EFFECT OF ACTH ON GLUCOSE UPTAKE BY HEPATIC CELLS AND ITS EFFECT ON HEPATIC ENZYMES IN PRESENCE OF INSULIN AND ACETYLCHOLINE

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(Received on September 9, 1985)

Summary : An intrinsic hypoglycaemic activity has been attributed to ACTH. This action of ACTH is essentially mediated by increased insulin secretion. In the present study, the di ect action of ACTH on glucose uptake by pigeon hepatocytes has been studied by *in vitro* technique. The results have shown that ACTH has a direct influence on glucose uptake and this action is not additive in presence of insulin. Glucose uptake in presence ACTH with Acetylcholine was not any more than what was obtained with ACTH alone. These observations have been taken to indicate a non-existence of synergistic action of ACTH with insulin or acetylcholine in promoting glucose uptake by avian liver cells.

Key words : ACTH, acetylcholine, glucose, insulin, liver, pigeon

INTODUCTION

Hypoglycaemic activity of pituitary extracts has been noted for over 30 years. The independent demonstrations by Westermeyer and Raben (16) and Engel and Engel (1) that Oxy-cell ACTH could lower the blood sugar in intact and adrenalectomized mice and rats suggested that corticotrophin itself had intrinsic hypoglycaemic activity; and that the hormone is hypoglycaemic in action was strengthened further by the observation that when ACTH was taken alongwith insulin glucose uptake was greately enhanced. Certain characteristics of the ACTH induced hypoglycaemic suggest that it is mediated through an increase in insulin secretion. Engel *et al.* (2) in a series of studies with adrenalectomized, intact and eviscerated rats were able to show that corticotrophin caused an increase in brown adipose tissue glycogen content of glucose treated rats and opined that this glycogen deposition was probably attributable to insulin action and that corticotrophin did not

potentiate the glycogen depositing effect of a small dose of insulin. Miller and Krake (10) have reported that oxy-cell ACTH causes no hypoglycaemia in alloxan diabetic mice. Oxy-cell ACTH has been shown to protect adrenalectomized mice from insulin convulsions and a hyperglycaemic diabetogenic effect of corticotrophin has been observed in adrenalectomized rats in which insulin secretion has been maximally stressed by force feeding a high carbohydrate diet and administering a sub-diabetogenic dose of cortisone. The hypoglycaemic action of corticotrophin under *in vivo* conditions is the result of an increase in insulin secretion which in turn results from a direct effect of corticotrophin on the endocrine pancreas. Apart from the influence of ACTH on insulin secretion this hormone is also known to cause rapid increase in phosphorylase activity in adrenals (6) and this effect is mediated through 3′—5′ cAMP as in the stimulation of hepatic phosphorylase by glucagon.

The effects of ACTH on glycaemic have been investigated mainly in mammals. In mammals, insulin which is secreted in greater quantity than glucagon, is involved in regulating blood sugar level to a high degree of precision. In birds, however, insulin has a lesser role to play in glucose homoeostasis and as such it is glucagon that is secreted more than insulin (7). Mammals are highly sensitive to hypeglycaemia while birds are highly sensitive to hypoglycaemia. Birds for that matter tolerate a very high blood sugar level and pancreatectomy causes hypoglycaemia rather than hyperglycaemia (14). Pilo and Patel (11) suggested that the prime hepatic glucose uptake inducer in birds is acetylcholine (vagal mediation). Although insulin is effective in this respect in birds, due to the fact that the rate of insulin release is poor in birds and the insulin release response is very sluggish as well as it takes place in response to a very high concentration of glucose in blood (7), the vagal cholinergic mediated uptake of glucose by liver assumes greater importance. It is not clear why insulin mediated control of glucose uptake is not preferred or not efficient in birds. The absence of insulin sensitive glucokinase in liver may be one reason. Other reasons may be absence of synergistic actions or presence of inhibitory actions by other hormones. To understand whether ACTH has any direct or synergistic influence on glucose uptake by hepatic cells of birds in *in vitro* conditions, the present study was undertaken.

MATERIAL AND METHODS

Ten adult pigeons (*Columba livia*) weighing 180-250 grams maintained in laboratory conditions on balanced diet were used for the present experiments. Pigeons were sacrificed by decapitation after 24 hours of starvation. The liver from each pigeon was quickly excised and washed with cold Krebs-Ringer-bicarbonate (KRB) medium. The liver

was placed on ice and cut into slices of 100-200 *mg* weight and were placed in 10 *ml* flasks containing 5 *ml* of KRB medium aerated before use. The liver slices were incubated for 30 min at 37°C in a water bath shaker adjusted for 120 ascillations/min. The slices were incubated in media containing 3 *mg/ml* D-glucose, 2 *mg/ml* albumin either with or without various additives. Six sets of media were prepared accordingly. They were :

(1) With no additives

(2) With Insulin (1 *Unit/ml*)

(3) With Acetylcholine (15 *mg/ml*)

(4) With ACTH (0.1 *I.U./ml*)

(5) With ACTH (0.1 *I.U./ml*) + Insulin (1 *Unit/ml*)

(6) With ACTH (0.1 *I.U./ml*) + Acetylcholine (15 *mg/ml*)

Each experiment was repeated twice and data were pooled for liver slices from the same pigeon. Fresh liver slices and incubated ones were washed with chilled KRB medium and homogenized in redistilled water. The homogenates were used for assaying enzymes and protein. Glycogen was estimated in the slices by the method of Seifter *et al.* (12). In order to assess glucose uptake by liver slices, glucose content of the medium prior to and after incubation was estimated by the micro method of Folin and Malmros (3). Acetylcholinesterase (AChE) activity was measured colorimetrically by the method of Guenther and Klaus (5). Na^+ -K^+ -ATPase activity was assayed according to the method described by Stastny (15), using ouabain as inhibitor and substracting the value obtained for Mg^{++}ATPase from total ATPase activity. Acid and alkaline phosphatase activities were measured using Sigma Phosphatase Kit No. 104 with p-nitrophenyl phosphate as substrate (14). Succinate dehydrogenase activity was quantitatively estimated according to the method of Kun and Abood (7), using TTC as the electron acceptor and the formazan formed was measured at 420 nm. The lactate dehydrogenase (E.C. 1.1.1.27) was estimated employing the colorimetric method of Kings as described by Varley (16). The protein content of the homogenate was estimated by the method of Lowry *et al.* (9).

RESULTS

The data on the effect of ACTH on glucose uptake, glycogen content in the liver and enzymes are presented in the Tables I and II.

When insulin and acetylcholine (ACh) were alone as additives in the medium, glucose uptake took place. Corticotrophin (ACTH), when present alone in the incubation medium, also induced a moderate uptake of glucose. In the presence of insulin, ACTH did not induce a glucose uptake by the liver cells more than what was observed when insulin was present alone in the medium. In combination with ACh, ACTH did not induce any further increase in the uptake of glucose than that was effected by ACTH or Ach alone in the medium.

TABLE I : Effect of ACTH on glucose uptake by pigeon liver slices in presence of insulin and acetylcholine (ACh). Mean ± S.E.

Additives	*Glucose uptake (a)*	*Glucose release (a)*	*Glycogen prior to incubation*	*Glycogen after incubation*
No additives	—	0.805±0.28	3.925±0.99	3.529±0.55
Insulin	1.145±0.22	—	3.563±0.53	3.434±0.42
Acetylcholine	0.577±0.09	—	3.943±0.33	3.533±0.03
ACTH	0.542±0.06	—	3.609±0.02	3.040±0.12
ACTH + Insulin	1.100±0.04	—	3.859±0.09	3.030±0.13
ACTH + Acetylcholine	0.600±0.08	—	3.278±0.08	2.829±0.08

(a) *mg* glucose taken up or released by 100 *mg* liver/30 min

ATPase activity showed a significant reduction in the presence of ATCH, which was so even when the media contained insulin or ACh also. Of the two dehydrogenases studied, succinate dehydrogenase showed a significant decrease whether ACTH was present in the medium alone or in combination with insulin or ACh. Lactate dehydrogenase on the other hand showed a slight increase in all the three combinations of additives (ACTH, ACTH+ insulin, ACTH + ACh). The activities of AChE, acid phosphatase and alkaline phosphatase showed no variations from what was observed in the liver slices prior to incubation. Glycogen content of liver slices showed a decrease in all experimental set ups.

TABLE II : Effect of ACTH, alone or in combination with insulin or acetylcholine on the enzyme activities in pigeon liver slices under *in vitro* conditions.

Enzymes	*Fresh liver*	*ACTH*	*ACTH+Insulin*	*ACTH+Acetylcholine*
Na^+-K^+-ATPase (a)	26.264±0.57	11.185±0.53****	18.138±0.23***	17.686±0.16***
Acetylcholinesterase (AChE) (b)	0.703±0.06	0.158±0.01**	0.572±0.11^{NS}	0.589±0.07^{NS}
Acid Phosphatase (c)	176.29±12.57	133.36±5.02^{NS}	163.65±14.79^{NS}	144.46±14.38^{NS}
Alkaline phosphatase (d)	8.919±0.26	6.438±0.45^{NS}	6.082±0.30^{NS}	6.722±0.14^{NS}
Lactate dehydrogenase (LDH) (e)	47.874±3.79	71.054±6.76^{NS}	116.261±5.41*	72.465±2.94^{NS}
Succinate dehydrogenase (SDH) (f)	9.685±0.17	6.605±0.44**	5.412±0.19***	4.202±0.32***

(a) μg phosphorus released/*mg* protein/10 min.

(b) μM ACh hydrolysed/*mg* protein/10 min.

(c) μM p-nitrophenol released/100 *mg* protein/30 min.

(d) μM p-nitrophenol released/100 *mg* protein/30 min.

(e) μM lactate oxidized/*mg* protein/15 min.

(f) μg formazan formed/*mg* protein/10 min.

*P<0.05; **P<0.02; ***P<0.01 ****P<0.001; NS - Not significant.

DISCUSSION

All trophic hormones exert their effect on basic cellular processes which in turn subserve the specific function of the target cells on which they act. Adrenals are the natural target organ of ACTH. But non-target organ like liver can be expected to show some response when sufficient concentration of the trophic hormone is present in the circulating fluid. Many reports have indicated that ACTH has direct action on several tissues independent of its actions via adrenocortical hormones. Hypoglycaemic action is one of them. Corticotrophin administration under *in vivo* conditions increased the plasma level of insulin (4) and it could be this reason that was mainly responsible for the hypoglycaemic action of ACTH in *in vivo* experiments, However, in the present study, ACTH also increased the uptake of glucose by pigeon liver slices in *in vitro* conditions. Hence, it is reasonable to surmise that ACTH has some direct action on the liver slices. No synergistic action was shown by ACTH in the uptake of glucose by liver slices in the presence of insulin or acetylcholine. The fact that neither acid phosphatase nor Na^+-K^+-ATPase showed any increase in the level of activities in the liver slices when ACTH

was present with insulin or ACh in the medium, indicates that enzyme dependent or permeability dependent glucose movement across the plasma membrane was not activated by ACTH. The increased LDH activity in the liver slices incubated in medium containing both ACTH and insulin indicates that enhanced lactic acid production which leads to a reduction in glucose-6-phosphate concentration could facilitate a chemiosmotic pull of glucose into the hepatocytes. Predominancy of glucagon in birds might have also affected the synergism to be expressed fully. All these facts, finally, explain the obvious difference between mammalian and avian mechanisms of blood sugar regulation.

REFERENCES

1. Engel, F.L. and M.G. Engel. 1945. Cited from Genuth, S. and Lebovitz, H.E. (1965).
2. Engel, F.L., J.F. Lopez and T. Albertson. 1958. Cited from Genuth, S. and Lebovitz, H.E. (1965).
3. Folin. O and H. Malmros. Micromethod for glucose estimation in blood. *J. Biol. Chem.*, **83 : 115**, 1929.
4. Genuth, S. and H.E. Lebovitz. Stimulation of insulin release by corticotrophin. *Endocrinology*, **76** : 1093, 1965.
5. Guenther, V. and S. Klaus. Red cell and plasma cholinesterase activities in microsamples of human and animal blood determined simultaneously by a modified acetylcholine/DTNB procedure. *Toxicology Appl. Pharmacol.*, **16** : 764-772, 1970.
6. Haynes, R. C. Jr. The activation of adrenal phosphorylase by the adrenocorticotropic hormones. *J. Biol. Chem.*, **233** :
7. Hazelwood, R.L. The avian endocrine pancreas. *Am. Zool.*, **13** : 699-706, 1973.
8. Kun, E. and L.G. Abood. Colorimetric estimation of succinic dehydrogenase by triphenyl-tetrozolic chloride. *Science*, **109** : 144-146, 1949.
9. Lowry, D.H., N.J. Rosenbrough. A. Farr and J. Randall. Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193** : 265-275, 1951.
10. Miller, W.L, and J.J, Krake. 1963. Cited from Genuth, S. and Lebovitz, H.E. (1965).
11. Pilo, B. and P.V. Patel. Influence of insulin and acetylcholine on transport of glucose and glycogen deposition in liver slices of pigeon and rat. *Indian J. Exptl, Biol.*, **16** : 929-932, 1978.
12. Seifter, S., S. Dalton, S. Novic and B. Muntwyler. The estimation of glycogen with anthrone reagent. *Arch. Biochem.*, **25** : 191-200, 1950.
13. Sigma Technical Bulletin, No. 104, Sigma Chem. Co., 3500-Dekalb St., St. Louis 18, Mo., U.S.A.
14. Sitbon, G. and P. Mialhe. Le pancreas endocrine des oiseaux. *J. Physiol. Paris*, **76** : 5-24, 1980.
15. Stastny, F. Hydrocortisone as a possible inducer of Na^+ -K^+ -ATPase in the chick embryo cerebral hemisphere. *Brain. Res.*, **25** : 397-410, 1971.
16. Varley, H. Colorimetric Method: In: "Practical Clinical Biochemistry", Arnold Heinemann Publishers, *Ind. Edn.*, 1975.
17. Westermeyer, V.W. and M.S. Raben, 1954. Cited from Genuth, S. and Lebovitz, H.E. (1965).