CHAPTER - VII

A COMPARISON OF THE EFFECTS OF CHEMICAL SYMPATHECTOMY ON LIVER AND KIDNEY OF RAT AND PIGEON

The metabolic functions have to be precisely controlled and balanced to achieve homeostasis of blood sugar level. This control is achieved through coordinated actions of both nervous and endocrine systems. The neural control is mediated through autonomic nervous system while insulin, glucagon, corticosteroids, thyroid hormones and growth hormones are involved in the endocrine regulation of metabolic activities.

Most visceral organs are innervated by parasympathetic (PNS) and sympathetic (SNS) nerve fibers. Liver and kidney, the most metabolically active organs are also innervated by fibers from PNS and SNS. Both these autonomic fibres are capable of stimulating metabolic activities in liver and kidney especially those concerned with carbohydrate metabolism.

The autonomic nerve fibres can also influence the output of endocrine pancreas as well as hormones from adrenals (Wodds and Porte, 1974). Both these autonomic fibres also have antagonistic actions in the release of insulin and glucagon. Stimulation of SNS inhibits the insulin release while stimulation of PNS inhibits the release of glucagon (Kaneto et al., 1973; Miller, 1975).

The control of insulin and glucagon secretion excerted through the autonomic nervous system is vested with hypothalamus. Immediately after lesions of hypothalmic nuclei (VMH and LH), there were corresponding changes in vagal and splanchnic nerve activity (Yoshimatsu et al., 1984). The autonomic centre in the hypothalamus probably receives a constant influx of glucose related signals from the liver, which may interact with hypothalamic mechanism controlling centrifugal influence upon hepatic carbohydrate metabolism and provide feed-back control of glucose homeostasis (Shimazu, 1981). The remarkable stability of blood glucose level, despite changes in supply of glucose indicates precise regulatory mechanism which allow liver to oscillate between balanced uptake and release of glucose (Teutsh, 1981; Teutsch and Lowry, 1982). The integrity of the sympathetic nervous system is essential for an efficient homeostatic response to insulin-induced hypoglycaemia (Sacca et al., 1977). There are at least three separate mechanisms by which the sympathetic nervous system can activate hepatic glycogenolysis and rapidly supply the circulating blood with glucose; first directly via the hepatic innervations, second, by the release of epinephrine from the adrenal medulla and third, by the release of glucagon from pancreatic islets. All these mechanisms are presumed to be integrated in the hypothalamus and act in coordination (Mondon and Burton, 1971). Sympathetic modulation is more potent in glucose homeostasis in birds which could occur even in the absence of vagus (Pilo and Mehan, 1987).

6-Hydroxydopamine (3,4,6-trihydroxy phenyl thylamine), 6-OHDA, an isomer of norepinephrine (NE) selectively inhibits NE synthesis at noradrenergic nerve terminals (Tranzer and Thoenen, 1967; Di Bana, 1978) and causes almost total depletion of noradrenaline in sympathetically innervated tissues. Chemical sympathectomy of rats with 6-OHDA caused a reciprocal alteration of postsynaptic and presynaptic autonomic receptors in adrenergically innervated tissues such as heart and spleen (Yamada et al., 1980e,b; 1982b). Autonomic nerves are found to have significant direct influence on metabolic activities in liver (Lautt, 1983; Shimazu, 1983). Since kidney too has these innervations, it is possible that the kidney metabolic activities could also be regulated by autonomic nerves. Studies on sympathetic nerve supply to kidney have been done by Ninomiya et al. (1989). Previous studies have highlightened the fact that, metabolic activities in avian kidney are influenced by autonomic nerve fibres. There are biochemical evidences for the possible localization of postsynaptic $\alpha 1$ $\alpha 2$ and adrenoreceptors and muscarinic cholinoreceptors in the rat kidney and also for the regulation of these adrenoreceptors by sympathetic nervous system (Yamada et al., 1986). The denervated adrenal gland is insensitive to the action of insulin (Comline and Silver, 1961) and it has been suggested that, regions in the hypothalamus might be responsible for controlling the rate of secretion of adrenal catecholamines when the blood sugar was abnormally high (Duner, 1953). It

is suggested that insulin accelerates the in vivo conversion of inactive renin into active renin probably through a stimulation of the sympathetic nervous system (Nakamaura et al., 1983). Studies on experimental animal models have shown that reserpine, a drug that causes striking depletion of catecholamines is associated with sedation and hypoactivity. Reserpine and 6-Hydroxydopamine, both cause a profound and long-lasting depletion of noradrenaline from sympathetically innervated tissues (Porter et al., 1963; Laverty et al., 1965) but the mechanism involved is different for these two drugs. Reserpine treatment itself results in overall depletion of NE from sympathetic nerve (Mukherjee et al., 1989).

The autonomic neuropathy or dysfunction could thus severely affect the regulation of blood sugar level. Autonomic neuropathy is very common amongst diabetic patients (Diani et al., 1979, 1981; Schmidt et al., 1981, 1982, 1983). It is well-known that the activities of the key enzymes of neurotransmitter metabolizing pathway - acetylcholinesterase are severely affected in diabetes. The degree of neuropathy in liver and kidney may vary according to the severity of diabetes (Pabke et al., 1988). It is also well-established that in mammals the final output of catecholamine following insulin treatment depends on the behaviour of the splanchnic nerve (Khalli et al., 1986a,b) and various enzymes namely tyrosine hydroxylase, dopamine tetrahydroxylase and

phenylethanolamine -N-methyltransferase which are essential for the synthesis of catecholamines (Sietzen et al., 1987). However, it is also reported that autonomic neuropathy could probably lead to diabetes or enhance the severity of diabetic condition (Lautt, 1978a).

Studies after vagotomy in pigeons revealed that PNS dysfunction leads to (1) hyperglycaemia (2) reduced glucose uptake (3) increased glucose release and (4) increased gluconeogenesis (Pilo and Mehan, 1988). The role of SNS in the regulation of carbohydrate metabolism, however, is more complicated because of the involvement of adrenal gland. To understand the effect of SNS dysfunctions on glucose homeostasis, pigeons were subjected to chemical sympathectomy through administration of 6-OHDA. Glucose homeostasis was studied through GTT and the trophic action of nerves on liver and kidney were determined by the analysis of protein and nucleic acid contents.

MATERIALS AND METHODS

The rats and pigeons were divided into two separate groups of 6 each. Chemical sympathectomy was achieved by the administration of 6-ODHA. Hbr intraperitoneally at a dosage of 30mg/kg body wt. in rats and 40 mg/kg body wt in pigeons (chapter-1). Control groups received vehicle alone. Reserpine was injected ip. 10 hrs before sacrificing at a dosage of 5mg/kg body wt in rat and 1.2 mg/kg body wt in pigeon. The

control and experimental animals of both groups were sacrificed by decapitation 48 hrs after injection of 6-ODHA.

Histofluorescence of catecholamines

The extent of sympathectomy was assessed through the histofluorescent localisations of catecholamines in cornea of rat and pigeon by the method of Terro (1977) (Chapter I).

Glucose Tolerance Test (GTT)

Glucose tolerance test was carried out after 48 hours of 6-OHDA treatment following 4 to 6 hours fast (see Chapter I). Blood samples were drawn slowly through orbital sinus puncture in rats at 0, 30, 60, 90, 120 and 150 minutes following an ip. injection of 30% glucose solution. In pigeons it was detemined by sampling blood from branchial vein and later analysed by the method of Folin Malmros (1929) (Chapter I).

Tissue preparation

Immediately after sacrifice, the liver and kidney were excised, blotted and weighed. A small portion of the tissues were minced and homogenised in ice-cold phosphate buffer (pH-7). The homogenate was centrifuged at 12,000 xg for 40 min at $4^{\circ}C$. The supernatant was stored at $4^{\circ}C$ for AChE assay (Chapter I). For the estimation of the nucleic acid content, pieces of the liver and kidney were homogenised in chilled water, precipitated in 10% trichloroacetic acid (TCA)

and centrifuged at different intervals. The final hydrolysed supernatant was used for DNA and RNA estimation (Chapter-I). The corresponding crude homogenate was used for protein assay.

Data were statitically analysed using Student's t' test at 95 % confidence level.

RESULTS

Histofluoresence studies showed complete depletion of catecholamine from the cornea of rat and pigeon after 6-OHDA treatment (figs. 1,2,3,4).

6-OHDA and reserpine caused an increase in the activity of AChE in the liver of rat (fig.5). However, the enzyme activity in the liver of pigeon did not show any variation from that of control (fig.5). After chemical sympathectomy, the activity of AChE in the kidney was significantly declined in the pigeon, but no such difference was noticed in the rats (fig. 5).

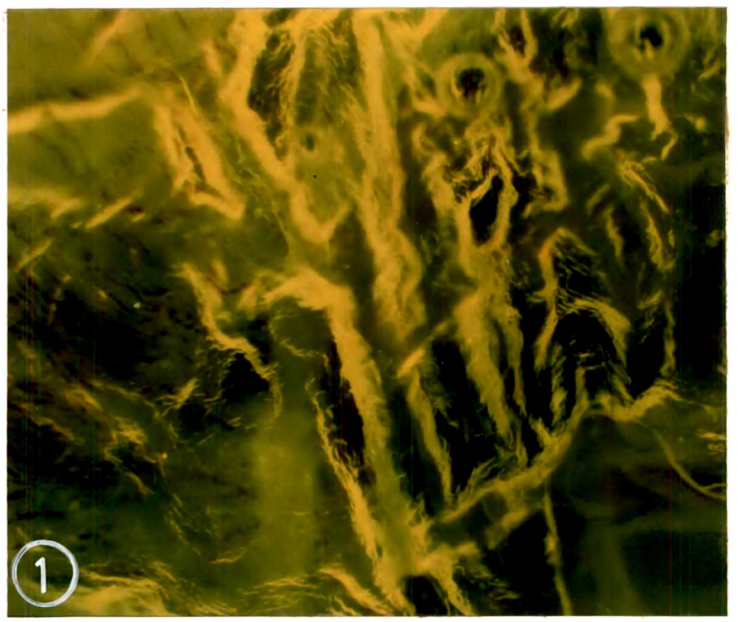
Chemically sympathectomised pigeon and rat and their controls were subjected to glucose load to attain the glucose tolerance curve. In pigeon, 6-OHDA + reserpine treatment caused an elevation of blood sugar level while in rat a slight decrease in glycaemic level was noticed (fig.6). Glucose loading in rat and pigeon (both experimentals and controls) produced a peak hyperglycaemic level by 30 minutes.

Explanation For Figures

Fig.1 & 2 Histofluorescence localisation of catecholamines in the cornea of the rat. 72x

Fig.1 Cornea of control rat. The yellow fluorescence of catecholamines are intense.

Fig.2 Cornea of the rat after 48 hrs. of 6-OHDA administration. The intensity of yellow flourescence of catecholamines are greately reduced.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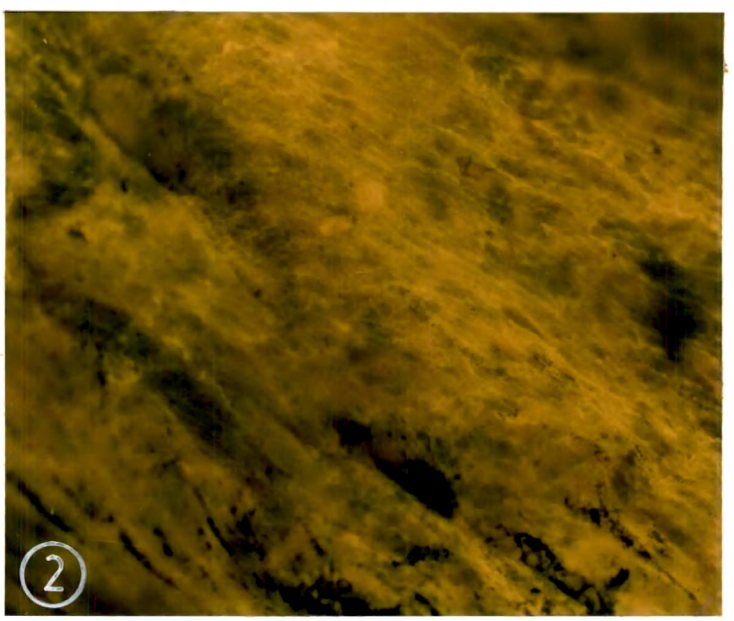
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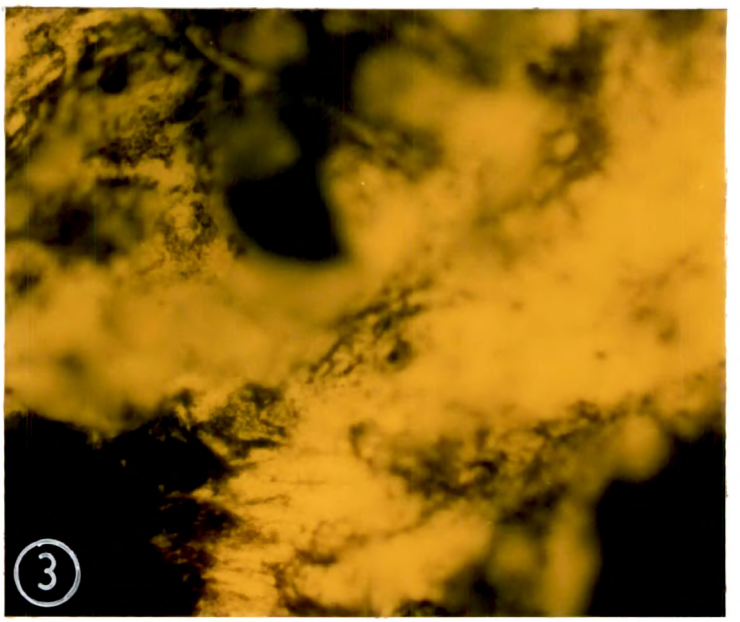
Explanation For Figures

Fig.3 & 4 Glyoxalic acid induced fluorescence of catecholamines in the cornea of the pigeon. 72x

Fig.3 Cornea of the control pigeon. Characteristic yellow fluorescence of catecholamines is intense.

Fig.4 Chemical sympathectomy using 6-OHDA reduces the catecholamine content in the cornea. (48 hrs after 6-OHDA treatment).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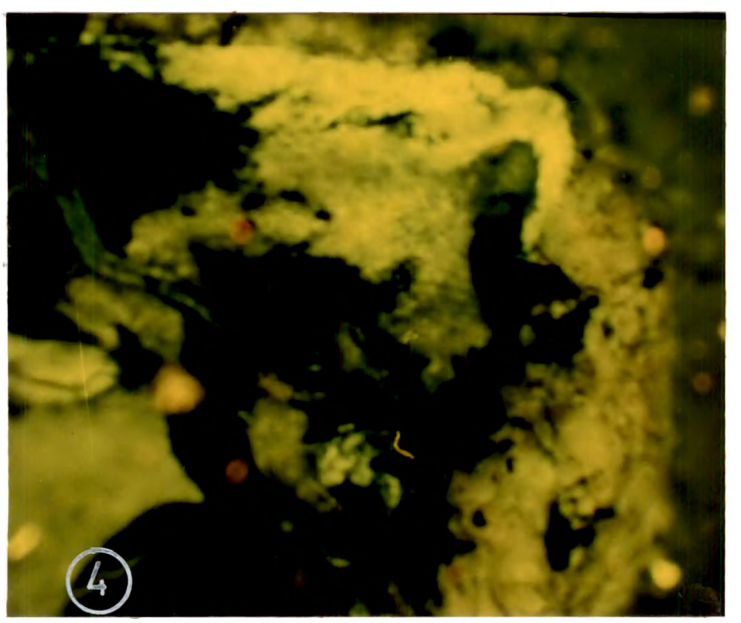
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Table I **Effect of chemical sympathectomy on AChE actitivity in the liver and kidney of rat and pigeon. (Mean ± SEM)**

Treatment	Rat		Pigeon	
	Liver	Kidney	Liver	Kidney
Control	0.0031 ± 0.0001	0.0015 ± 0.00006	0.0118 ± 0.001	0.0051 ± 0.0007
6-OHDA	0.0038**** ± 0.00013	0.0014 NS ± 0.00009	0.0108 NS ± 0.0008	0.0033 ** ± 0.0004

Values are expressed as µg substrate hydrolysed/mg protein/min

NS - Not significant, ** - $P < 0.02$; **** $P < 0.001$

Table II **Effect of chemical sympathectomy on DNA and RNA content in the liver and kidney of rat and pigeon. (Mean ± SEM)**

Treatment		Rat		Pigeon	
		Liver	Kidney	Liver	Kidney
DNA	Control	0.0954 ± 0.0028	0.1137 ± 0.0025	0.0693 ± 0.0025	0.1074 ± 0.0036
	6-OHDA	0.1130**** ± 0.002	0.1523**** ± 0.008	0.0443**** ± 0.0015	0.0944* ± 0.0051
RNA	Control	0.0825 ± 0.0005	0.0412 ± 0.0005	0.0635 ± 0.0006	0.0503 ± 0.0012
	6-OHDA	0.0976**** ± 0.0016	0.0479**** ± 0.0007	0.0687* ± 0.0016	0.054** ± 0.0008

Values expressed as mg/100mg tissue

* - $P < 0.05$; ** $P < 0.02$; **** $P < 0.001$

Table III Effect of 6-OHDA Administration On Glucose tolerance in rat and pigeon (Mean ± SEM).

Interval in Minutes	Rat		Pigeon	
	Control	6-OHDA	Control	6-OHDA
0	114.618 ± 6.339	86.579 ± 6.307	212.656 ± 8.185	324.891 ± 8.520
30	296.728 ± 6.082	437.347 ± 6.844	266.707 ± 12.604	407.167 ± 11.073
60	144.281 ± 7.353	246.225 ± 5.501	236.516 ± 9.500	348.018 ± 14.042
90	184.486 ± 6.882	177.342 ± 4.90	216.406 ± 7.618	346.507 ± 9.440
120	174.828 ± 7.767	170.874 ± 6.873	228.581 ± 12.43	341.308 ± 9.10
150	142.828 ± 6.868	152.527 ± 7.237	216.0741 ± 6.585	363.996 ± 11.369

Glucose mg/100ml blood

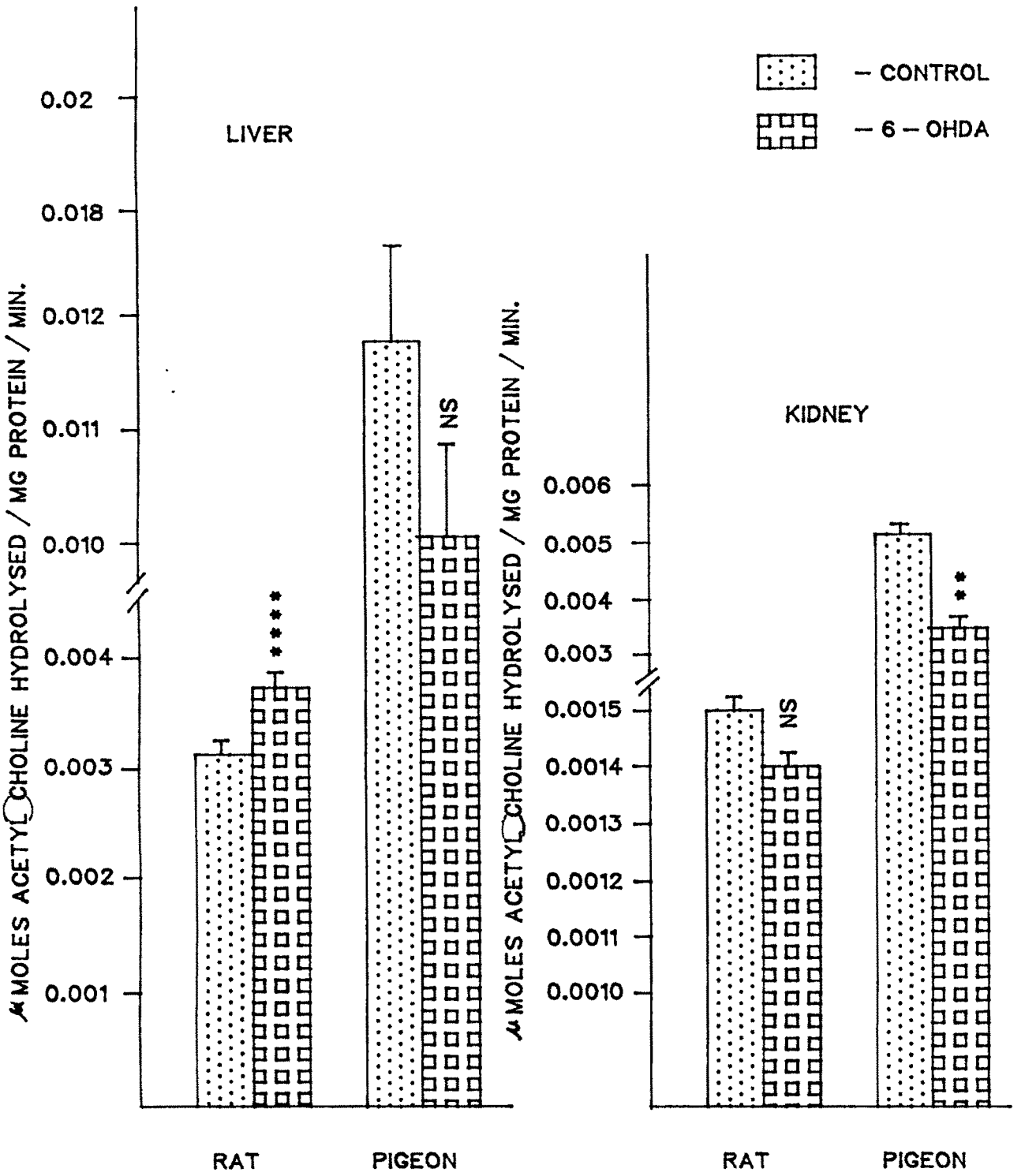

FIG. 5 EFFECT OF 6 — OHDA ADMINISTRATION ON ACHE IN THE LIVER AND KIDNEY OF RAT AND PIGEON. EACH BAR REPRESENTS THE MEAN ± SEM OF AT LEAST SIX ANIMALS. NONSIGNIFICANT; ** $P < 0.02$; **** $P < 0.001$.

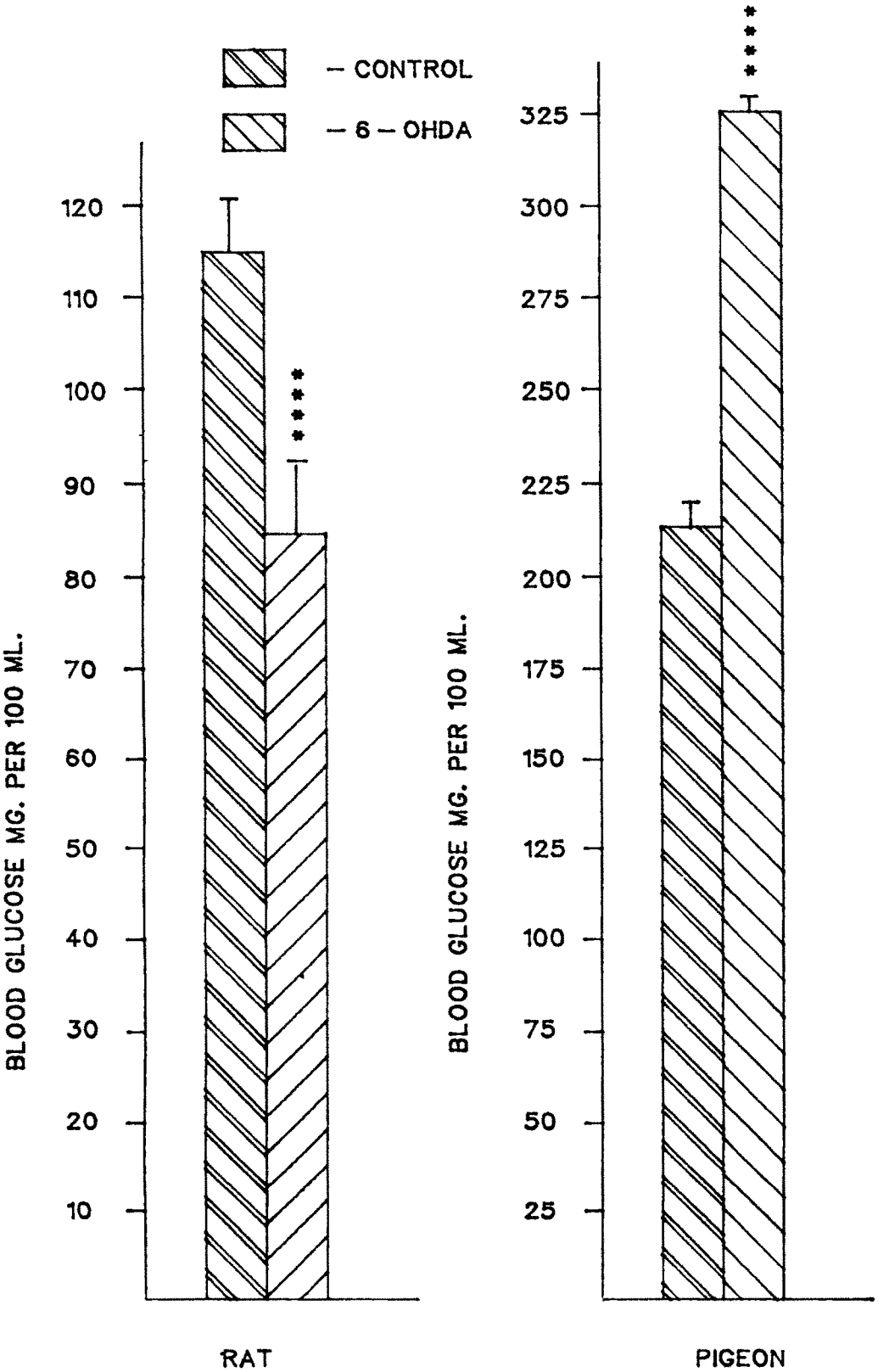

FIG. 6 BLOOD GLUCOSE LEVEL IN SYMPATHECTOMIZED RAT AND PIGEON EACH BAR REPRESENTS THE MEAN ± SEM OF AT LEAST SIX ANIMALS. **** $P < 0.001$.

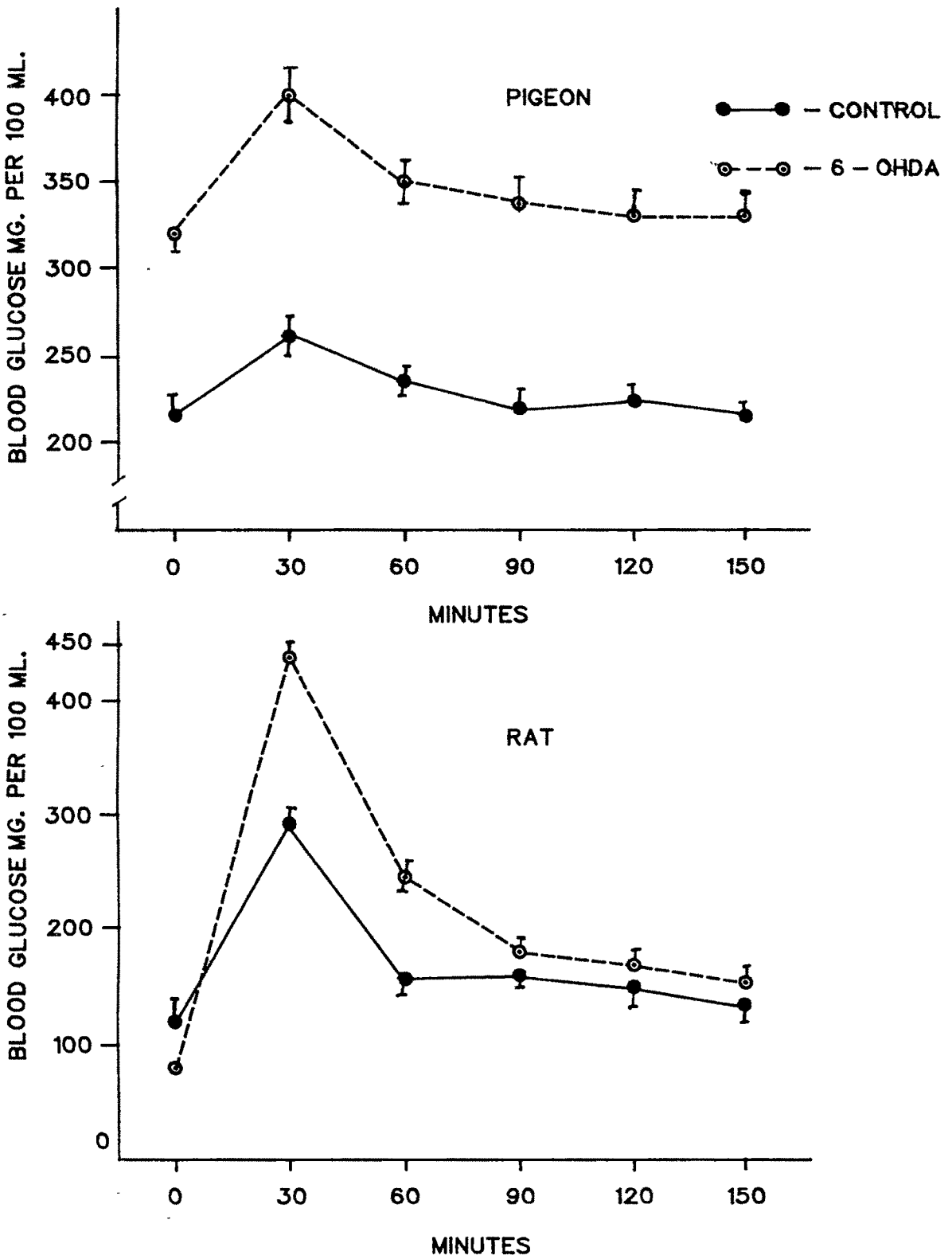

FIG.6 GLYCAEMIC RESPONSE TO 6 — OHDA TREATED RAT AND PIGEON.(N = 6).

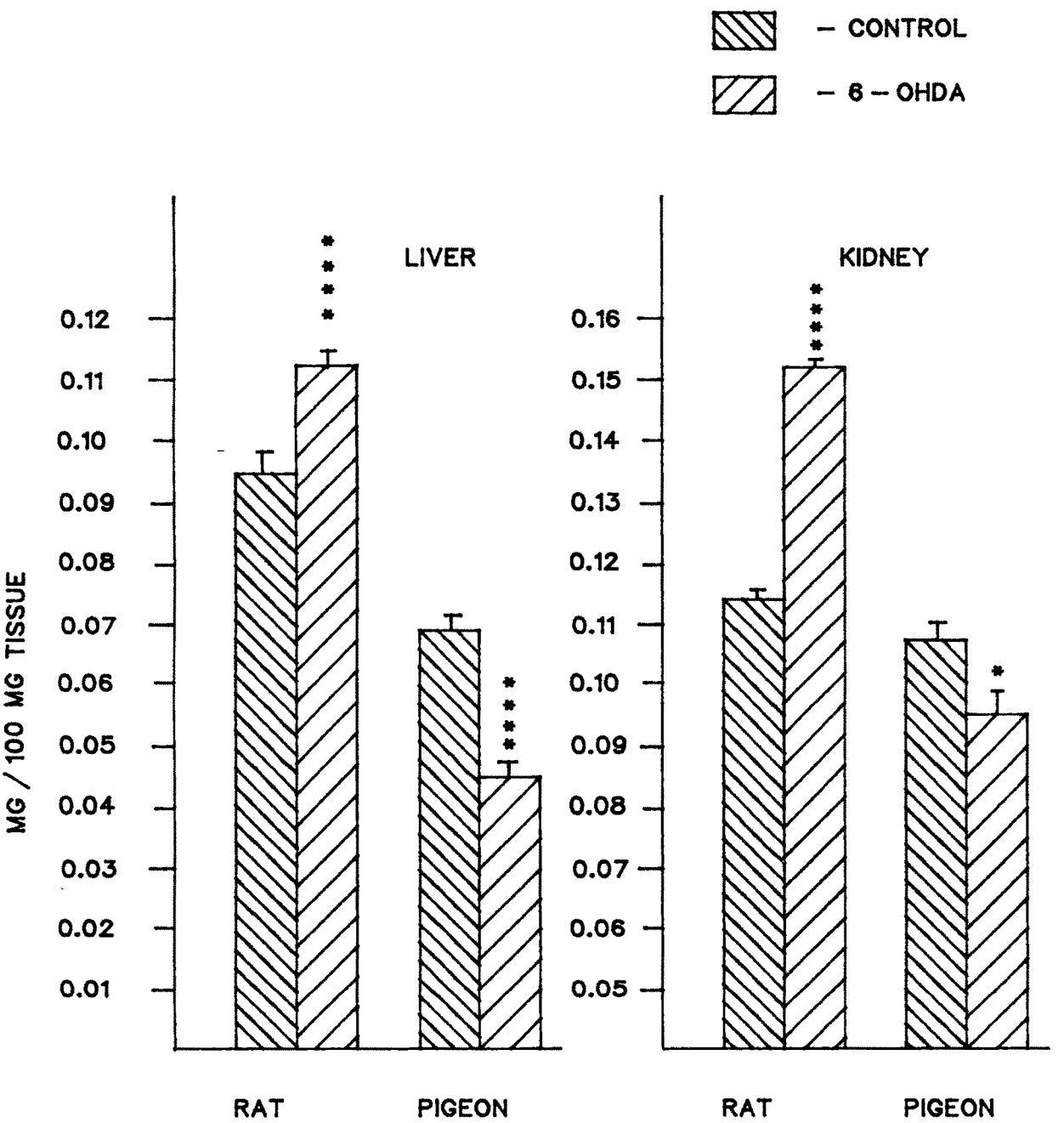

Fig.7 EFFECT OF 6 – OHDA ADMINISTRATION ON DNA CONTENT IN THE LIVER AND KIDNEY OF RAT AND PIGEON. THE MEAN ± SEM OF AT LEAST SIX ANIMALS. * $P < 0.05$; **** $P < 0.001$.

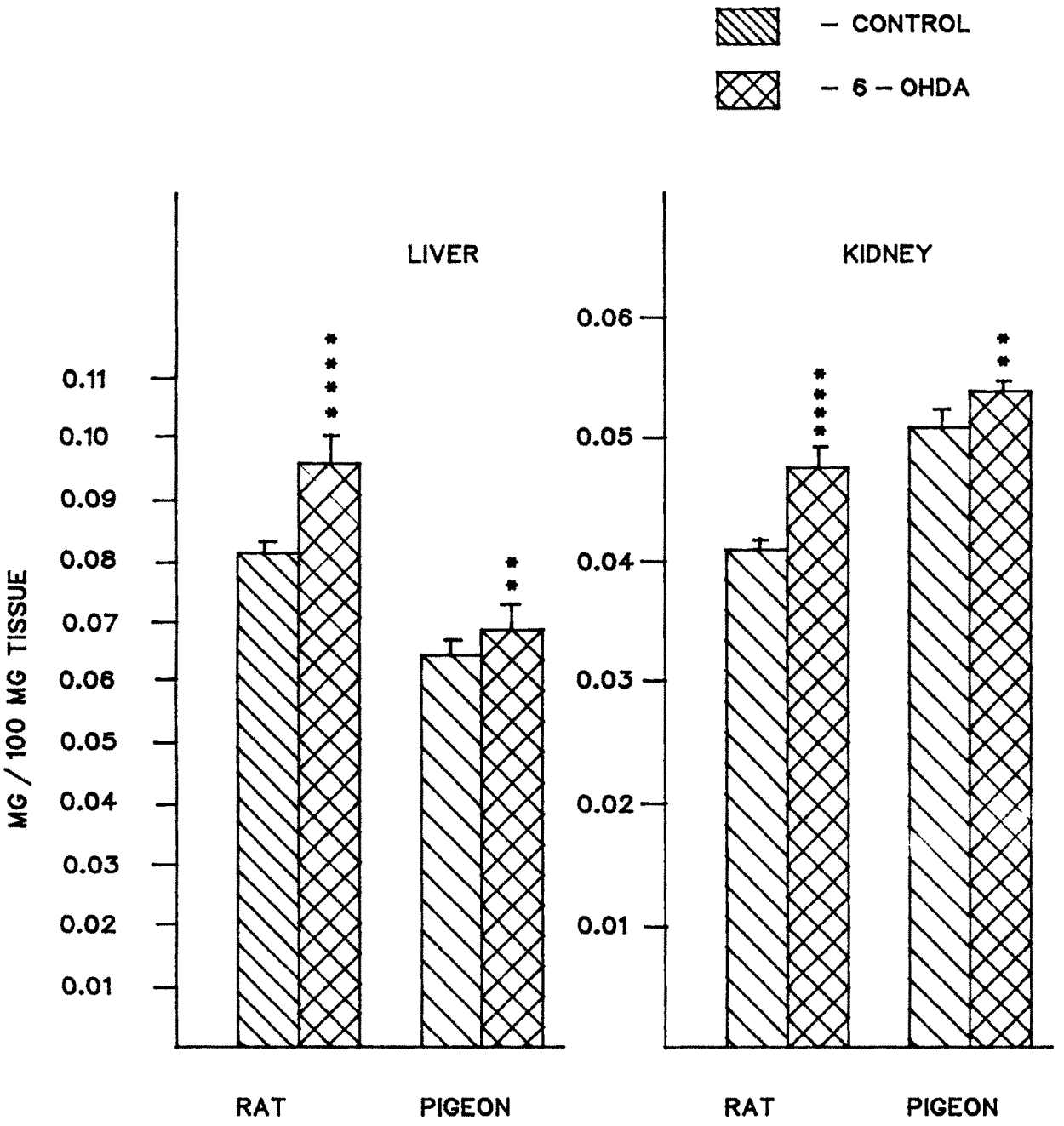

Fig.8 EFFECT OF CHEMICAL SYMPATHECTOMY (6 — OHDA) ON RNA CONTENT IN LIVER AND KIDENY OF RAT AND PIGEON. EACH BAR REPRESENTS THE MEAN ± SEM OF AT LEAST SIX ANIMALS. ** $P < 0.02$; **** $P < 0.001$.

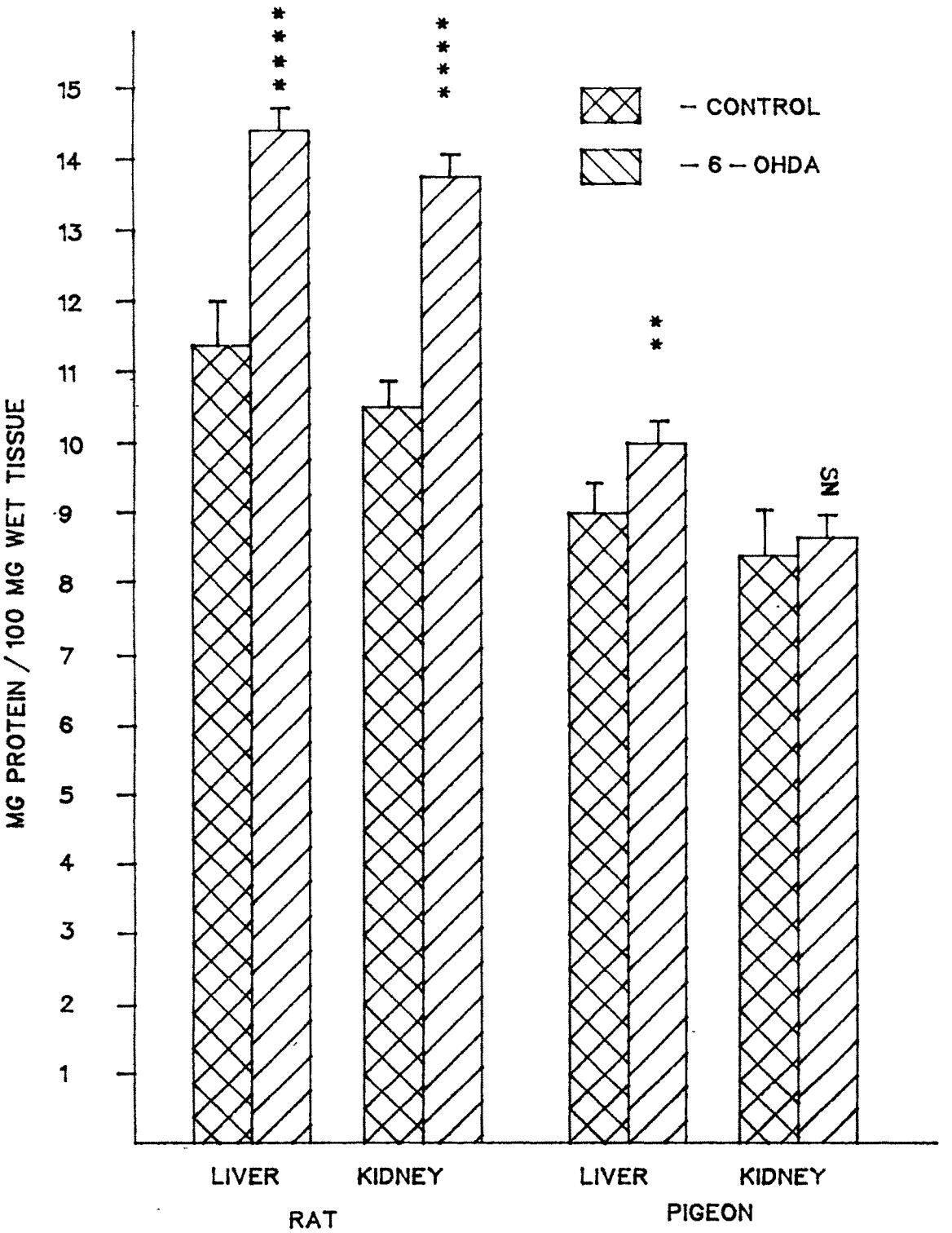

FIG. 9 EFFECT OF 6—OHDA ADMINISTRATION ON PROTEIN CONTENT IN LIVER AND KIDNEY OF RAT AND PIGEON. EACH BAR REPRESENTS THE MEAN ± SEM OF AT LEAST SIX ANIMALS NS — NON SIGNIFICANT; ** $P < 0.02$; **** $P < 0.001$.

The hyperglycaemic response of the rat was much more than that of pigeons. The glycaemic levels started declining from 60 minutes onwards. The GTT curves in the experimental and control animals were similar in both rat and pigeon. However, in 6-OHDA treated pigeons an increased basal level of glucose was maintained. (fig. 6).

Administration of 6-OHDA and reserpine in the rat resulted in an increased protein content and DNA/RNA levels in the liver and kidney (figs. 7 & 8). The nucleic acid metabolism was not much affected by chemical sympathectomy in pigeon. In the liver and kidney of 6-OHDA treated pigeons DNA content showed a decrease while RNA content showed slight increase, compared to that of control pigeons (fig. 7 & 8).

DISCUSSION

Studies conducted in our laboratory (Pilo and Patel, 1978) clearly showed that avian and mammalian liver cells take up glucose in the presence of acetylcholine (ACh). This action of ACh in avian liver cells was much more prominent than in mammalian hepatocytes. Chemical sympathectomy (using 6-OHDA) increased AChE activity significantly in the liver of rat indicating an increased vagal cholinergic action (tone). Administration of 6-OHDA into the portal vein selectively disturbs the hepatic sympathetic nerves (Lautt and Cotte, 1976, 1977) and also prevent the neuronal stimulation of glucose release (Lautt and Wong, 1978). Chemical sympathectomy by 6-OHDA did not affect the AChE activity in

the liver of pigeons. Chemical sympathectomy significantly reduced the dopamine (DA) concentration in muscle and NE in muscle, mucosa, pylorus and duodenum (Orloff et al., 1985). Resperine treatment results in an overall depletion of NE from the sympathetic nerves (Mukherjee et al., 1989). The depletion of NE by high dose of resperine does not influence the total activity of cholinesterase in nerve cell bodies of locus coeruleus of rat (Sket et al., 1985). Avian kidney is quite distinct from that of mammal in several aspects. AChE in the kidney of pigeon showed a decrease after 6-OHDA treatment indicating that chemical sympathectomy did not result in cholinergic stimulation in kidney. The action of vagal cholinergic fibres could somewhat be judged indirectly by the cholinesterase activity. Chemical sympathectomy has an adverse effect on the biochemical and functional development of the kidney in rat and pigeon (Mehta and Pilo, 1987; Slotkin et al., 1988)

The responses of glycaemic level in sympathectomy were different in rat and pigeon; the rat showed a decrease in glycaemic levels while pigeon showed a high level of glycaemia. An increased vagal tone after chemical sympathectomy will naturally bring about increased glucose uptake by the liver and a lower glycaemic level in the blood. Vagal action could stimulate the glucose uptake and glucogen synthesis while sympathetic action could induce glycogenolysis and glucose release. The glycaemic reponse in

birds is different from that of mammals especially with reference to the role of insulin and glucose (Pilo and Patel, 1978). The stimulation of peripheral end of vagus nerve results in an increase in the concentration of insulin in the portal vein of dog (Frohman et al., 1966). Both sympathetic and parasympathetic nervous system may be intimately involved in the mechanisms which control insulin secretion in the normal subject (Malaisse et al., 1967). The reduced NE and E secretion should also reduce the glucose release and a reduced blood sugar level in rat. In mammals, the circulating level of adrenaline is too low to stimulate glycogenolysis in the liver and hence the adrenergic effect on the mobilisation of glucose from liver must be mediated through sympathetic stimulation (Bentley, 1976). The suppression of sympathetic activity after VMH lesions could be one factor that modulate the release of insulin from the pancreas (Wood and Porte et al., 1974). In Birds, however, the insulin has a lesser role to play in glucose homeostasis and as such it is glucagon that is secreted more than glucose (Hazelwood, 1973). Inspite of the absence of sympathetic activity, pigeons maintained a high glucose level probably due to the activity of hormones such as growth hormone (GH) and corticosteroids (Glucocorticoids) due to stress. Glucocorticoid excess and glucocorticoid deficiency in vivo result in characteristic changes in the binding of insulin to its receptors and in the degree of biological actions of insulin (Conn et al., 1956; Munck, 1962; Munck and Kuritz

1962; Mc Kiddie, 1968; Modigliani et al., 1970; Benett et al., 1972; Olefsky et al., 1980; Yusuda, 1980). Glucocorticoid hormones are important in the regulation of gluconeogenesis and glycogenolysis (Baxer and Forhan, 1972). Increased action of GH and corticosterones would ensure a hyperglycaemia and a low response to insulin. Glucosteroids have been shown to have distinct effect on carbohydrate metabolism including promotion of gluconeogenesis, deposition of glycogen in the liver and elevation of blood glucose concentration (Parikh, 1992). Glucocorticoids play a major role in maintaining blood glucose level and most of its actions are manifested in the liver.

The reduced sympathetic tone in pigeon could have stimulated the release of more glucocorticoids as a response to stress. Glucocorticoids are known to have gluconeogenic and diabetic action in mammals (Ingle, 1952). Corticoid administration has been reported to produce hyperglycaemia in birds like pigeon and White Leghorn chicken (Pilo and Mehta, 1985). In laying hens the increase in NE level was influenced by the corticoid infusion which suggests that corticosterone mainly affects peripheral sympathetic nervous activities rather than adrenal medullary secretion (Pilo et al., 1985). Thus the hyperglycaemic response of pigeons following chemical sympathectomy are probably due to the increased release of hormones such as growth hormone (GH) and corticosterone. The GTT response in sympathectomized rat and pigeon was similar

to that of respective control animals which means that the glucose uptake mechanism was not affected by chemical sympathectomy.

The results (Table 11) demonstrate that sympathectomy does affect the DNA and RNA content in the liver and kidney of rat. The DNA and RNA content was enhanced in the liver and kidney of rat after chemical sympathectomy. In the liver, the major influence of insulin is attributed to the augmentation of RNA synthesis (Rannels et al., 1977). The insulin is known to promote protein synthesis through accelerating amino acid transport into cells (Innu and Ishioka, 1983a,b). Since the blood sugar level in 6-OHDA treated rat is low, it is reasonable to believe an increased release of insulin from B cells (Kleitonam and Helzworth, 1985). The decreased DNA content in the liver and kidney of sympathectomised pigeon could be correlated to the excess release of corticosterone during chemical sympathectomy. Injection of normal animals with high doses of corticosteroids results in an inhibitory effect on protein synthesis (Manchester, 1959; Rannels et al., 1978). RNA content in the liver and kidney of pigeon showed no insignificant response to chemical sympathectomy.

Thus, the present study has unravelled the mechanisms involved in glucose regulation after sympathectomy in the liver and kidney of avian and mammalian models. The glycaemic response of these two models were found to be different after

the sympathectomy and thus has thrown some light on the action of pancreatic hormone in regulation of glucose homeostasis. Another noteworthy aspect is the increase in vagal tone as indicated by AChE activity in chemically sympathectomised rats. This enhanced AChE levels were absent in sympathectomised pigeons. Protein and nucleic acid levels were found to be increased in rats while these parameters were not much affected in pigeon. The increase in proteins, DNA and RNA levels in rats were well-correlated with the multiple actions of insulin on tissues. The insignificant role of insulin in maintaining the glycaemic level in avian model was also reflected on the levels of protein, DNA and RNA. The decrease in these parameters in pigeon could be primarily due to the release of glucocorticoids which in excess suppress the protein, DNA and RNA levels in tissues.