CHAPTER - II

VAGAL CHOLINERGIC ACTION ON LIVER AND KIDNEY OF VAGOTOMIZED AND CISPLATIN TREATED RAT AND PIGEON

One of the main functions of liver is to maintain the blood glucose level. This function is achieved by its unique ability to release or remove glucose from the blood stream depending on the concentration of this sugar and the hormonal status of the organism (Lautt, 1983; Shimazu, 1983). Glucose sensitive vagal afferent fibres from the liver are believed to play a significant role in control of food intake and maintenance of blood glucose levels (Nijima, 1983). In the rat liver, stimulation of the hepatic parasympathetic nerves has direct effects on glucose metabolism, synergistic with insulin and antagonistic to glucagon (Grademann et al., 1986). Birds maintain a very high glucose level in blood compared to mammals. Pilo and Patel (1978b) suggested that in birds, the parasympathetic nerves may be the predominant inducer of glucose uptake by liver. It is reasonable to believe that avian kidney is also involved in blood sugar homeostasis and vagal cholinergic fibres, through ACh, are involved in the regulation of kidney metabolism (Pilo and Mehta, 1988).

The cholinergic fibres secrete acetylcholine while sympathetic fibres secrete adrenaline and noradrenaline at their respective nerve endings. Vagal parasympathetic nerves, which contain mainly cholinergic fibres, innervate liver and

kidney. Acetychloinesterase (AChE) hydrolyses acetylcholine to choline and acetate, thereby inactivating the neurotransmitter. AChE activity is localized intracellularly in cholinergic neurons, as well as extracellularly adjacent to cholinergic receptors at sites such as the neuromuscular junction (Silver, 1974). The pathway of an ACh system which is related to the hypothalamus has been elucidated. AChE containing neurons are found in the LHA, but ACh fibres pass only sparsely to the dorsal part of the VMH (Shute and Lewis, 1966). The histochemical demonstration of AChE in the renal nerves has been considered to indicate the existence of a distinct cholinergic innervation (Weitsen and Norvell, 1969; Norvell et al., 1970). The AChE activity in the pigeon liver was almost double than that was measured in the rat liver (Patel and Pilo, 1977; Pilo and Patel, 1977). The nerve fibres present in liver and kidney may have afferent branches that take information to the hypothalamus.

Ban (1966) suggested that there is parasympathetic zone (Zone A and C) and sympathetic zone (Zone B) in the hypothalamus. He considered that ventromedial hypothalamic nuclei (VMH), known as satiety centre is included in the B sympathetic zone and lateral hypothalamic area (LHA), the location of the feeding centre is included in the parasympathetic zone. Taking these findings into consideration, it is quite reasonable that decrease in liver enzyme activity and increase in kidney enzyme activity after bilateral VMH lesions are brought about by changes in the relative

excitatory state of the parasympathetic zone in the LHA (Nagai et al., 1983). The autonomic centre in the hypothalamus probably receives a constant afferent flux of glucose related signals from the liver which may interact with hypothalamic mechanisms controlling influence upon hepatic carbohydrate metabolism and provide a feed-back control of glucose homeostasis (Shimazu, 1981). The afferent fibres are known to be projected to hypothalamus and hence may have important role to play in the regulation of blood sugar level, food intake and water intake (Lautt, 1980a).

Apart from the direct action of efferent fibres on the liver and kidney metabolic activities, the autonomic fibres could also control metabolism through controlling the release of hormones such as insulin and glucagon. Both sympathetic and parasympathetic nervous system are intimately involved in the mechanisms which control insulin secretion in the normal subject (Malaisse et al., 1967). It is well-known that the electrical stimulation of the vagus nerve of normal rats favours not only insulin but also glucagon secretion; both processes are inhibited by the cholinergic anatagonist atropine (Miller, 1981; Helman et al., 1982).

Dysfunction of both afferent and efferent fibres may have tremendous influence on the metabolic regulation in liver and kidney. Drugs that inhibit nervous activity or that bring about neuronal dysfunction can also disturb the metabolic regulation. There are several antitumor drugs in this

category; cis-diamminedichloroplatinum (CDDP) is one of the most widely used chemotherapeutic agents in the treatment of neoplasia (Rosenberg, 1985). Administration of cisplatin causes distribution of the drug throughout the body with higher concentration found in kidney, liver, lung and ovary (Litterest et al., 1976; Pretorius et al., 1981). Cisplatin has differential affinities for kidney and liver. Cisplatin also shows preferential localizations within subcellular sites in the kidney (Choie et al., 1980). Nephrotoxicity is the most important side effect as it is the limiting factor in the chemotherapeutic uses of this drug (Goldstein and Mayor, 1983; Goldstein et al., 1981; Gottlieb and Drewinko, 1975). Administration of CDDP results in hyperglucagonemia in rats (Goldstein et al., 1982) and this result may be related to the decreased glucagon degradation associated with impaired renal function (Goldstein et al., 1983). Several divalent metal cations influence carbohydrate metabolism by their ability to alter insulin and/or glucagon metabolism (Eaton, 1973; Ghafghazi and Mennear, 1975; Horak and Sunderman, 1975a, 1975b; Ithakissios et al., 1975). There are also some relevant recent reports of peripheral neuropathies. A significant acceleration in the onset of neurotoxicity especially peripheral neuropathy was noticed in the high dose cisplatin treated groups (Mollman, 1990; Hainsworth et al., 1990; Zambetti et al., 1990). The present experiment was aimed to investigate the effects of vagotomy

and CDDP treatment on the parasympathetic nervous system and thus on glucose metabolism and also to compare these effects in rat with that in pigeons.

MATERIALS AND METHODS

Cis-diamminedichloroplatinum (Cisplatin), Acetylthiocholine Iodide and 5'5' Dithiobis (2-Nitrobenzoic acid) (DTNB) were purchased from Sigma chemical company, USA. All other chemicals were of reagent grade.

Animals and Treatment :

The animals used in this study were male albino rats (Charls Foster strain) and domestic pigeons (Columba livia). For experimental purpose all animals were divided into eight groups of six animals each. These animals were housed in cages and maintained under a controlled 12L:12P light schedule and temperature (26°C) and allowed ad libitum access to food and water.

Cisplatin was dissolved in physiological saline (0.9%) just before use. The rats were given a single ip. injection of cisplatin in a dosage of 7mg/kg body weight. Control animals were injected with saline only and treated in an identical manner. Group 3 pigeons were administered with single dose of 5mg/kg of cisplatin in 0.85% saline and its control group of pigeons received the same dose of vehicle. Under light ether anaesthesia, vagotomy (VgX) was performed at the

subdiaphragmatic level in the rats, and the pigeons underwent bilateral cervical vagotomy. Sham (VgS) operated animals were used as respective controls (Chapter I). During pair fed control experiments, intake of food by the animals was limited to that consumed by drug treated and vagotomized animals. This pair feeding was done because cisplatin administration is known to decrease food intake in rats. (Killer and Aggarwal, 1983). The cisplatin treated rats and controls were sacrificed on the 4th day of injection. Blood was obtained by puncturing heart under mild anaesthesia for the analysis of blood glucose (Folin and Malmros, 1929) (Chapter I). The vagotomized and sham operated pigeons were sacrificed at 48 hrs by decapitation under mild anaesthesia and liver and kidney were rapidly extirpated. The tissues were weighed and homogenized in chilled buffer for the assay of AChE by the method of Ellman et al. (1961). The specific activity was represented as µg substrate hydrolysed/mg protein/min. The protein values of enzyme extract was estimated by the method of Lowry et al. (1951) by using bovin serum albumin as standard.

Expression of Data

All experiments were performed at least five times. All data were statistically analysed by Student's 't' test. Data were expressed as means ± SEM.

RESULTS

When rats were injected with a therapeutic dose levels of cisplatin and sacrificed three days later, it was noticed that the stomach was extremely swollen and filled with food (fig. 1 & 2). Nausea and vomiting did not appear in cisplatin treated rats because they were incapable of vomiting. The rats and pigeons were not free from the side effects like diarrhoea after CDDP treatment and vagotomy. CDDP induced a significant loss of body weight in rats (Parikh, 1992). The reduction of body weight was also noticed in vagotomized rat and pigeon. Animals subjected to subdiaphragmatic vagotomy and fed conventional diet have shown reduced weight gain which is associated with marked hypophagia and stomach distention (Andrews et al., 1985).

The data obtained from the studies on liver of sham operated and vagotomized rats and pigeons are presented in Table 1-2. The AChE activity was seen to undergo a tremendous decline after vagotomy in the liver and kidney of rat and pigeon (fig. 5 & 6). Cisplatin administration also brought about a significant decrease in the activity of AChE in the liver and kidney of pigeon. On the other hand, AChE in the liver of the CDDP treated rat did not show any significant variation compared to control animals. A significant diminution was observed in AChE activity in the kidney of rat following CDDP administration (fig. 6). In rat and pigeon, both vagotomy

Explanation For Figures

Fig.1 Sham operated (control) and vagotomized rats. In vagotomized rats the stomach is bloated.

Fig.2 Saline (control) and cisplatin treated rats. In cisplatin treatment, the stomach became distented to form a 'balloon like' appearance.

Compare the appearence of stomach in vagotomized and cisplatin treated rats.


CONTROL VAGOTOMY
①


②
CISPLATIN CONTROL

Table I **Comparative effect of vagotomy and cisplatin on AChE activity on liver and kidney of rat and pigeon (Mean ± SEM)**

Treatment	Rat		Pigeon	
	Liver	Kidney	Liver	Kidney
Saline	0.0048 ±0.0004	0.0041 ±0.0003	0.0156 ± 0.0006	0.0046 ± 0.0005
Cisplatin	0.0054 ±0.0002NS	0.0026*** ±0.0001	0.0113**** ± 0.0003	0.0026* ± 0.00009
Sham	0.0040 ±0.0001	0.0014 ±0.0001	0.0195 ± 0.0004	0.0049 ± 0.0002
Vagotomy	0.0025**** ±0.0001	0.0009*** ±0.00002	0.0131*** ± 0.0003	0.004* ± 0.0001

Values expressed as µg Substrate hydrolysed/mg protein/min.

* - $P < 0.05$; *** $P < 0.01$; **** $P < 0.001$

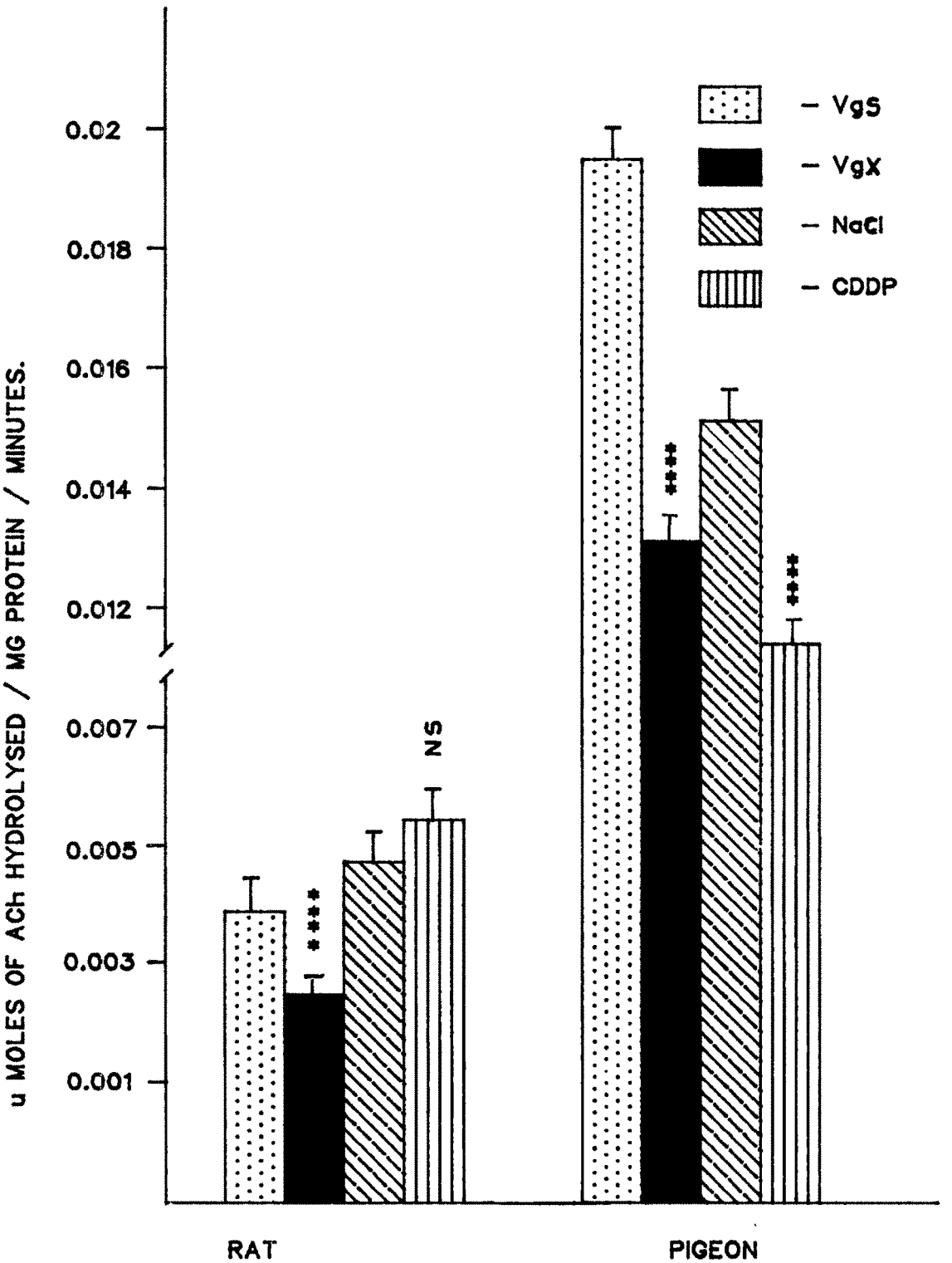

Fig. 5. EFFECT OF VAGOTOMY AND CISPLATIN ON AChE ACTIVITY IN LIVER OF RAT AND PIGEON. RESULTS ARE EXPRESSED AS MEAN ± STANDARD ERROR OF THE MEAN. **** CORRESPONDS TO $P < 0.001$. ($n = 6$).

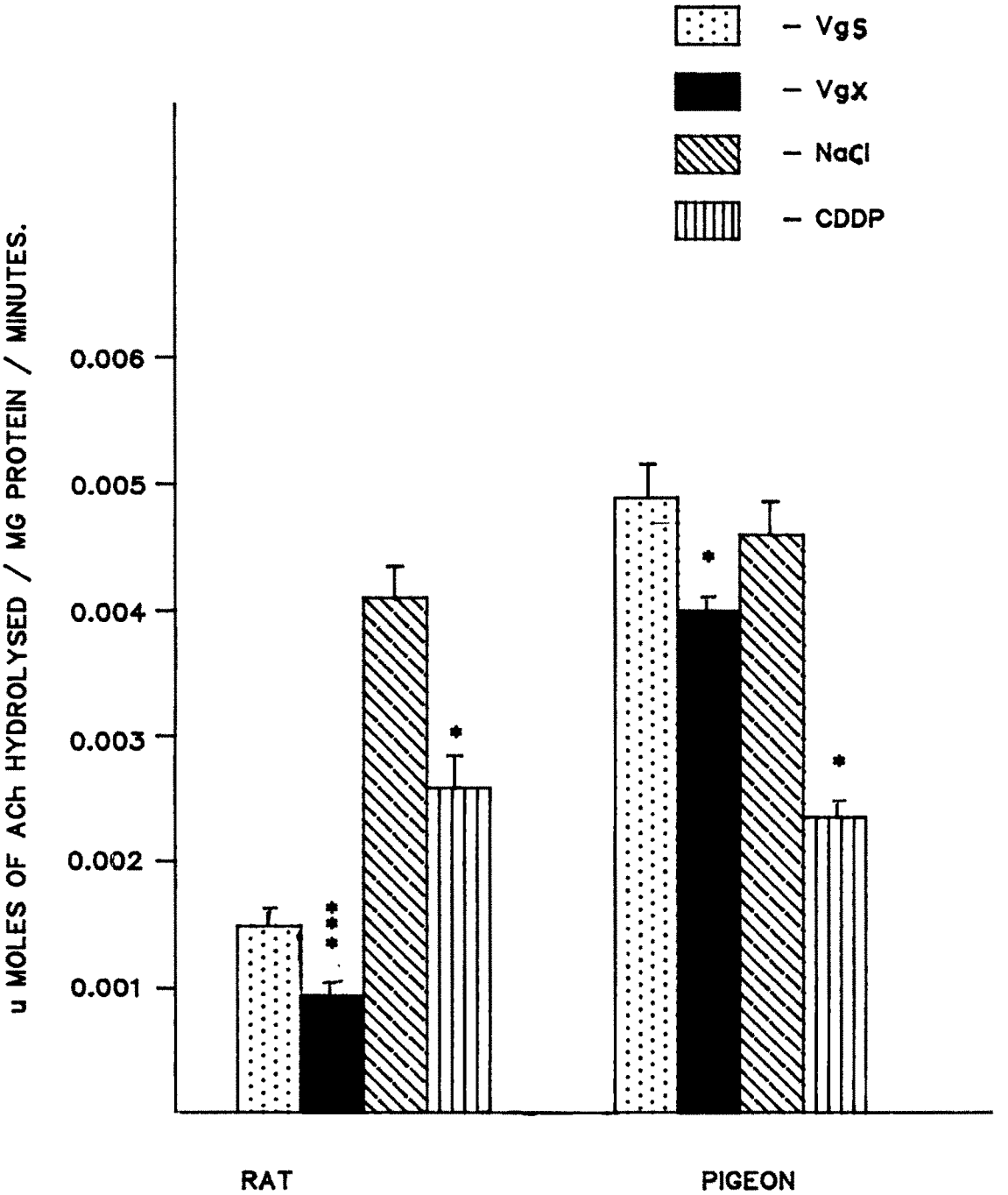

Fig. 6. EFFECT OF VAGOTOMY AND CISPLATIN ON AChE ACTIVITY IN KIDNEY OF RAT AND PIGEON. RESULTS ARE EXPRESSED AS MEAN ± STANDARD OF THE MEAN.
*** $P < 0.01$; * $P < 0.05$. ($n = 6$).

Table II Effect of Vagotomy and Cisplatin on blood glucose level in rat and pigeon (Mean ± SEM)

	Rat	Pigeon
Sham	135.989 ± 3.740	261.46 ± 8.30
Vagotomy	173.63**** ± 5.34	312.18** ± 7.47
Saline	140.64 ± 3.48	215.42 ± 8.76
CDDP	211.05**** ± 3.60	263.25** ± 6.44

(Glucose mg/100 ml blood)

** P 0.02; **** P 0.001

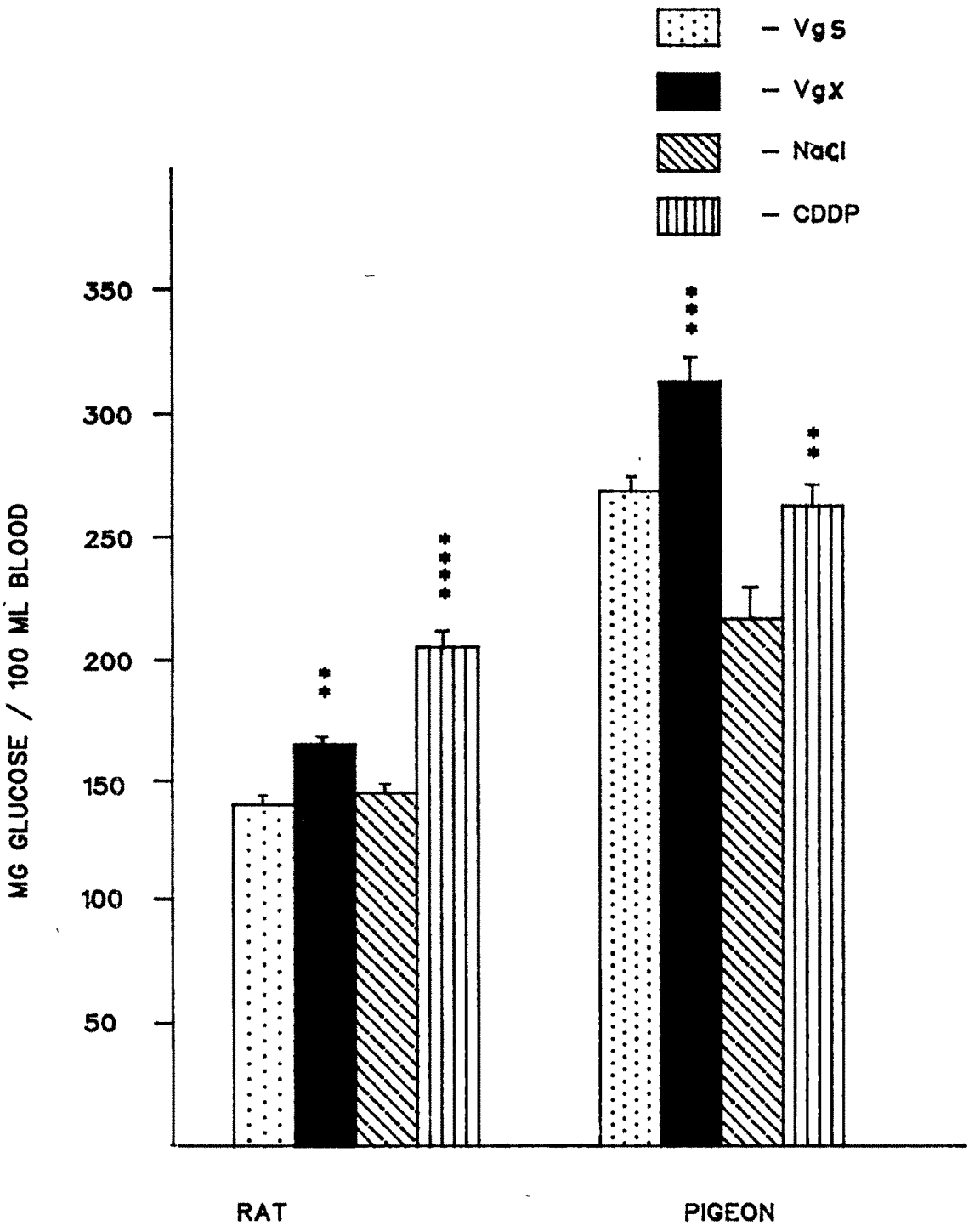

Fig. 7. COMPARISON OF THE EFFECT OF VAGOTOMY AND CISPLATIN ON BLOOD GLUCOSE LEVEL IN RAT AND PIGEON. MEAN VALUES ± SEM ARE SHOWN. (n = 6).
**** P < 0.001; *** P < 0.01; ** P < 0.02.

and cisplatin treatment caused an elevation of blood sugar level when compared with that of control animals.

DISCUSSION

Cervical vagotomy in mammals, owing to the interruption of laryngeal innervation through recurrent laryngeal nerves, results in paralysis of the larynx, while in birds it does not lead to any such respiratory tract dysfunction. In view of this fact, subdiaphragmatic vagotomy was performed in rats and cervical vagotomy was done in pigeon. Vagotomy in rats caused stomach distension, which could be due to the lack of motility of stomach muscles in the absence of vagal innervation. Same phenomenon was observed in cisplatin treated rats which could be interpreted as due to the cholinergic inhibition. Neural denervation or inhibition could lead to the digestive and absorptive disruption. This must be the reason for reduction in food intake and loss of body weight in CDDP treated and vagotomized animals. Parasympathetic feed-back from hepatic and gastrointestinal (GI) tract vagal branches are also necessary for modulating appetite and food intake (Sakaguchi et al., 1988).

Vagal cholinergic dysfunction or inhibition could be discerned from the level of AChE activity in various visceral organs. Both liver and kidney showed reduction in AChE activity following vagotomy. AChE activity in the liver of pigeon was much more than in the rat liver. Likewise the

reduction in AChE due to vagotomy was also greater in pigeon liver. In pigeon, kidney AChE activity was much less than in liver. Vagotomy, however, decreased AChE activity equally in kidney of both rat and pigeon.

Cisplatin treatment caused a tremendous decrease in AChE activity in the liver and kidney of pigeon. However, in the rat liver CDDP treatment did not elicit the expected reduction, although the kidney AChE was affected adversely. The difference between vagotomy and CDDP has to bring about cholinergic dysfunction by neurotoxicity either at peripheral nerve level or at hypothalamic level. Autonomic neuropathy is observed after CDDP treatment (Richardson, 1990; Hansen, 1990). Symptoms and signs of CDDP neuropathy is usually developed during cisplatin treatment (Hoop et al., 1984; Thompson et al., 1984; Hancock, 1986; Pomes et al., 1986; Lele et al., 1987; James et al., 1988; Mollman et al., 1988;), which could be mainly due to its direct interaction on neurons (Hoop et al., 1990).

Vagal (parasympathetic) dysfunction can affect metabolic activities. One of the functions of cholinergic parasympathetic fibres is to facilitate glucose uptake by liver through stimulation of glucose uptake machinery including membrane bound enzymes. Another effect of parasympathetic neuropathy is the lack of insulin release response to a glucose load. The release of insulin and glucagon from the pancreas, which occurs in normal conscious

calves in response to changes in plasma glucose concentration is mediated largely via parasympathetic innervation (Edwards, 1984). Impaired glucose uptake mechanism can adversely affect the glycaemic level. Vagotomy as well as CDDP treatment elevated the blood sugar level in both rat and pigeon.

Elevated blood sugar level was more prominent in CDDP treated rat than in VgX rat. In pigeon, both CDDP treatment and VgX produced similar glycaemic effects. The difference between rat and pigeon in glucose metabolism is due to the fundamental difference in the finer controlling mechanisms involved in glucose regulation. In other words, in mammals paraympathetic dysfunction, due to hypothalamic lesions or due to peripheral nerve neuropathy, could lead to reduced insulin release response. It is well-known that in mammals insulin plays a predominant role in blood sugar regulation and these animals quickly respond to hyperglycaemia (by insulin release). Birds, on the other hand, tolerate hyperglycaemia but quickly respond (by glucagon release) to hypoglycaemia (Pilo and Verma, 1985). Significant fall in plasma insulin levels following vagotomy has already been reported (Frohman et al., 1967). Vagotomy in pigeon did not alter the insulin level (John et al., 1985). On the contrary, the plasma levels of NE and corticosterone were found to be very high in VgX pigeons (Viswanathan et al., 1987). The role of parasympathetic fibers in mammals in the

release of insulin from B cells has been well explained (Kaneto et al., 1981; Edwards, 1984).

In conclusion, it could be stated that vagotomy as well as CDDP treatment causes parasympathetic dysfunction and this in turn causes profound adverse effect on metabolism, especially those concerned with glucose. Either impairment of glucose uptake mechanism in liver or absence of insulin release response could be the reason for the elevated blood sugar level in VgX and CDDP treated rat and pigeon. In pigeon liver, the glucose uptake and in rat, the insulin release, could be the mechanisms that were affected most.