CHAPTER 9

COMPARATIVE STUDIES ON THE INFLUENCE OF INSULIN AND ACETYLCHOLINE ON THE TRANSPORT OF GLUCOSE AND GLYCOGEN DEPOSITION IN LIVER SLICES OF PIGEON AND RAT

The hypoglycaemic effect of insulin is due to its actual influence on different metabolic tissues. The effect of insulin on mammalian carbohydrate metabolism is known for long. However, in birds, ~~the~~ insulin is reported to be playing only a secondary role in the regulation of carbohydrate metabolism (Hazelwood, 1973). In this respect, both these groups of vertebrates are quite different; one (mammals) is more insulin dependent and the other (birds) is more glucagon dependent (Hazelwood, 1973). Although, the avian insulin, when injected into intact chicken, lowered blood sugar level (Hazelwood *et al.*, 1968), the control of carbohydrate metabolism by insulin in birds is of minor significance only.

In support of this contention, Hazelwood (1973) lists paucity of insulin reserve within the pancreas, the sluggish response of B-cells to release insulin in face of glucose challenge and persistent hyperglycaemia (225-300 mg% glucose as opposed to 90-120 mg% in mammals). The avian insulin is believed to act synergistically with glucagon accentuating the latter's role in adjusting carbohydrate metabolim. In fact the action of insulin in *in vivo* conditions are different from its *in vitro* effect as in the former case other endocrine secretions and even the nervous elements could modulate insulin's action on tissues.

The recent findings denote that the effect of insulin on the sugar transport is amplified by the synergistic participation of nervous elements and their neurotransmitters (Mondon and Burton, 1971). Shimazu (1967) has reported that the stimulation of cholinergic nerve fibres activates the glycogen synthetase enzyme. In the previous *in vivo* studies, an elevated cholinesterase activity has been observed in the liver, following glucose administration in both pigeon (Chapter 8a) and rat (Chapter 8b) and these findings were suggestive of participation of acetylcholine (ACh) in the transport of glucose across hepatic cell membrane. The

cyclic variation of hepatic cholinesterase (ChE) during feeding and fasting also suggest that ACh-AChE system might be involved in the mechanism of assimilation (Gerebtzoff, 1959). An intense reactivity of AChE at the sinusoidal linings of hepatic cords of birds (Pilo, 1969; Shah et al., 1972b and Chapter 2) was also reported to be suggesting an active participation of ACh-AChE system in the transport of glucose and perhaps other metabolites like amino acids.

The previous experiments have clearly shown that glucose administration elicites AChE activity in the liver of pigeon much ~~more~~ faster than in the liver of rat (Chapters 8a and 8b). The obvious inference was that in the avian liver, the concerted action of insulin and acetylcholine is more favourable for glucose uptake than insulin alone. To substantiate these findings, a further attempt is made at present to see, in in vitro condition, the synergistic effect of insulin and acetylcholine on the hepatic uptake of glucose and the resultant glycogenesis in an avian species and for comparison in rat.

MATERIALS AND METHODS

Adult pigeons (Columba livia) weighing 180-200 gms and male albino rats (Haffkine strain) weighing 160-180 gms,

maintained in laboratory conditions on balanced diets were used for experiments. Animals were sacrificed by mild anesthesia after 24-hr starvation period. A piece of liver was quickly excised and 0.5 mm thick slices weighing about 50-60 mg, were cut and washed in chilled Krebs Ringer bicarbonate (KRB) medium. The slices were incubated in a KRB medium, previously gased with air mixture for 15 minutes, containing 2.0 mg bovine albumin per ml. The pH was adjusted to 7.4. The incubation was carried out for 90 minutes at 37°C in a water bath shaker with 120 oscillations/min. The slices were incubated in media of following different categories.

1. 3 ml Krebs Ringer bicarbonate medium alone.
2. 3 ml KRB medium + D-glucose (2.5 mg/ml for rats and 5.0 mg/ml for pigeons).
3. 3 ml KRB medium + D-glucose + insulin (1 unit/ml, The Boots Co. (India) Ltd.).
4. 3 ml KRB medium + D-glucose + acetylcholine chloride (E. Merck), (0.5 mg/ml for rats and 1.0 mg/ml for pigeons).
5. 3 ml KRB medium + D-glucose + insulin + acetylcholine chloride.

After the incubation, the slices were quickly washed in KRB medium and were subjected to the estimation of glycogen

using anthrone reagent according to the method described by Seifter et al. (1950).

RESULTS

The results obtained from the experiments on pigeon and rat liver slices are presented in Table 1.

In the pigeon liver, the insulin stimulated the uptake of 0.5496 μ mole glucose/gm liver while in rat liver it stimulated 30.9 μ mole glucose/gm liver. Whereas, the pigeon liver slices treated with ACh incorporated 36.201 μ mole glucose/gm liver into the glycogen, the rat liver took up only 6.92 μ mole glucose/gm liver in the presence of acetylcholine. When incubated in the medium containing both insulin and acetylcholine, pigeon liver slices deposited 38.482 μ mole glucose/gm liver into glycogen, while rat liver slices deposited 57.28 μ mole glucose/gm liver glycogen. (The glucose uptake was calculated from the amount of glycogen deposited when incubated in medium having only glucose). When the medium contained no glucose, the liver slices were found to lose glycogen; from the pigeon liver slices 105.64 μ mole glucose/gm liver was released into the medium while from rat liver only 16.52 μ mole glucose/gm liver was released.

TABLE 1

Effects of insulin and acetylcholine, either individually or combined, on net glycogen deposition and glucose uptake in liver slices of 24-hr starved male albino rats and domestic pigeon.

Incubation time 90 minutes	Glycogen in slice incubated in medium containing				
	Only medium	Glucose[1] (CONTROL)	Glucose[1] + insulin[2]	Glucose[1] + acetylcholine[3]	Glucose[1] + insulin[2] + acetylcholine[3]
PIGEON	0.8787 ±0.2316	1.0625 ±0.1146	1.0912 ±0.1148	1.1625 ±0.1142	1.1671 ±0.1149
µg glycogen/100 gm liver depleted (-) or deposited (+)	(-) 211.30 ±164.00		(+) 1.10 ±0.10	(+) 72.40 ±17.10	(+) 76.96 ±17.74
µ mole glucose/gm liver depleted (-) or deposited (+)*	(-) 105.64 ± 81.99		(+) 0.5496 ±0.0499	(+) 36.201 ± 8.560	(+) 38.482 ± 8.870
RAT	0.1606 ± 0.0163	0.1937 ±0.0242	0.2555 ±0.0286	0.2074 ± 0.0232	0.3083 ± 0.0241
µg glycogen/100 mg liver depleted (-) or deposited (+)	(-) 33.10 ± 14.10		(+)61.80 ±22.80	(+) 13.70 ± 1.40	(+)114.60 ±24.40
µ mole glucose/gm liver depleted (-) or deposited (+)*	(-) 16.52 ± 7.04		(+)30.90 ±11.40	(+) 6.92 ± 0.70	(+) 57.28 ±12.19

1. Glucose conc. 2.5 mg/ml for rats and 5.0 mg/ml for pigeons.
2. Insulin conc. 1 unit/ml (The Boots Co., (India) Ltd.).
3. Acetylcholine chloride 0.5 mg/ml for rats and 1.0 mg/ml for pigeons.
* values are calculated from glycogen using conversion factor (1.11).

DISCUSSION

From the results it could be seen that in pigeon liver slices the insulin has only a negligible effect on the uptake of glucose or glycogen deposition. Whereas the liver slices treated with acetylcholine enhanced tremendously the uptake of glucose and glycogen deposition, the insulin and acetylcholine together, increased glycogen deposition only slightly more than that seen in the liver slices treated with acetylcholine alone. Acetylcholine alone stimulated the deposition of 72.40 µg of glycogen/gm liver while insulin alone stimulated the deposition of 1.10 µg of glycogen/gm liver in the slices.

In the rat liver slices, the effect of insulin and acetylcholine were different from that seen in that of pigeon liver slices. Here, the insulin is found to play a significant role in the uptake of glucose and glycogen deposition by the hepatic cells, while acetylcholine alone had only a negligible influence on the glucose uptake. Insulin stimulated the deposition of 61.80 µg glycogen while ACh influenced the deposition of only 13.70 µg glycogen/gm liver. However, the combined effect of insulin and acetylcholine resulted in a deposition of 114.60 µg glycogen or the uptake of 57.28 µ mole glucose/gm liver by the slices. Interestingly, in the rat liver slices, ACh-insulin combination accentuated the uptake of glucose while in the pigeon liver slices such effect was

only a negligible.

Mondon and Burton (1971) have clearly shown that in the rat liver, the uptake of glucose molecules and the deposition of glycogen were enhanced by acetylcholine when added along with insulin. An increase in the acetylcholinesterase activity has been observed in the liver of rat following glucose administration (Chapter 8b) and from that it was suggested that an active release of ACh from the cholinergic fibres present at the sinusoidal linings of the hepatic cords, ensued following glucose loading. Similar observation was also made in the liver following glucose challenge in pigeon (Chapter 8a). In fact, the liver of birds consuming mainly carbohydrate rich food showed more AChE activity in the sinusoidal linings (Shah et al., 1972b). In the liver of growing pigeons, the AChE activity was maximum when the parents fed them with carbohydrate rich food (Chapter 2). It is conceivable that the ACh through its action on the membrane, facilitates the movement of glucose into the cells. The acetylcholine alters the membrane permeability, releases the membrane bound Ca^{++} and raises the concentration of c-AMP by inhibiting phosphodiesterase (Rasmussen, 1975). The movement of ions across the cell membrane increases when membrane permeability is altered by acetylcholine (Augustinsson, 1950). Since sugar transport

can be mediated through flow coupled transport (Wilbrandt, 1975), it could be reasoned that ACh can induce sugar transport across hepatic membrane which utilizes the same energy that is expended during ionic movements. Na^+/K^+ dependent sugar transport is also reported in the intestinal mucosa (Riklis and Quastel, 1958; Crane, 1962 and Schultz and Curran, 1970).

Although, acetylcholine has an obvious influence over the sugar uptake by hepatic cells in pigeon, such effect is not seen in the case of rat liver cells. However, it is the insulin that has a tremendous influence over the sugar transport in rat liver cells. The reason for this paradoxical trend in the influence of ACh and insulin in rat and pigeon liver cells could be that (1) the pigeon hepatocytes have less insulin receptors on the plasma membrane whereas rat liver have a predominant insulin receptors, or (2) the glucokinase in the pigeon liver is not sensitive to insulin as in the case of rat liver.

In this connection it is pertinent to mention that the rat tissues contain three isoenzymes of hexokinase, the distribution of which are different in different tissues (McGilvery, 1972). Liver contains all the three types, the type III phosphorylating more glucose when ATP concentration is especially high, while the other two types function in conditions when glucose-6-phosphate is depleted compared to

the glucose available. Type II is sensitive to insulin (skeletal muscles and adipose tissue have more of this type) while type I is insensitive to insulin. Brain and kidney have type I and heart contains both type I and II. Perhaps the liver of pigeon contains more type I and III hexokinases compared to rat liver which may contain more type II hexokinase than the other two. If this contention rings true, then in the pigeon liver transport of glucose could be partly linked to the active transport of ions expending energy by spliting ATP in which case the phosphorylation of glucose by hexokinase (type I and III) would be taking place only after the glucose enters the cells. A small part of the glucose that enters the liver of birds could also be through diffusion especially during post absorptive hyperglycaemia. The dependency of extracellular (blood) sugar concentration in maintaining sugar movement into the hepatic cells is envisaged from the observation of seepage of glucose out of the cells (211.30 μ mole glucose/gm liver) when the slices were incubated in a medium without glucose, as compared to a negligible 16.52 μ mole glucose/gm liver that leaked out in the case of rat liver slices.

The general lack of response of avian adipose tissue (Langslow and Hales, 1969) and liver cells (Goodridge and Ball, 1966 and 1967) to insulin, perhaps is indicative of

the absence of type II hexokinase in these avian tissues.

On the other hand insulin in rat liver, by activating membrane bound glucokinase (Salas et al., 1963) and glycogen synthetase activity in the cytosol (Gold, 1970) favours the glucose uptake, as well as its utilization in glycogen synthesis. Perhaps the action of insulin in rat liver is more concerned with the activation of glucokinase since together with acetylcholine (which not only influences the uptake but also activates glycogen synthetase), insulin enhances glycogen deposition in liver slices (twice as much as insulin alone). The quantum of glycogen deposited in the rat liver slices is found to be very much higher than in the pigeon liver slices. In the pigeon liver, the glucose that enters the cell might be getting converted into lipids rather than glycogen. An increase in the activity of important lipogenic enzymes such as 'malic enzyme' and ATP-citrate lyase in the liver of chick after hatching was also found to take place under the influence of heavy carbohydrate rich diet (Pearce and Brown, 1971). When challenged with glucose load, pigeon liver also showed ~~an~~ increased G-6-PDH and 'malic' enzyme activities (Chapter 10). From this fact, it could be deduced that the glucose taken up by the avian liver is more rapidly converted into lipids than into glycogen. The rapid conversion of carbohydrate to lipid in the liver

is also evident in the young ones at a time when large quantity of grains are fed to them by parents (Chapter 1). During the time of plentiful supply, the glucose taken up by the liver cells is stored as triglycerides or glycogen (McGilvery, 1972). Perhaps, in the pigeon liver more of glucose is converted into triglycerides, while in the rat liver more of it is diverted to glycogenesis.