CHAPTER VIII

EFFECT OF CATECHOLAMINES ON KIDNEY OF BLUE ROCK PIGEON (*COLUMBA LIVIA*)

It has become almost customory to classify catecholamines responses as either α or β-responses. Such categorizations are convenient to place the data into neat slots of understanding. But in reality, such clear cut and distinct α & β-responses seldom exist in tissues like liver. Hepatic catecholamine receptors are believed to be non-specific (Fleming and Kenny, 1964; Guthrie and Murphy, 1975; Himms-Hagen, 1967; Hornbrook, 1970). The current view point is that the metabolic actions of catecholamines are seperate from their stimulatory effects on adenylate cyclase (Krans-Friedman, 1984). Some of the actions of epinephrine (E), norepinephrine (NE) and α-agonists are mediated through an increase in cytosolic concentrations of Ca^{++}. Hence, discussion on the effect of catecholamines on metabolic activities could dispense with characterization of the responses into α and β types, or such attempt may prove futile.

Characteristic metabolic actions of catecholamines are hyperglycaemia and depletion of glycogen stores and gluconeogenesis. In birds these responses are age dependent (Freeman, 1966; Heald, 1966). Of the two catecholamines, epinephrine is more potent as hyperglycaemic agent than norepinephrine (NE).

Epinephrine is known to inhibit pyruvate kinase by a cAMP-independent mechanism which is reported to be the most prevalent method by which it activates gluconeogenesis (Feliu et al., 1976; Chan and Exton, 1978). Kneer et al.(1974) reported that catecholamines stimulates the rate of gluconeogenesis from substrates through pathways not involving mitochondria. Epinephrine has been shown to stimulate the rate of glucose synthesis from pyruvate and lactate in perfused livers (Exton and Park, 1968; Menahan et al., 1968; Rose et al., 1967; Williamson et al., 1969), liver slices (Rinard et al., 1969) and suspension of liver cells (Johnson et al., 1972; Garrison and Haynes, 1973). In kidney, NE stimulates Na^{+}-K^{+}-ATPase both in vivo and in vitro (Krut'Ko, 1982). In chicken liver NE stimulation is mediated through a α-adrenergic and Ca^{++} dependent mechanism in which redox changes of mitochondrial pyridine nucleotides are involved (Tsukasa et al., 1982). NE also stimulates SDH activity in kidney, heart, liver and brown adipose tissue (Sivaramakrishnan and Ramasarma, 1982). Even cytochrome 'C' oxidase was activated by both E and NE in kidney, liver and brain tissues (Tapbergenov, 1982).

Catecholamines, thus, exert considerable influence on carbohydrate metabolism whether through an α-adrenergic cAMP independent, or through β-adrenergic cAMP dependent mechanisms. Both these mechanisms activate protein kinases that phosphorylate enzymes involved in carbohydrate metabolism. Thus both

α-and β-agonists may exhibit identical pattern of activation or inactivation of enzymes.

With a view to understand the effect of E and NE on the metabolic functions of avian kidney, these catecholamines were administered in pigeon and kidney enzymes were estimated.

MATERIAL AND METHODS

Adult domesticated variety of Blue rock pigeons (Columba livia) of both sexes weighing 250-300 gms were used in the experiment. The birds were acclimated to laboratory conditions for two weeks and fed ad-libitum. The birds were divided into two groups. Experimental group was injected (i.p) with epinephrine (Harson Laboratories) 0.1 mg/day for two days, 24 hours apart and were kept in starved conditions. Norepinephrine (Unichem Laboratories) was also injected (i.p) in the dose of 0.1 mg/day for two days 24 hours apart and they were also kept in the starved conditions. Control birds received same amount of R.D.W. Twenty four hours after the last injection, both experimental and control pigeons were sacrificed by decapitation. Prior to decapitation, for glucose estimation, the blood was drawn from the wing vein. Kidney was quickly excised and used for glycogen and enzyme estimations. The enzymes estimated were alkaline and acid phosphatases, G-6-Pase, phosphorylase, Na^+-K^+-ATPase, Transaminases (GOT and GPT), and LDH and AChE. Glycogen and

protein were also estimated. The methods followed for these estimations are given in Chapter 1.

RESULTS

The results are presented in Table I and Figs. 1 to 6.

The data show that both E and NE produced a significant hyperglycaemic response in the pigeon. At the same time kidney glycogen content showed an increase. Alkaline phosphatase showed differential response to E and NE. The activity increased when NE was administered while it decreased when E was administered. Acid phosphatase showed a decrease in response to E but failed to show any variation in response to NE administrations. Phosphorylase activity showed a decrease in response to both the catecholamines. G-6-Pase showed a decreased activity level in the kidney with E administration while NE failed to induce any change in the activity. Na^+-K^+-ATPase activity increased significantly due to NE administration but with E, the activity only decreased. LDH activity similarly showed a decrease in E administered pigeon kidney, while in NE administered pigeon kidney, it showed a significant increase. Of the two transaminases alanine aminotransferase (GOT) showed enhanced activity only in the E treated pigeon kidney, while GPT did not show any change in the level of activity. In NE treated pigeon kidney both GOT and GPT failed to show any variation.

Table I: The effect of catecholamine injection on metabolic activities of pigeon kidney. (Mean ± S.E).

Parameters	Normal	Control	Epinephrine	Norepinephrine
Protein	13.956 ± 1.038	16.510 ± 1.550	7.05 ± 1.07	8.86 ± 1.04
Alk Pase	1.379 ± 0.078	0.296 ± 0.023	0.117 ± 0.030 **	0.55 ± 0.070 *
Acid Pase	0.834 ± 0.046	0.271 ± 0.021	0.117 ± 0.008 ***	0.267 ± 0.023 NS
GOT	90.400 ± 8.695	57.290 ± 5.860	75.630 ± 1.430 **	64.140 ± 3.90 NS
GPT	151.620 ± 8.346	75.220 ± 2.600	73.670 ± 2.880 NS	68.860 ± 6.900 NS
Na^+-K^+-ATPase	133.300 ± 18.088	53.000 ± 8.380	14.720 ± 0.791 **	78.320 ± 5.070 *
Phosphorylase	233.566 ± 21.963	262.600 ± 18.980	87.730 ± 9.250 ***	144.190 ± 6.540 ***
G-6-Pase	0.113 ± 0.022	0.114 ± 0.022	0.257 ± 0.035 **	0.102 ± 0.004 NS
AChE	3.370 ± 0.077	0.55 ± 0.018	1.790 ± 0.022 ***	1.801 ± 0.275 **
LDH	16.000 ± 1.696	17.410 ± 1.230	11.310 ± 1.110 **	22.730 ± 1.150 *
Glucose	120.000 ± 5.262	76.510 ± 4.871	322.800 ± 7.860 ***	351.120 ± 6.250 ***
Glycogen	0.033 ± 0.009	0.024 ± 0.001	0.056 ± 0.006 ***	0.035 ± 0.001 ***
Body weight	290 ± 4.47	250 ± 14.50	224 ± 10.29	242 ± 8.59
Total kidney weight	1.59 ± 0.07	1.27 ± 0.03	1.12 ± 0.07	0.92 ± 0.04

* $P < 0.02$, ** $P < 0.01$, *** $P < 0.001$, NS - Not significant.

EXPLANATIONS TO GRAPHS - CHAPTER VIII

Fig.1. Graphs showing the effect of catecholamines administration on blood sugar level in the kidney of blue rock pigeon.

Fig.2. Graphs showing the effect of catecholamine administration on GOT and GPT activities in the kidney of blue rock pigeon.

Fig.3. Graphs showing the effect of catecholamine administration on acid Pase and G-6-Pase activities in the kidney of blue rock pigeon.

Fig.4. Graphs showing the effect of catecholamine administration on Alk Pase and Na^{+}-K^{+}-ATPase activities in the kidney of blue rock pigeon.

Fig.5. Graphs showing the effect of catecholamine administration on AChE and LDH activities in the kidney of blue rock pigeon.

Fig.6. Graphs showing the effect of catecholamine administration on phosphorylase activity and protein content in the kidney of blue rock pigeon.

FIG. 1 : EFFECT OF CATECHOLAMINES

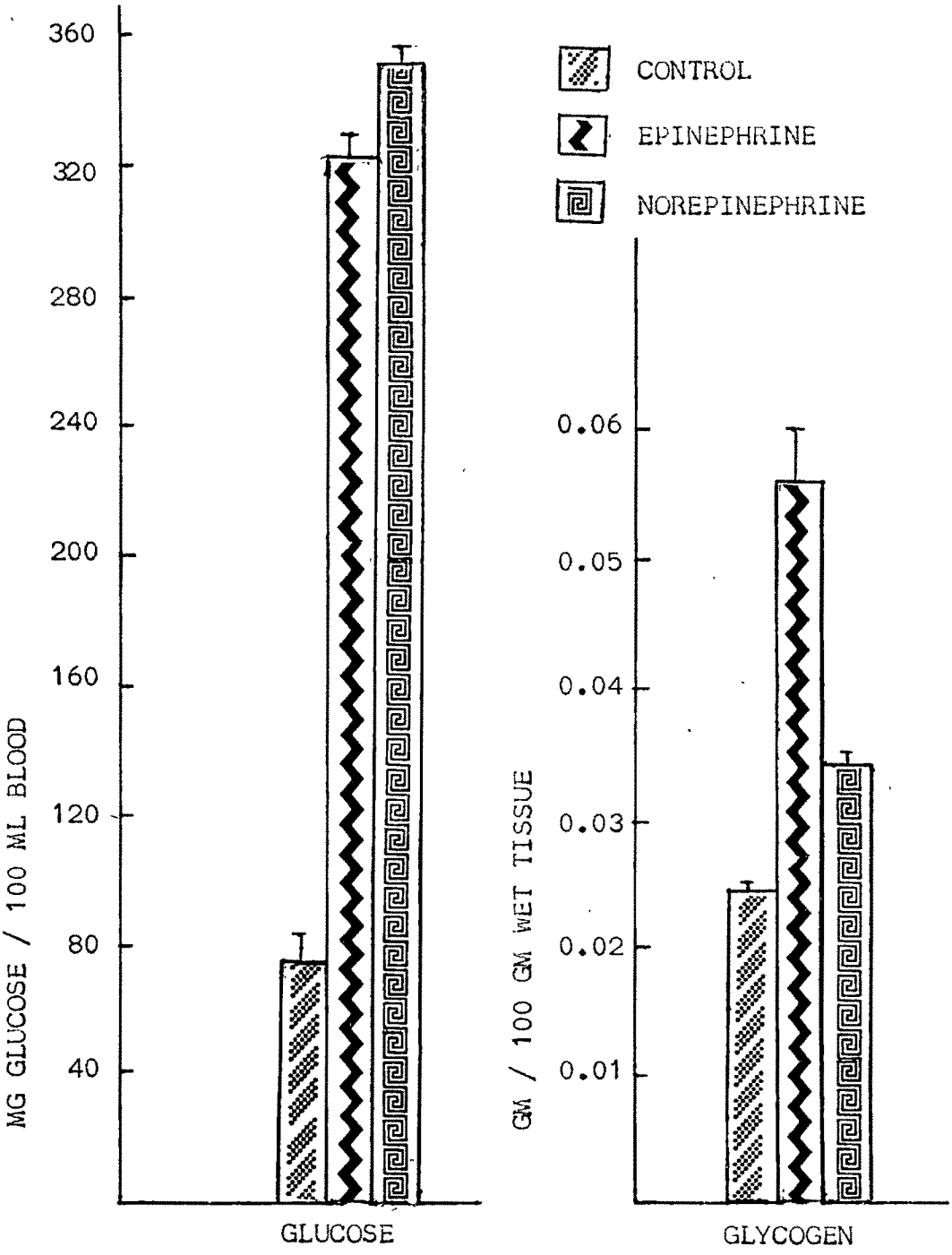


FIG. 2 : EFFECT OF CATACHOLAMINES

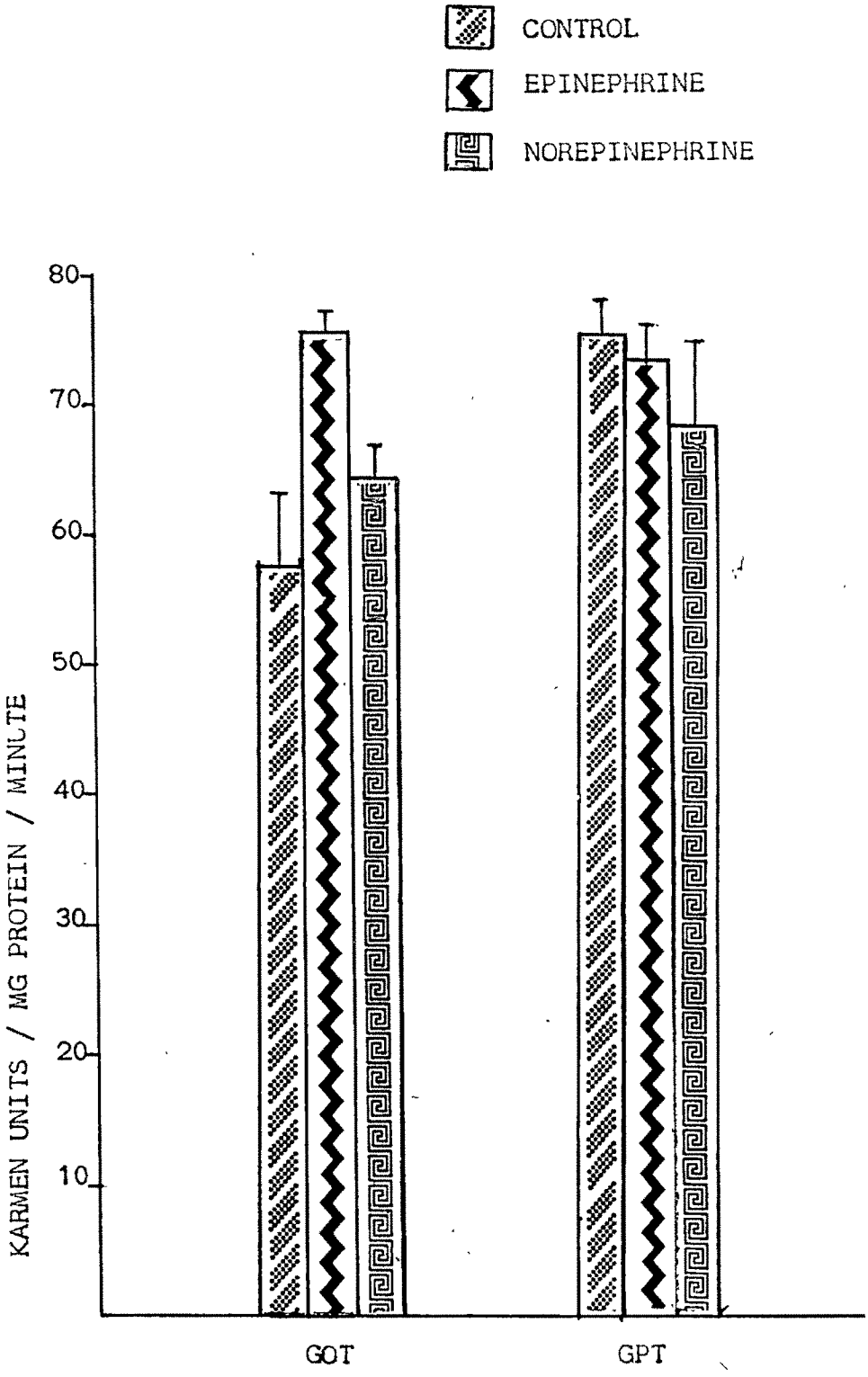


FIG. 3 : EFFECT OF CATACHOLAMINES

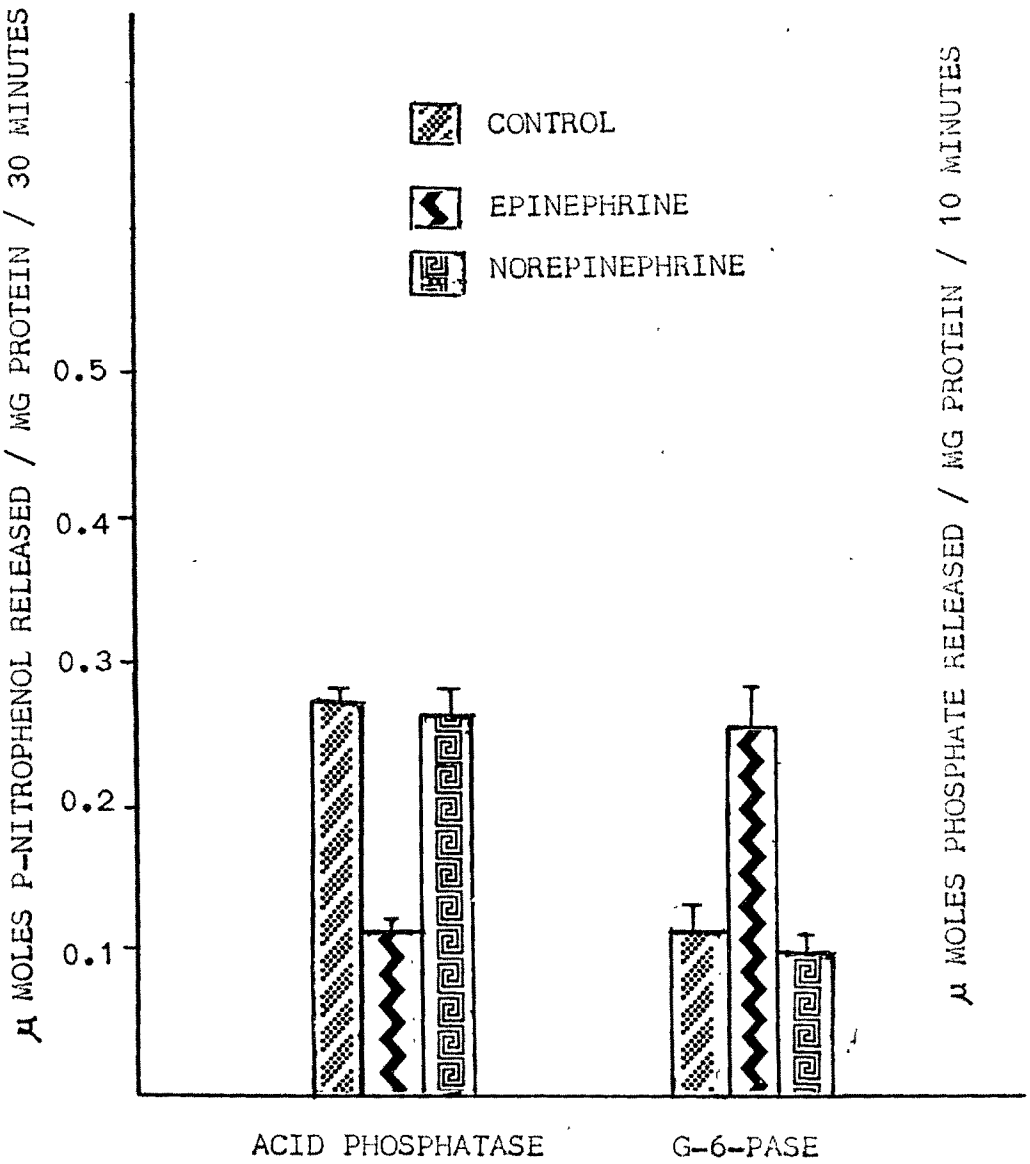


FIG. 4 : EFFECT OF CATACHOLAMINES

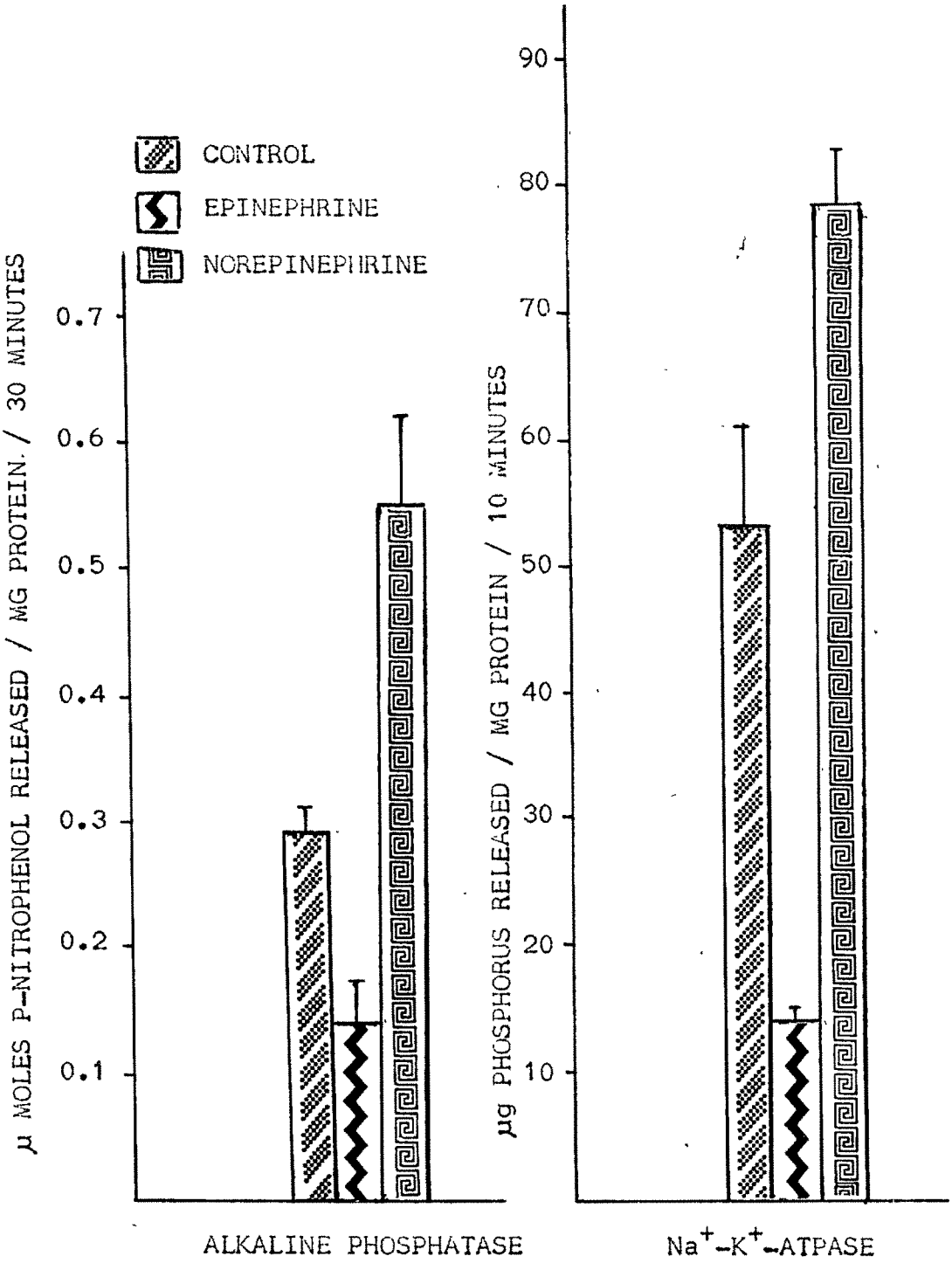
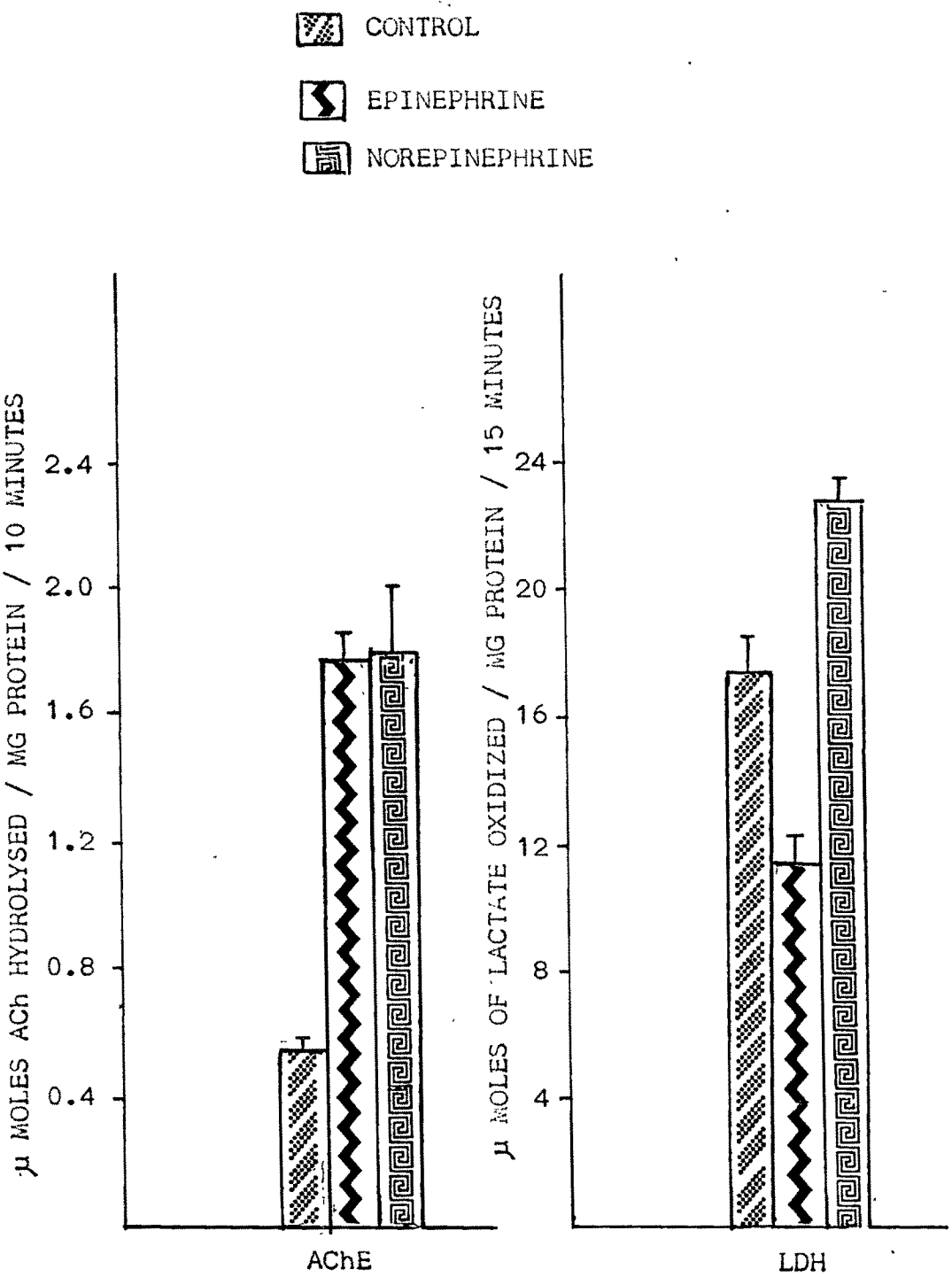


FIG. 5 : EFFECT OF CATACHOLAMINES



The protein content of kidney exhibited reduction in both E and NE treated pigeon. Similarly, both the catecholamines produced a reduction in general body weight and total kidney weight. Both E and NE increased the activity of AChE in the kidney.

DISCUSSION

Gluconeogenic activities in liver and kidney may have several similarities, but patterns of responses are not always similar. In mammalian kidney, gluconeogenic condition increases the activities of gluconeogenic enzymes but not the activities of enzymes concerned with amino acid catabolism (Szepesi et al., 1970). In birds, the pattern of responses in liver and kidney differ further due to the presence of distinct cytosolic PEPCK enzyme in the kidney, while liver contains a mitochondrial PEPCK (Ogata et al., 1982; Watford et al., 1981). In chickens, the liver synthesizes glucose from lactate and the kidney produces it from substrates such as pyruvate and amino acids (Watford, 1985). The cytosolic form of PEPCK is subject to regulation by hormones such as glucagon, c-AMP, E and glucocorticoids which increase the activity of this enzyme (Iynedjian et al., 1978). Effects of hormones on gluconeogenic activities in liver and kidney could also be expected to be different.

Administration of E or NE produced hyperglycaemia

without parallel reduction in kidney glycogen content indicating that glucose released into the blood comes from liver. This is also evident from the fact that both E and NE caused a decrease in the activity of phosphorylase in the kidney. Increase in G-6-Pase was seen only in the kidney of E administered pigeon. Probably some amount of glucose production and release do take place in E treated pigeon kidney. Gluconeogenesis in response to E in kidney must be taking place from precursors such as alanine as GOT activity was significantly high. Gluconeogenesis from aminoacids is not activated in NE treated pigeon kidney where both GOT and GPT showed no significant variations in the activities. In all probability, NE stimulated the lactate release or utilization in the kidney by increasing LDH activity. In mammalian liver, it was E that stimulated gluconeogenesis from lactate and alanine (Luigi et al., 1983). The stimulation of gluconeogenesis in liver or kidney by catecholamines, thus depends on the enzyme profile of tissues and species. In the avian kidney, E may be stimulating gluconeogenesis from aminoacids, while NE may be stimulating it form lactate. As mentioned by Chiscko et al. (1983) catecholamines play dual roles in the regulation of amount of enzymes by suppressing the synthesis of some enzymes while inducing the synthesis of others. In the avian kidney also, catecholamines may be involved in inducing the synthesis of some key gluconeogenic enzymes, while inhibiting the synthesis of glycolytic enzymes.

The mechanism of catecholamine effect on mammalian hepatic gluconeogenesis from substrates that enter the pathway prior to phosphoenolpyruvate or from reduced substrate that enter the pathway at triose phosphate level is reported to be through their ability to mobilize Ca^{++} to the cytosol (Kneer and Lardy, 1983). NE also activates gluconeogenesis from oxidized substrates that enter the pathway at triose phosphate level and this effect is mediated through a mechanism that does not involve either changes in c-AMP or cytosolic Ca^{++} concentrations (Kneer and Lardy, 1983).

In vivo actions of E and NE on the gluconeogenic activity in the avian kidney are somewhat different. E favours gluconeogenesis from precursors such as alanine and pyruvate while NE favours gluconeogenesis from lactate. However, administration of catecholamines may not bring about all characteristic actions in tissues in in vivo condition. Simultaneous increase in reciprocating antagonists such as insulin or vagal cholinergic activity, may suppress some of the catecholamine actions in the kidney. Catecholamine administration produced an increase in acetylcholine secretion in the kidney. This could be deduced from the fact that AChE activity increased almost 3 fold in the kidney after E and NE administration.