CHAPTER 3

IN VITRO EFFECT OF IONS IN THE PRESENCE OF INSULIN AND ACETYL CHOLINE ON THE GLUCOSE UPTAKE OR RELEASE BY LIVER OF DOMESTIC PIGEONS

Importance of Na^+ and K^+ in the metabolic regulation of glycogen synthesis and lysis was recognised in the fourties (Buchanan and Hastings, 1949). Incubation of isolated liver preparations with supraphysiological concentrations of K^+ resulted in an inhibition of glycogenolysis followed by a stimulation of glycogen synthetase (Cahill et al., 1957; Hue et al., 1975). When isolated hepatocytes were incubated in K^+ predominant medium, an increase in intracellular K^+ and a decrease in intracellular Na^+ have been noted (Van de Werve, 1981). However, it is the presence of more intracellular K^+ or the decrease of Na^+ inside the cell, that brings about inactivation of phosphorylase and activation of glycogen synthetase. The changes in intracellular Na^+/K^+ ratio in turn alter the concentration of Ca^{++} which is the basis of such metabolic control (Nedergaard and Cannon, 1980; Hughes et al., 1980). In hepatocytes incubated with Ca^{++} in the presence of Na^+, phosphorylase enzyme became highly active, while in hepatocytes incubated with Ca^{++} in the presence of K^+ the enzyme did not show any activation (Assimacopoulos-Jeannet et al., 1977). An interplay of Ca^{++}, Na^+ and K^+ ions in the cell could, thus, regulate glycogenolysis or glycogen synthesis by activating or inactivating the key enzymes. The importance of interactions of K^+, Na^+ and Ca^{++} in the uptake or release of glucose from the pigeon liver

slices was clearly established in the experiments previously described (Chapter 2). In the medium containing hypertonic Na^+, liver slices showed an uptake of glucose. Hypertonic K^+ or Ca^{++} did not elicit this response (on the contrary a release was observed). Ca^{++} with Na^+ or K^+ stimulated glucose uptake. Even when the medium contained both Na^+ and K^+ glucose uptake by liver slices was not observed. The effect of these ions on the isolated hepatocytes was reported to be dependent on the metabolic state of the liver. Isolated liver cells from fed or starved animals responded differently (Van de Werve, 1981). The post-prandial and pre-prandial conditions are characterised by the plasma concentrations of hormones such as insulin, glucagon, catecholamines and glucocorticoids. Recently, the autonomic nerve activity was also reported to be different in fed or starved state (Shimazu, 1983). In other words, the effect of ions could be modified or modulated by hormonal or humoral factors. In this context, the present experiment was planned to obtain information regarding the effect of ions in the presence of insulin or ACh on the glucose uptake or release by the pigeon liver slices.

MATERIALS AND METHODS

Adult pigeons (Columba livia) weighing 180-250 grams maintained in laboratory conditions on balanced diet were used for the present experiment. The birds were sacrificed after an overnight starvation period. The liver was perfused with cold Krebs Ringer Bicarbonate medium and then was quickly excised.

The liver was placed on ice and cut into slices, weighing 40-50 mg and the slices were incubated in 10 ml flask with 5 ml of KRB Buffer containing glucose and albumin. The liver slices were incubated for 90 minutes at 37°C in a water bath shaker with 120 oscillations/min. The slices were incubated in media of following categories.

(1) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + Insulin (1 unit/ml). The Boots Co.,(India) Ltd.

(2) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + ACh (15 mg/ml)

(3) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + Insulin (1 unit/ml) + NaCl (5 mg/ml)

(4) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + Insulin (1 unit/ml) + KCl (5 mg/ml)

(5) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + Insulin (1 unit/ml) + $CaCl_2$ (5 mg/ml)

(6) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) +ACh (15 mg/ml) + NaCl (5 mg/ml)

(7) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + ACh (15 mg/ml) + KCl (5 mg/ml)

(8) 5 ml KRB Medium + D-Glucose (3 mg/ml) + Albumin (2 mg/ml) + ACh (15 mg/ml) + $CaCl_2$ (5 mg/ml).

After the incubation, the slices were quickly washed with chilled KRB Medium and were digested in KOH for glycogen estimation. The glycogen was estimated in slices immediately after killing as well as in the slices that were subjected to incubation. Glycogen was estimated as per the method described in Chapter 1. The glucose concentration was determined (Chapter 1) in the medium before and after incubation and the differences were calculated.

RESULTS

The data obtained are presented in Table 3-1 as well as in Figs. 3-1 and 3-2.

It is clear from the table that the presence of insulin stimulated glucose uptake (3.144 mg/100 mg tissue) by liver slices. When ACh was an additive in the incubation medium, 0.5772 mg glucose per 100 mg tissue was taken up by the liver slices. When insulin was present in combination with NaCl or KCl in the incubation medium, glucose uptake was same as when insulin was alone. Insulin in combination with $CaCl_2$ stimulated a glucose uptake of 0.9728/100 mg tissue.

ACh also stimulated glucose uptake in combination with NaCl, KCl and $CaCl_2$. ACh in combination with NaCl stimulated 2.4480 mg/100 mg tissue glucose uptake. When ACh was present with KCl, glucose uptake (2.1866 mg/100 mg tissue) took place.

Fig. 3-1. Effect of cations in combination with acetylcholine (ACh), choline chloride or insulin on glucose uptake by pigeon liver slices under *in vitro* conditions.

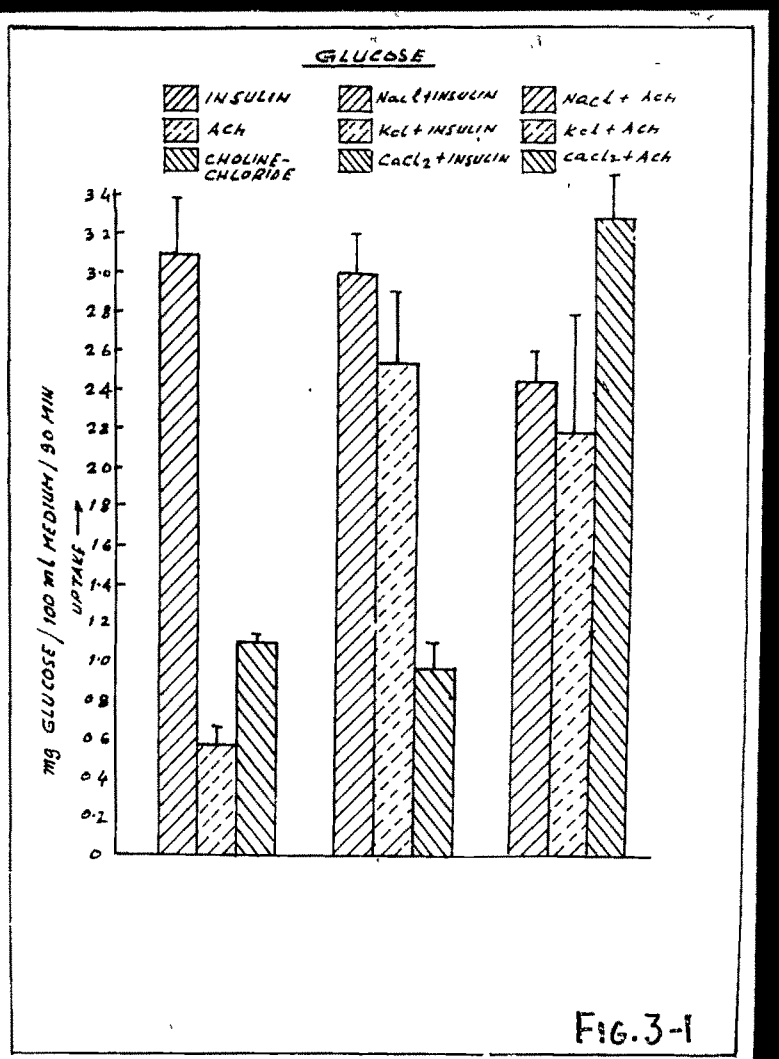
GLUCOSE
INSULIN
ACH
CHOLINE-CHLORIDE
NaCl+INSULIN
KCl+INSULIN
CaCl2+INSULIN
NaCl+ACH
KCl+ACH
CaCl2+ACH
mg GLUCOSE / 100 ml MEDIUM / 90 MIN
UPTAKE
34
32
30
28
26
24
22
20
18
16
14
12
10
08
06
04
02
0

FIG. 3-1

Fig. 3-2. Effect of acetylcholine (ACh), choline chloride and insulin on glycogen content in pigeon liver slices under *in vitro* conditions.

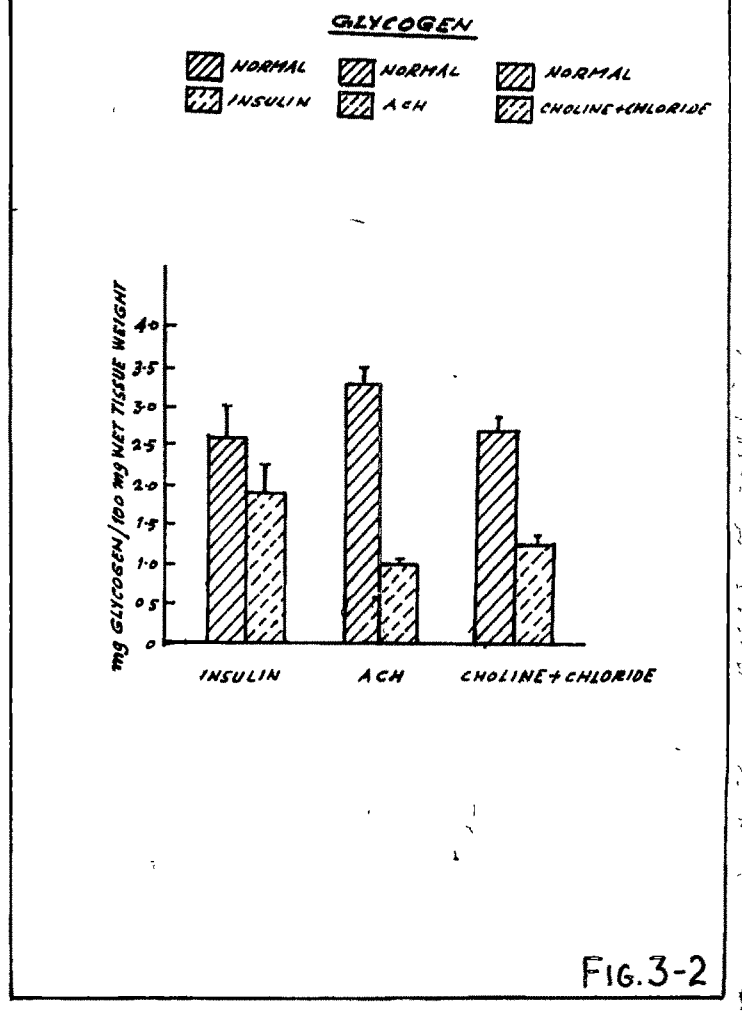
GLYCOGEN
NORMAL
INSULIN
NORMAL
ACH
NORMAL
CHOLINE+CHLORIDE
mg GLYCOGEN/100 mg WET TISSUE WEIGHT
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
INSULIN
ACH
CHOLINE+CHLORIDE

FIG. 3-2

Fig. 3-3. Effect of cations in combination with acetylcholine (ACh), choline chloride or insulin on glycogen content in pigeon liver slices under *in vitro* conditions.

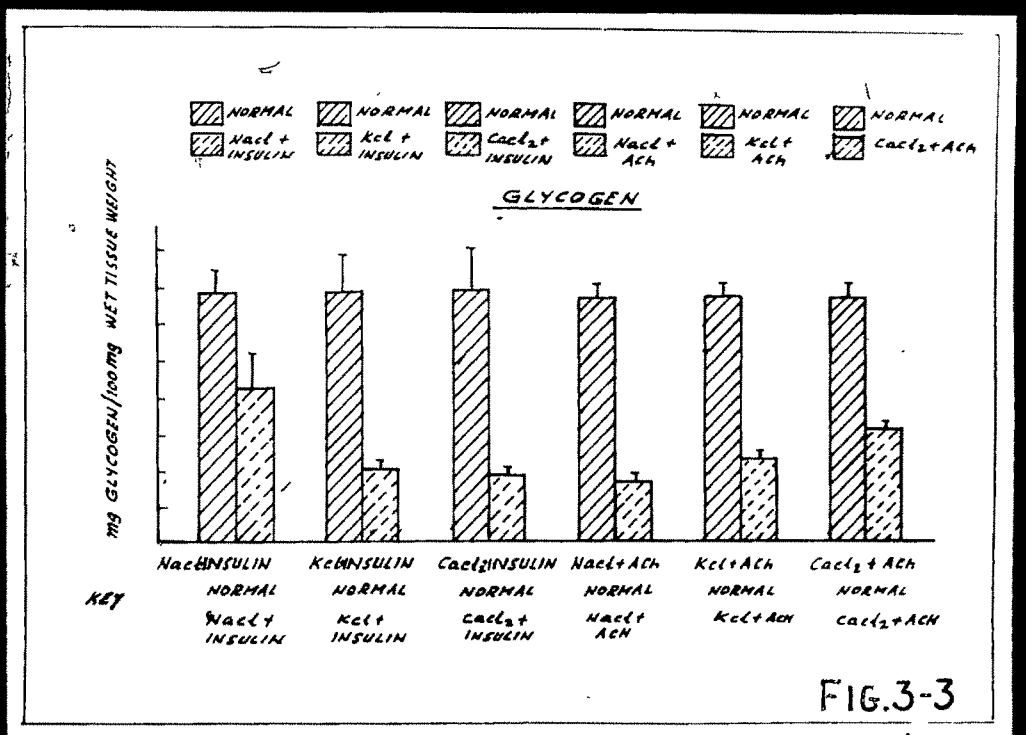
NORMAL
NaCl + INSULIN
NORMAL
KCl + INSULIN
NORMAL
CaCl2 + INSULIN
NORMAL
NaCl + ACh
NORMAL
KCl + ACh
NORMAL
CaCl2 + ACh
GLYCOGEN
mg GLYCOGEN/100mg WET TISSUE WEIGHT
NaCl+INSULIN
KCl+INSULIN
CaCl2+INSULIN
NaCl+ACh
KCl+ACh
CaCl2 + ACh
KEY
NORMAL
NaCl + INSULIN
NORMAL
KCl + INSULIN
NORMAL
CaCl2 + INSULIN
NORMAL
NaCl + ACh
NORMAL
KCl + ACh
NORMAL
CaCl2 + ACh

FIG.3-3

Table 3-1

In vitro effect of ions together with insulin or acetylcholine on the uptake or release of glucose by pigeon liver slices (Mean ± SEM)

Additives	Glucose		Glycogen Depletion (2)
	Uptake (1)	Release(1)	
Insulin	3.1446 ±0.3189	-	0.7390 ±0.0364
Acetylcholine	0.5772 ±0.1025	-	1.2789 ±0.1274
NaCl + Insulin	3.0116 ±0.2542	-	1.2695 ±0.1987
KCl + Insulin	2.5488 ±0.5103	-	2.2873 ±0.4339
$CaCl_2$ + Insulin	0.9728 ±0.1355	-	2.5450 ±0.4765
NaCl + ACh	2.4480 ±0.2178	-	2.4894 ±0.1110
KCl + ACh	2.1866 ±0.2472	-	2.2118 ±0.0605
$CaCl_2$ + ACh	3.2742 ±0.3439	-	1.8494 ±0.0180

(1) Difference between the glucose concentration in the medium before and after the incubation. Mg glucose/100 mg liver.

(2) Difference between glycogen values of fresh liver slices and liver slices after incubation. Mg glycogen/100 mg liver.

DISCUSSION

The most interesting observation was that ACh in the presence of Na^+, K^+ or Ca^{++} induced glucose uptake by the liver slices much more than what was observed when ACh was alone in the medium. On the other hand, Na^+ or K^+ with insulin did not enhance the glucose uptake any further than what was obtained with insulin alone. Insulin, however in the presence of Ca^{++} showed a reduced glucose uptake. The results clearly indicate that Na^+, K^+ or Ca^{++} could act synergestically with ACh but not with insulin. Of the three cations, only Na^+ in hypertonic concentration could induce glucose uptake (Chapter 2). Hence the synergism of Na^+ and ACh is understandable. However, that of K^+ or Ca^{++} with ACh is puzzling. There are so many variable physiological conditions, such as metabolic state of the hepatocytes, presence or absence of right amount of hormones or a specific ratio of hormones in the blood, level of metabolites, neural activation or inactivation of membrane bound systems, receptor activation or down regulation and multitudes of other parameters that could effect the response of liver to a given stimulus. Permutation and combination of all these factors are difficult to experimentally reproduce in in vitro conditions. Isolated hepatocytes from fed or starved animals respond differently to glucose or hormones. Even some sort of 'priming' of the cells by low concentration of hormones, such as insulin was observed, although at that concentration insulin does not produce any detectable physiological or biochemical changes within the liver cells (Felig and Wahren, 1971;

Brown et al., 1978; Best et al., 1981; Proietto et al., 1983). The rate of glucose uptake is reported to be a function of both glucose and insulin concentrations. Best et al. (1981) studied the effect of variable glycaemia on glucose uptake at different predetermined insulin concentrations. Their data show that the low concentration of insulin have an essential role in normal glucose disposal (after a carbohydrate meal) by priming the tissues so as to allow the glucose to enter along its concentration gradient. The mechanism of this priming action of insulin at its low concentration is not clear, but it was suggested to be related to the acute lowering of FFA levels. Alternatively, the priming effect may reflect the well known in vitro action of insulin in recruiting glucose carriers (Ludvingsen and Jarrett, 1979; Czech, 1980). At higher glucose concentration, glucose transport or metabolism is saturated at low glucose concentration, therefore there is little increase in the peak rate of uptake when plasma glucose rises. At higher insulin level, glucose transport or metabolism begins to saturate only at higher glucose concentration. When insulin level is high, rate of glucose uptake is eventually linearly related to the plasma glucose concentration. In other words, more insulin is effective only when more glucose is available and when both are high, more glucose transporters are recruited (activated) so that the rate of glucose uptake increases further.

If such differential responses by liver cells to low or high concentrations of insulin in relation with glycaemic level

are observed, similar responses could also occur with respect to acetylcholine (vagal activity) and ions. The glucose uptake response of liver slices to high K^+ medium (Chapter 2) was an inhibitory one; that of ACh was only 0.5772 mg/100 mg tissue/90 min.; but both together could induce an uptake of 2.1866 mg glucose/100 mg tissue/90 min. Since similar glucose uptake response was obtained with Na^+ and ACh or Ca^{++} and ACh, it could be reasoned that ACh in the presence of a hypertonic condition (irrespective of which cation caused the hypertonicity) could induce greater glucose uptake. This fact some what points to the mechanism of ACh action. Ach could induce permeability changes in the plasma membrane and in the presence of hypertonic conditions, the ionic efflux might be more. More the efflux, more the pump activity. The glucose enters the cells through the flow coupled mechanism.

In conclusion it could be stated that the action of insulin and ACh on glucose uptake by liver cells could be very much influence by the ionic concentration in the ambient environment.