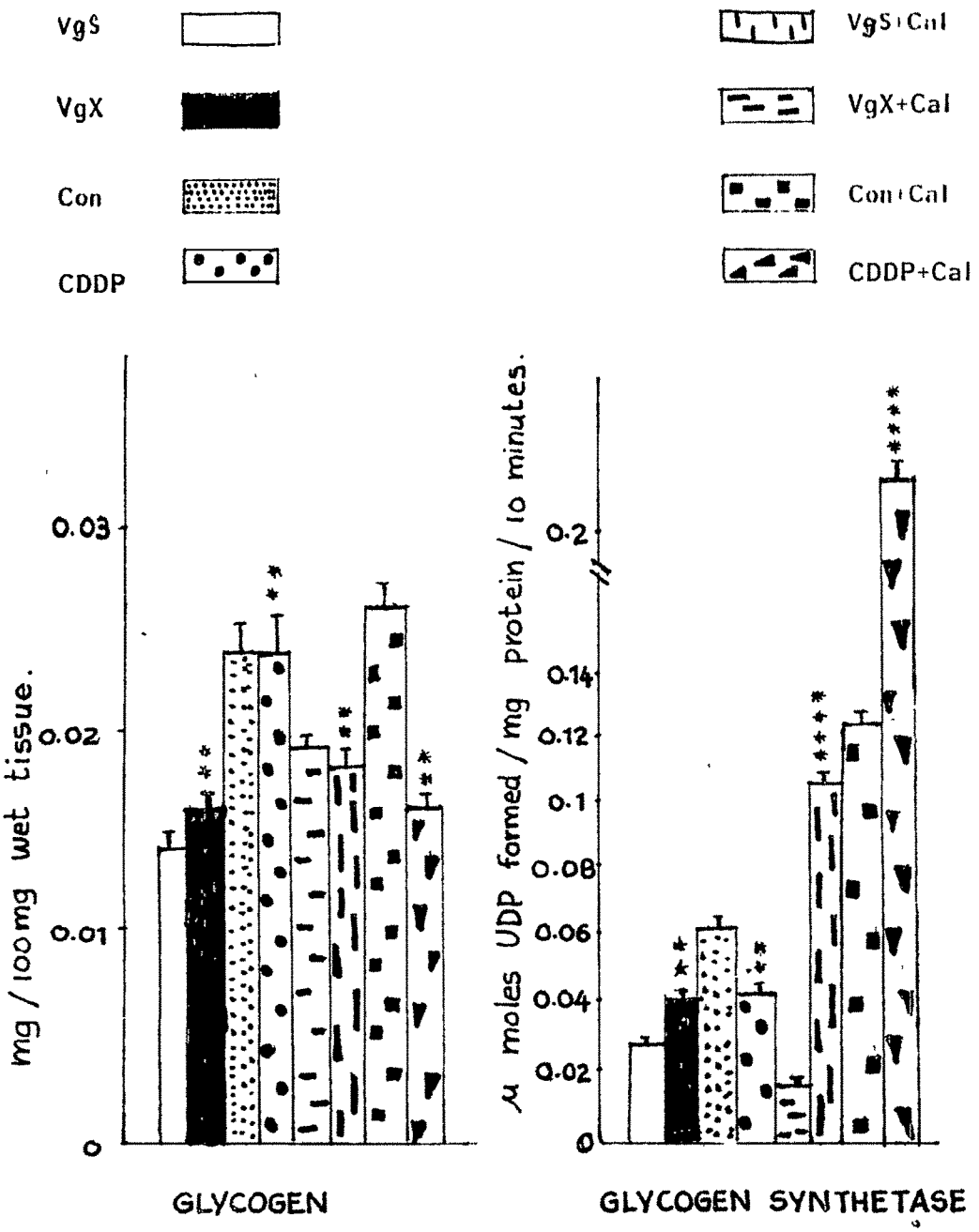

Fig : 1 Results given as mean ± SEM six experiments
$P<0.02^{*}$, $P<0.05^{**}$, $P<0.01^{***}$, $P<0.001^{****}$

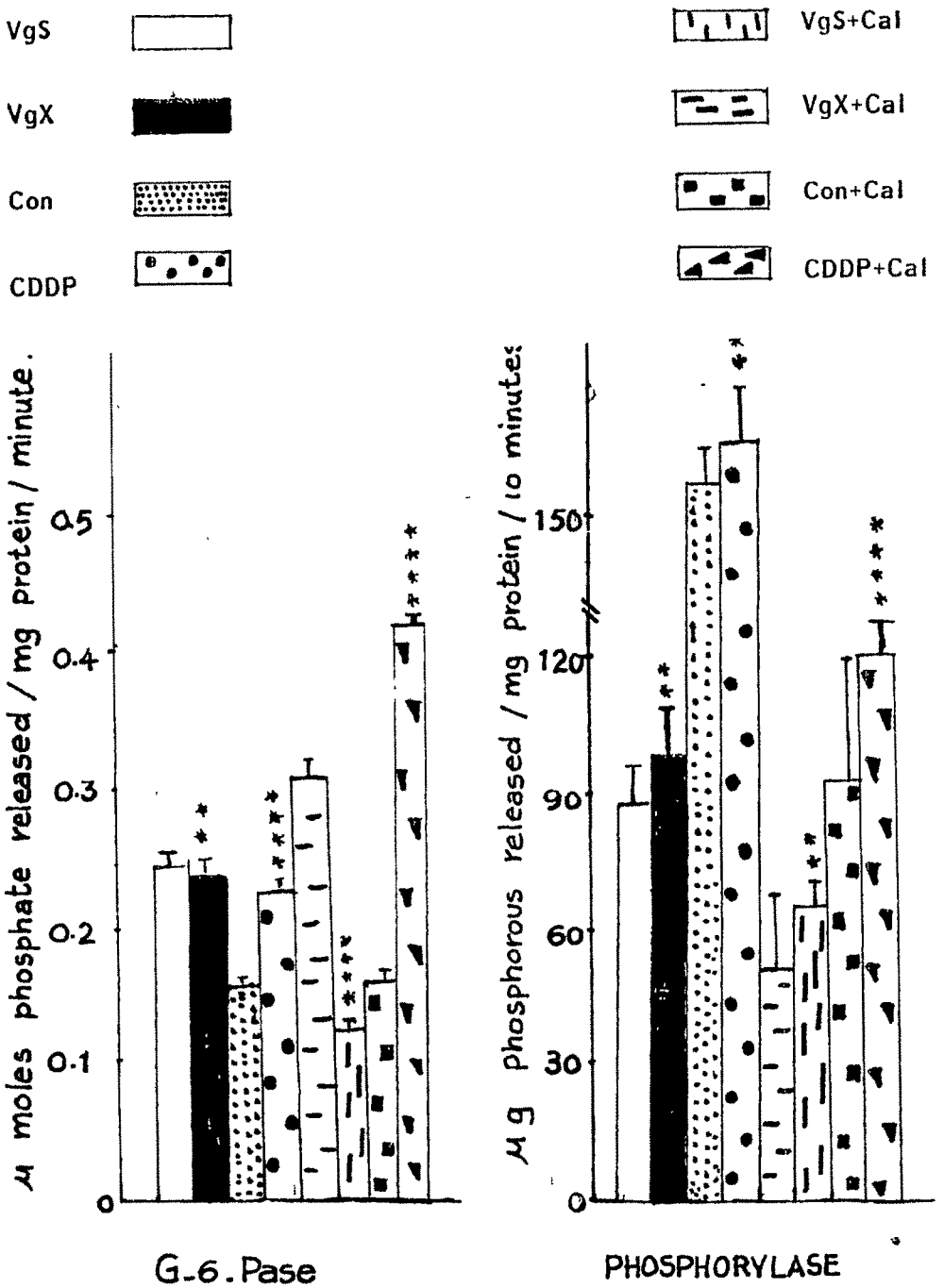

Fig : 2 Results given as mean ± SEM of six experiments.
$P < 0.02^{*}$, $P < 0.05^{**}$, $P < 0.01^{***}$, $P < 0.001^{****}$

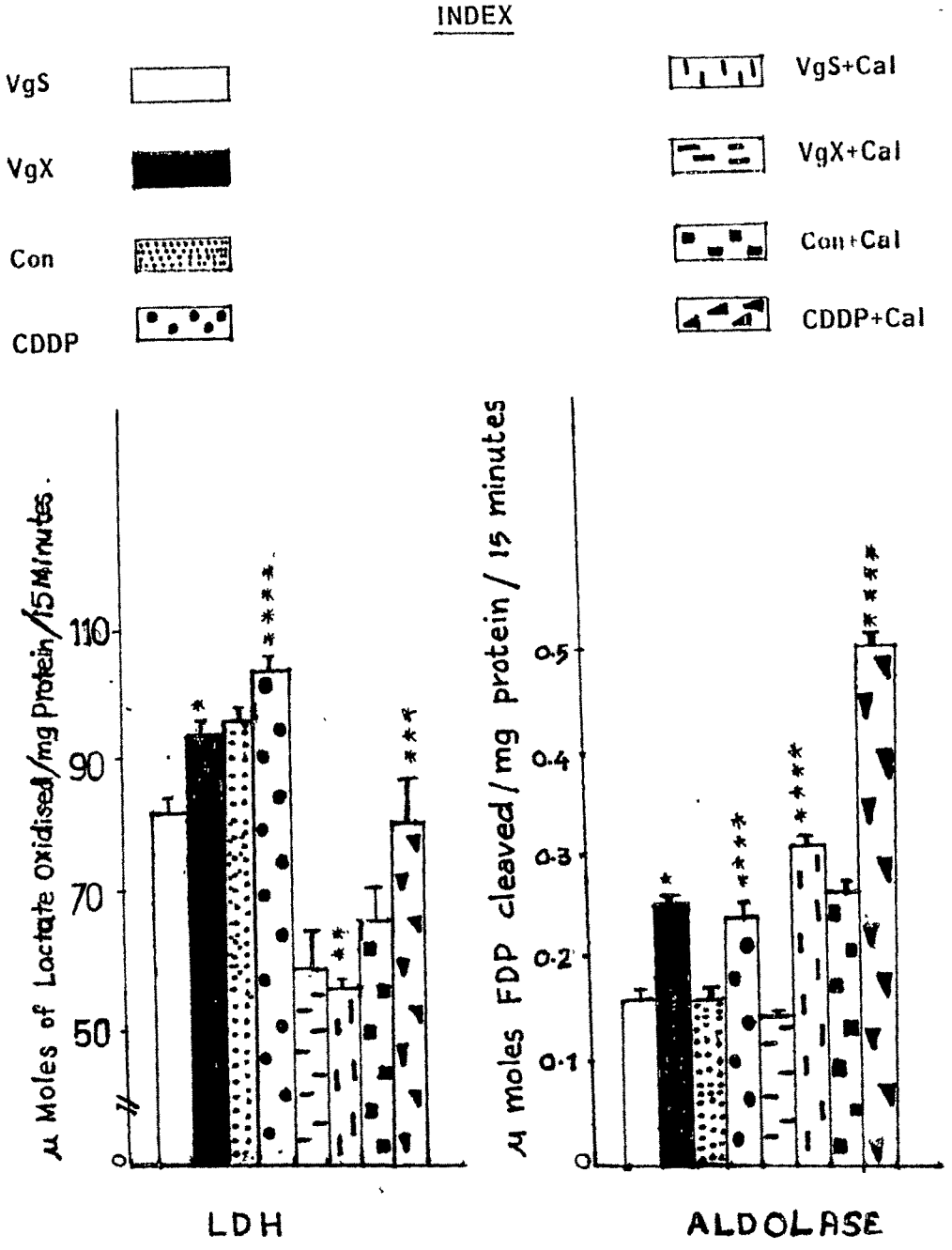

Fig: 3 Results given as mean ± SEM of six experiments. $P<0.02^{*}$, $P<0.05^{**}$, $P<0.01^{***}$, $P<0.001^{****}$

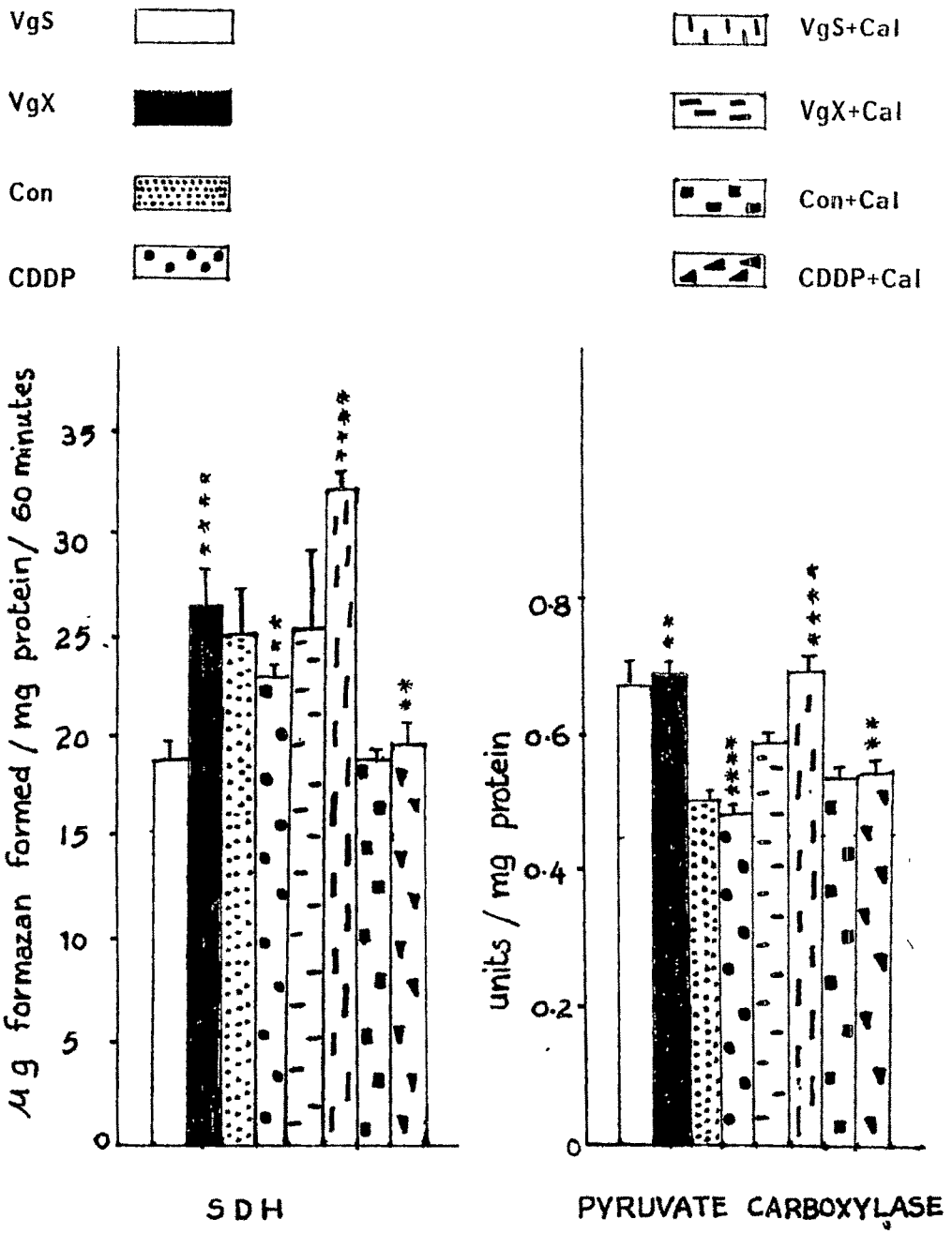

Fig:4 Results given as mean ± SEM of six experiments. $P<0.02^{*}$, $P<0.05^{**}$, $P\leq 0.01^{***}$, $P<0.001^{****}$

Vagotomy with Calcium Chloride (VgX + $CaCl_2$) :

$CaCl_2$ treatment to VgX rat brought about reversal in the response of glycogen content, glycogen synthetase, LDH while response of aldolase and PC only showed further enhancement in their activity. Glycogen synthetase showed a very high increase in the activity. G-6-Pase showed more than 50% reduction in VgX rat kidney with $CaCl_2$.

Cisplatin Treatment :

Cisplatin administration did not cause any change in the glycogen content in the kidney compared to that of control rat kidney. Glycogen synthetase however, showed a reduction. Both G-6-Pase and phosphorylase showed increased responses and so was the case with LDH and aldolase. SDH activity and PC showed slight reduction in the activities in the kidney.

Cisplatin with Calcium Chloride (CDDP + $CaCl_2$) :

$CaCl_2$ treatment to cisplatin administered rat reversed the trend only in the case of G-6-Pase and LDH. However, a perceptible reduction in the affect was noticed with respect to aldolase and SDH. Phosphorylase enzyme became more active in the kidney of $CaCl_2$ administered CDDP treated rat.

Discussion

Vagotomy and cisplatin treatment have caused hyperglycaemia in rats (Oommen, 1992; Parikh 1992). The hyperglycaemic response could be due

to several reasons. The main cause could be the autonomic dysfunction leading to decreased insulin release. Although Langerhans in 1869 described nerves lying closer to islet of pancreas, its association with endocrine cells was demonstrated only in 1937. Pancreas is known to have neural innervations belonging to sympathetic and parasympathetic system and these neural fibres control the release of glucagon and insulin respectively. These fibres arise from hypothalamus, which is an important integrative station for neural and hormonal regulation of peripheral metabolism (Shimazu et al., 1977; Shimazu, 1979; 1981). More accurately ventromedial hypothalamus (VMH) and lateral hypothalamic areas (LHA) are the centres specific for the coordination of metabolic activities with food intake. Stimulation or denervation of the fibres arising from the above mentioned centres cause alterations in metabolic activities. Stimulation of vagus elicited an increased glycogen synthetase activity in pancreatectomized rabbits. Shimazu and Amakawa (1968) have shown that enzymes implicated in hepatic glycogen metabolism are under the influence of autonomic nervous system. This system is involved in the regulation of metabolic activities in liver and kidney.

The glycogenolytic activity as such is also under hormonal control. Glucagon as well as adrenomedullary activation can increase the glycogenolytic activity.

Increased activity of phosphorylase in the kidney of VgX rat indicated increased glycogenolysis. However, there was no parallel reduction in content or in the glycogen synthetase activity in the kidney of VgX rat.

The peripheral autonomic nervous system has direct influence on pancreatic hormone secretions. It has been reported that vagal nerves have excitatory (Frohman et al., 1964; Bergman et al., 1973) and splanchnic nerves have inhibitory (Girardier et al., 1976) effects. Thus the hypothalamic control of pancreatic hormone secretion is mediated by the autonomic nervous system. Various reports have implicated the role of VMH and LHA in pancreatic hormone secretion (Nijima et al., 1981). Dysfunction of either centers may bring out neuropathological effects. Hyperinsulinemia is observed after a VMH lesion in rats (Frohman et al., 1968; Yoshimatzu et al., 1984). Another study by Iguchi (1988) reported that CNS mediated hyperglycemia was independent of epinephrine, glucagon secretion or insulin deficiency and was thought to be mediated in part by direct neural effects at tissue level.

Hyperglycemic levels were also observed in patients receiving CDDP against various tumours. Gastrointestinal, renal toxicities and autonomic neuropathies were reported along with hyperglycemia in these patients (Goldstein et al., 1981; Mollman, 1990). Peripheral neuropathy as a toxic side effect of CDDP was reported by Kedar et al. (1978). Hyperglucagonemia was observed after CDDP treatment in rats (Goldstein et al., 1982).

Current study of CDDP treated rat revealed that, kidney glycogen synthesis was reduced while glycogenolysis and glucose release was slightly on the increase. Glycogenolytic pathway (aldolase and LDH was more active) was enhanced.

Acute cervical vagotomy resulted in a marked reduction in glycogen deposition in the liver and on elevation of glucose concentration in portal veins (Mondon and Burton, 1971). They opined that loss of parasympathetic stimulation may also affect glycogen synthesis by decreasing secretion of gastrointestinal hormones or limiting the cholinergic stimulation of liver. Stimulation of vagus nerve resulted in significant increase in the activity of G-6-P dependent glycogen synthetase (Shimazu, 1967).

In general, it could be suggested that the glycogenolytic pathway was activated in the kidney of both vagotomized and CDDP treated rats.

Enhanced levels of LDH and aldolase activities were observed in vagotomized and CDDP treated rat kidney which suggested the possibility of glucose formation from lactate. In vagotomized rats SDH activity was also found to be very high. Satisfactory tissue oxygenation is a must for active gluconeogenesis (Krebs, 1963). During vagotomized situation an increase in the sympathetic release can be expected. This could activate the glucagon secretion which stimulates pyruvate metabolism. Glucagon can cause a separate stimulation of intramitochondrial carboxylation (Chan et al., 1979). Activity of pyruvate carboxylase is also influenced by acetyl CoA concentration (Scrutton and Griffths, 1981). In CDDP treated rats however, pyruvate carboxylase level showed a decrease. Rise in blood sugar after CDDP treatment might have occured through inhibition of glucose uptake and glycogenolysis through the action of glucagon. Hyperglucagonemia in rats after CDDP treatment is reported by Goldstein

et al. (1983). They opined that hyperglucagonemia after CDDP treatment is related in part due to decreased hormone degradation which may be associated with the nephrotoxicity of the drug. The mechanism contributing to renal extraction of glucagon involves glomerular filtration by tubular reabsorption and uptake from postglomerular capillaries (Emmanuoel et al., 1976). Glomerular uptake and reabsorption occurs in proximal tubular cells which contain glucagon degrading enzymes in both their brush border and cytosol (Duckworth, 1976). Thus it could be accounted that the rise in glucagon might have initiated a glycogenolysis and glycolysis thereby contributing to hyperglycaemia after CDDP treatment.

Many authors have opined that cellular mortality resulting from CDDP is due to the damage caused at the membrane level, which is basically operated by calcium homeostasis. CDDP nephrotoxicity leading to capillary hypertonicity was reported by Safirstein et al. (1981). Vassilev et al. (1986) observed that calcium channel blockers can enhance the cytoxicity of different antitumour agents. Renal morphological studies by Gordon and Gattone (1986) implied that there was a reduction in mitochondrial calcium level. Various reports are at hand opining that chloride salts of various compounds can rectify these obstructions caused by CDDP at cellular levels. Usage of sodium chloride was postulated by Litterest (1981), and usage of ammonium chloride was demonstrated by Yates and McBrien (1985). Aggarwal and Hammouda (1980) reported that $CaCl_2$ was found to be effective in improving the functional status of kidney of rats treated with CDDP.

Present observation recorded a decrease in glycogen content, but rest of the enzyme activities remained unchanged after a $CaCl_2$ load. There was an opposite response in the enzyme activities except for G-6-Pase when compared to the non-calcium treated ones. The data thus directly points to an overall improvement brought out by calcium. In some cases $CaCl_2$ prevents alteration in the activities at enzyme level (eg. G-6-Pase). Inclusion of hypertonic $NaCl_2$ in the injection vehicle reduced nephrotoxicity in CDDP treated rats (Litterest, 1981). Possibility exists that $NaCl_2$ induces a significant diuresis that decreases the cisplatin toxicity in animals (Guarino et al., 1979; Litterest et al., 1979) and in human (Madias and Harrington, 1978). Ozols (1984) have also shown that hypertonic $NaCl_2$ reduces the nephrotoxicity caused by CDDP. Yates and McBrien (1985) showed that infusion of NH_4Cl_2 reduced the cytotoxicity of CDDP. Other ions such as phosphate and potassium may also play important role in maintaining calcium homeostasis. Metabolic de rangements in the pancreatic islets have elevated their resting levels of cytosolic calcium while impaired glucose metabolism have been observed in chronic renal failure (Fadda et al., 1991). These abnormalities were associated with impaired insulin secretion in response to glucose (Fadda et al., 1988; 1991) or potassium (Comunale et al., 1990). Phosphate depletion was observed in an experimental model where pancreatic islets display metabolic defects similar to those seen in chronic renal failure (Zhow et al., 1991) along with impaired glucose homeostasis. In vagotomized rats decrease in glycogen content and increase in several enzymes were observed except in case of G-6-Pase and LDH activity compared to that

of respective controls, while an opposite response was observed in case of G-6-Pase, phosphorylase and SDH levels compared to non-calcium treated rats after vagotomy.

To sum up, the data presented in this chapter reveal that vagotomized and CDDP treated rats produced a hyperglycemia. The similarity led to the conclusion that dysfunctioning of parasympathetic nervous system could alter blood glucose level. The $CaCl_2$ administration to both groups could minimize the side effects observed in the tissue thereby bringing the hyperglycemic index considerably to a lower level. Thus it could be concluded that the autonomic nervous system not only collectively integrates the pancreatic hormone secretions by modifying the balance of secretions of insulin and glucagon, but can also directly affect the metabolic activities in tissue and organs such as liver and kidney. Some of the side or toxic effects of CDDP are mediated through autonomic neuropathy which in turn causes parallel metabolic dysfunctions.