AN IN VITRO EFFECT OF CORTISOL ON PYRUVATE CARBOXYLASE AND GLUCONEOGENESIS

H.V. Henning*, W. Huth and W. Seubert
Institut für vegetative Physiologie der Universität Frankfurt/Main, Germany

Received October 26, 1964

The simultaneous rise of glycogen and pyruvate carboxylase activities within 6 hours in livers of cortisol treated rats (HENNING et al., 1963) suggested a hormone dependent activation or induction of pyruvate carboxylase as one of the primary actions of cortisol in regulating gluconeogenesis from pyruvate. To support this assumption a possible effect of the hormone on pyruvate carboxylase in tissue slices was investigated. Indirect evidence for an in vitro effect of glucocorticoids on this enzyme came from studies of OKUNO (1960), HAYNES (1962), UETE and ASHMORE (1963) and EISENSTEIN et al., (1964). These authors observed increased incorporation of pyruvate, alanine and CO_2 into glucose by liver slices in the presence of triamcinolone.

According to KREBS et al.,(1963), "the kidney offers major advantages over the liver as an experimental material for the study of gluconeogenesis." Since pyruvate carboxylase is also present in kidney, this organ was chosen to study an in vitro effect of cortisol on the enzyme. The results reported in this paper confirm the statement of KREBS. As will be shown, gluconeogenesis from pyruvate and succinate together with pyruvate carboxylase activities are

* Post doctoral fellow, Deutsche Forschungsgemeinschaft

significantly increased after a 1 hour incubation of rat kidney cortex slices with cortisol.

MATERIALS AND METHODS

Male albino rats of the Wistar strain, fed on rat pellets (Altromin GmbH, Lage/Lippe), were starved for 12 hours. The animals were killed under light ether anesthesia and kidneys immediately removed. Kidney cortex slices of an average thickness of 0.3-0.4 mm were cut with a STADIE-RIGGS microtome. Incubations were carried out at 38°C in the saline medium of KREBS and HENSELEIT (1932), equilibrated with 95% O_2 and 5% CO_2. 0.032 ml of 5 x 10^{-3}M cortisol in waterethanol (1:1) were added to 8 ml of incubation medium (end concentration: 2 x 10^{-5}M). Controls contained an equivalent amount of ethanol (0.016 ml).

The effect of the hormone on the rate of gluconeogenesis was determined in the following way: 100 mg slices (wet wt) in 8 ml medium were preincubated for 1 hour with and without hormone. 1.6 ml of 0.1 M substrate were added and incubation continued for another hour. After cooling to 0°C the slices were removed by centrifugation. Glucose in the medium was determined by the glucose oxidase method.

To study the effect of cortisol on pyruvate carboxylase 400 mg kidney cortex slices were incubated for 1 hour with and without hormone in 8 ml medium equilibrated with 95% O_2 and 5% CO_2. Hormone was added to an end concentration of 2 x 10^{-5}M. After incubation the slices were removed by centrifugation and homogenized in 8 ml 0.1 M Tris buffer pH 7.2 for 3 minutes. Subsequent treatment of the homogenates and assays of the enzyme were carried out as described previously by HENNING and SEUBERT (1964).

Table 1: Effect of cortisol on pyruvate carboxylase activities and glucose synthesis from pyruvate and succinate in rat kidney cortex slices

controls			2×10^{-5}M cortisol		
pyruvate-carboxylase units/g*	glucose formed from pyruvate µM/hxg	glucose formed from succinate µM/hxg	pyruvate-carboxylase units/g*	glucose formed from pyruvate µM/hxg	glucose formed from succinate µM/hxg
1,61	16.8	22.1	1.91	32.2	26.2
1.52	16.5	24.8	2.00	28.1	24.5
1.47	17.3	23.0	1.91	27.6	28.2
1.56	13.0	27.0	2.15	23.0	34.6
1.94	14.4	22.4	1.86	26.6	32.1
1.63	16.2	24.3	2.78	20.7	28.8
1.65	13.9	22.0	2.63	26.4	29.3
	16.4	21.6	2.14	26.6	21.5
	16.8	27.2	1.85	28.4	29.3
	14.7	26.9	2.0	19.1	31.7
	14.8			21.5	
$\bar{x}\pm s$ 1.63±0.15	15.5±1.4	24.1±2.2	2.12±0.32	25.4±3.9	28.4±3.8

* One enzyme unit catalyzes the carboxylation of 1 µM pyruvate to oxaloacetate per minute under the conditions described by HENNING and SEUBERT (1964)

RESULTS AND DISCUSSION

In table 1 the levels of pyruvate carboxylase and the rates of gluconeogenesis from pyruvate and succinate in kidney cortex slices after 1 hour of incubation with and without hormone are summarized. The activities of the enzyme in hormone treated slices are in all cases elevated when compared with controls ($p < 0.01$). Glucose synthesis from pyruvate and succinate is stimulated after prein - cubation of the slices with cortisol ($p < 0.001$ and $p < 0.01$, respectively).

Elevated activities of enzymes involved in glucose formation from pyruvate have been reported for glucose-6-phosphatase (ASHMORE et al.,1954; LANGDON et al.,1955; KVAM et al.,1960; WEBER et al.,1961), fructose-1,6-di - phosphatase (MOKRASCH et al.,1956; KVAM et al.,1960; WEBER et al.,1961), phosphoenolpyruvate carboxykinase (SHRAGO et al.,1963) and glycerophosphate dehydrogenase (MATZELT et al.,1963). For all these enzymes, however, the increase in activity is discernible only long after the early effect of adrenal corticoids on the rate of glucose synthesis (KVAM et al.,1960) and cannot be regarded as a primary effect of the hormone. The re - sponse of pyruvate carboxylase and of gluconeogenesis from pyruvate to cortisol within 1 hour in kidney supports again the carboxylation of pyruvate to oxaloacetate as a rate limiting step in gluconeogenesis which is regulated by glucocorticoids (HENNING et al.,1963; WAGLE,1964; PRINZ and SEUBERT,1964).

The early response of glucose synthesis from succinate to cortisol suggests pyruvate carboxylase also to be

involved in glucose formation from intermediates of the citric acid cycle. The reaction sequence leading from malate through oxaloacetate to phosphoenolpyruvate may be supplemented by an alternate pathway which involves oxidative decarboxylation of malate to pyruvate and subsequent carboxylation to oxaloacetate. This interpretation is strengthened by experiments of KREBS and de GASQUET (1964), who observed an inhibition of gluconeogenesis from various intermediates of the citric acid cycle by phenylpyruvic acid. The latter substrate is a potent inhibitor of pyruvate carboxylase (HUTH and SEUBERT,1964). A possible role of pyruvate carboxylase in the conversion of citric acid to oxaloacetate is under investigation.

ACKNOWLEDGEMENTS

We wish to thank Dr. Stephen C.Kinsky for stimulating discussions and the Deutsche Forschungsgemeinschaft for financial support.

REFERENCES

Ashmore,J., Hastings,A.B., and Nesbett,F.B., Proc.Nat. Acad.Sci.U.S., 40, 673 (1954)
Ashmore,J., Hastings,A.B., Nesbett,F.B., and Renold,A.E., J. Biol.Chem., 218, 77 (1956)
Eisenstein,A.B., Berg,E., Goldenberg,D., and Jensen,B., Endocrinology, 74, 123 (1964)
Haynes,R.C., Endocrinology, 71, 399 (1962)
Henning,H.V., Seiffert,I., and Seubert,W., Biochim. Biophys.Acta, 77, 345 (1963)
Henning,H.V.and Seubert,W., Biochem. Z., 340,160 (1964)
Huth,W.and Seubert,W., unpublished experiments
Krebs,H.A. and Henseleit,K., Hoppe-Seylers Z.physiol. Chem., 210, 33 (1932)
Krebs,H.A.,Benett,D.A.H., deGasquet,P., Gascoyne,T., and Yoshida,T., Biochem.J., 86, 22 (1963)
Krebs,H.A. and deGasquet,P., Biochem.J., 90,149 (1964)
Kvam,D.C. and Parks,R.E.,jr., Am.J.Physiol.,188, 21,(1960)
Langdon,R.G. and Weakly,D.R., J.Biol.Chem., 214, 167 (1955)
Matzelt,D., Oriol-Bosch,A., and Voigt,K.D., Biochem.Z., 335, 485 (1962)
Mokrasch,L.C., Davidson,W.D., and McGilvery,R.W., J.Biol. Chem., 222, 179 (1956)

Okuno,G., Med.J.Osaka Univ., 10, 483 (1960)
Prinz,W.and Seubert,W., Bioch.Bioph.Res.Com., in press
Shrago,E., Lardy,H.A., Nordlie,R.C., and Foster,D.O., J.Biol.Chem., 238, 3188 (1963)
Uete,T.and Ashmore,J., J.Biol.Chem., 238, 2906 (1963)
Wagle,S.R., Bioch.Bioph.Res.Com., 14, 533 (1964)
Weber,G., Banerjee,G., and Bronstein,S.B., J.Biol. Chem., 236, 3106 (1961).