IN VITRO STIMULATION OF GLUCONEOGENESIS FROM PYRUVATE AND OF $^{14}CO_2$-FIXATION BY DEXAMETHASONE PHOSPHATE AND VASOPRESSIN IN THE KIDNEY CORTEX OF ADRENALECTOMIZED RATS

B. Stumpf, A. Boie and W. Seubert

Physiologisch-Chemisches Institut der Universität,

Göttingen, Germany

Received July 24,1972

SUMMARY: Dexamethasone phosphate and vasopressin stimulate glucose synthesis and $^{14}CO_2$-fixation of adrenalectomized rat kidney cortex explants in vitro. 3',5'-cyclic-AMP demonstrates similar effects as vasopressin. In contrast to dexamethasone phosphate, vasopressin and 3',5'-cyclic-AMP act without any lag phase. Their stimulatory effects are additive to that of the adrenal steroid. All hormonal effects are suppressed by cycloheximide. The implications of these results concerning different mechanisms of action for the adrenal steroid and vasopressin (or 3',5'-cyclic-AMP) will be discussed.

It is proposed that regulation of gluconeogenesis by glucocorticoids involves de novo enzyme synthesis in liver and kidney cortex. This conception is based on experiments with inhibitors of protein synthesis (1,2,3) and on immunological investigations (4). Glucagon and epinephrine accelerate hepatic gluconeogenesis by elevation of intracellular 3',5'-cyclic-AMP-levels (5). However, the mode of action of the cyclic nucleotide is still not completely understood. 3',5'-cyclic-AMP-levels of kidney tubules are increased by parathyroid hormone and vasopressin (6,7). Stimulation of gluconeogenesis by parathyroid hormone and 3',5'-cyclic-AMP in kidney tubule fragments isolated by collagenase treatment has been recently reported (7,8). But effects of glucocorticoids are no longer observed in this system (9). In a search for an in vitro renal system suitable for studying the action of both

hormone groups, the effects of dexamethasone phosphate, vasopressin and 3',5'-cyclic-AMP on gluconeogenesis and $^{14}CO_2$-fixation by adrenalectomized rat kidney cortex explants were investigated.

MATERIAL AND METHODS

Male rats of the Sprague-Dawley strain, weighing 140-160 g, were used as kidney donors, 5 to 6 days after adrenalectomy. In order to lower control rates of glucose synthesis to the values reported in this paper, the animals received a carbohydrate diet almost free of fat and protein (Altrogge, Lage/Lippe) from the day of adrenalectomy and were kept at a constant room temperature of 25°C. Drinking water was supplemented by 0.9 % NaCl. The animals were killed under ether anesthesia. Kidney cortex slices of an average thickness of 1 mm were cut with a Stadie-Riggs microtome. For the purpose of uniformity of tissue samples, slices of all animals were mixed and forced through a nylon sieve with a mesh width of 1 mm. The resulting tissue particles were used for incubation.

Incubations were performed in siliconized 15 ml Warburg vessels at 37°C under oxygen. Tests contained 50 mg of kidney cortex particles, 40 µmoles of sodium pyruvate and 20 µmoles of $NaH^{14}CO_3$ (0.1 µCi/µmol) in 2 ml of the saline medium of Krebs and de Gasquet (10).

L'age et al. (3) reported that stimulation of renal gluconeogenesis by glucocorticoids <u>in vitro</u> is only observed in the presence of an aminoacid mixture, and for this reason all tests were supplemented with peptone (1 mg/ml). Experiments

	GLUCOSE µMOLES/G · H ± S.E.M.	N	P	$^{14}CO_2$-FIXATION I.P.M./G · H ± S.E.M.	N	P
CONTROLS	11.6 ± 0.38	3		378 · 10^3 ± 41 · 10^3	3	
VASOPRESSIN 25 MU/ML	16.0 ± 0.52	3	<0.001*	725 · 10^3 ± 6.4 · 10^3	3	0.001*
DEX.-PHOSPHATE 2 · 10^{-5} M	16.1 ± 0.91	3	<0.01*	663 · 10^3 ± 30 · 10^3	3	0.01*
DEX.-PHOSPHATE 2 · 10^{-5} M + VASOPRESSIN 25 MU/ML	25.2 ± 1.6	3	<0.001*	932 · 10^3 ± 57 · 10^3	3	0.001*
VASOPRESSIN 25 MU/ML + CYCLOHEXIMIDE 10^{-5} M	13.4 ± 0.1	3	<0.01**	387 · 10^3 ± 22 · 10^3	3	0.001**
DEX.-PHOSPHATE 2 · 10^{-5} M + CYCLOHEXIMIDE 10^{-5} M	14.0 ± 0.16	3	N.S.**	385 · 10^3 ± 4.6 · 10^3	3	0.001**
DEX.-PHOSPHATE 2 · 10^{-5} M + VASOPRESSIN 25 MU/ML + CYCLOHEXIMIDE 10^{-5} M	12.6 ± 0.3	3	<0.001**	356 · 10^3 ± 18 · 10^3	3	0.01**

Table 1: Effects of vasopressin, dexamethasone phosphate and dexamethasone phosphate plus vasopressin on glucose synthesis and $^{14}CO_2$-fixation and their inhibition by cycloheximide.

Duration of incubations 240 minutes. Addition of dexamethasone phosphate and cycloheximide at zero time, of vasopressin and substrates after 180 minutes. *related to controls, **related to values without cycloheximide. n.s.=not significant.

	GLUCOSE µMOLES/G · H ± S.E.M.	N	P	$^{14}CO_2$-FIXATION I.P.M./G · H ± S.E.M.	N	P
CONTROLS	13.7 ± 0.29	3		561 · 10^3 ± 25 · 10^3	3	
DEX.-PHOSPHATE 2 · 10^{-5} M	12.5 ± 0.54	3	N.S.	580 · 10^3 ± 10 · 10^3	3	N.S.

Table 2: No effect of dexamethasone phosphate on glucose synthesis and $^{14}CO_2$-fixation without preincubation.

Duration of incubations 60 minutes. Addition of dexamethasone phosphate and substrates at zero time. n.s.=not significant.

---

were started by the addition of substrates and terminated with 0.2 ml of 3N $HClO_4$. Excess $^{14}CO_2$ was absorbed with KOH. Glucose and $^{14}CO_2$-fixation were determined in the supernatants by the glucose oxidase method and liquid scintillation counting, respectively, after neutralization with $KHCO_3$.

RESULTS

INFLUENCE OF DEXAMETHASONE PHOSPHATE ON GLUCONEOGENESIS FROM PYRUVATE AND ON $^{14}CO_2$-FIXATION

When kidney cortex explants were preincubated for 3 hours with dexamethasone phosphate ($2 \cdot 10^{-5}$M), glucose synthesis from pyruvate was stimulated by 39 % and $^{14}CO_2$-fixation by 75 % (Table 1). The hormonal effect was completely suppressed by simultaneous addition of cycloheximide, an inhibitor of ribosomal protein synthesis (Table 1). Without preincubation, the glucocorticoid was ineffective (Table 2).

INFLUENCE OF VASOPRESSIN ON GLUCONEOGENESIS FROM PYRUVATE AND ON $^{14}CO_2$-FIXATION

Although the action of glucagon, epinephrine and 3',5'-cyclic-AMP in stimulating hepatic gluconeogenesis could be localized between pyruvate and PEP (11), the molecular mechanism of their action remained unclear. In order to approach this problem in experiments with kidney cortex explants, the influence of vasopressin and 3',5'-cyclic-AMP on gluconeogenesis from pyruvate and on $^{14}CO_2$-fixation, and its suppression by cycloheximide were investigated. In these experiments vasopressin (25 mU/ml) stimulated glucose synthesis by 38 % and $^{14}CO_2$-fixation by 92 % (Table 1). 3',5'-cyclic-AMP demonstrated similar effects in a concentration range between 10^{-5} M and 10^{-3} M (Table 3), supporting the view that the glucogenic effect of vasopressin was mediated by the cyclic nucleotide. In contrast to dexamethasone phosphate the stimulating action of vasopressin acted without any discernable lag phase (Tables 1 and 4), but was also suppressed by cycloheximide

3',5'-CYCLIC-AMP	DEX.-PHOSPHATE $2 \cdot 10^{-5}$ M	GLUCOSE µMOLES/G · H	$^{14}CO_2$-FIXATION I.P.M./G · H
-	-	11.5	$485 \cdot 10^3$
10^{-5} M	-	12.6	$521 \cdot 10^3$
10^{-4} M	-	14.9	$595 \cdot 10^3$
10^{-3} M	-	17.1	$895 \cdot 10^3$
-	+	15.8	$545 \cdot 10^3$
10^{-5} M	+	17.6	$723 \cdot 10^3$
10^{-4} M	+	17.2	$752 \cdot 10^3$
10^{-3} M	+	19.4	$840 \cdot 10^3$

Table 3: Effects of 3',5'-cyclic-AMP, dexamethasone phosphate and dexamethasone phosphate plus 3',5'-cyclic-AMP on glucose synthesis and $^{14}CO_2$-fixation.

Duration of incubations 24o minutes. Addition of dexamethasone phosphate at zero time and of 3',5'-cyclic-AMP and substrates after 18o minutes. Number of experiments in each group: 2.

	GLUCOSE µMOLES/G · H ± S.E.M.	N	P	$^{14}CO_2$-FIXATION I.P.M./G · H ± S.E.M.	N	P
CONTROLS	11.4 ± 0.52	3		$800 \cdot 10^3 \pm 36 \cdot 10^3$	3	
VASOPRESSIN 25 MU/ML	14.6 ± 0.23	3	<0.001*	$905 \cdot 10^3 \pm 39 \cdot 10^3$	3	<0.05*
VASOPRESSIN 25 MU/ML CYCLOHEXIMIDE 10^{-3} M	12.7 ± 0.23	3	<0.05**	$825 \cdot 10^3 \pm 42 \cdot 10^3$	3	<0.05**

Table 4: Inhibition of the effect of vasopressin on glucose synthesis and $^{14}CO_2$-fixation by cycloheximide.

Duration of incubations 9o minutes. Addition of cycloheximide at zero time and of vasopressin and substrates after 3o minutes. *related to controls, **related to values without cycloheximide.

(Tables 1 and 4) if the antibiotic was added at least 3o minutes prior to the hormone (Table 4). When vasopressin or 3',5'-cyclic-AMP was added after the tissue particles had been preincubated for 3 hours with dexamethasone phosphate an

addition of their stimulative effects could be observed (Tables 1 and 3).

DISCUSSION

These results clearly confirm earlier reports on glucocorticoid stimulation of gluconeogenesis from pyruvate in in vitro systems with rat liver and rat kidney cortex slices (12,3). Seubert and his colleagues (13,14) and Exton and Park (15) were able to localize the rate limiting step of gluconeogenesis between pyruvate and PEP. The acceleration of glucose synthesis and the concomitant rise of $^{14}CO_2$-fixation after the addition of both hormones or 3',5'-cyclic-AMP as well as the inhibition of their effects by cycloheximide indicate that dexamethasone phosphate, vasopressin and 3',5'-cyclic-AMP may act by induction of de novo synthesis of pyruvate carboxylase and/or phosphoenolpyruvate carboxykinase, yet do not exclude other explanations. The observation that vasopressin and 3',5'-cyclic-AMP accelerate glucose synthesis and $^{14}CO_2$-fixation in contrast to dexamethasone phosphate without any lag phase and that their effects are additive to those of the glucocorticoid, support the proposal of Wicks (16) that both groups of hormones induce de novo enzyme synthesis by two different mechanisms, namely the induction of the synthesis of a template by glucocorticoids and the acceleration of a posttranscriptional process by 3',5'-cyclic-AMP. Recently Chuah and Oliver (17) observed stimulatory action of 3',5'-cyclic-AMP on the release of tyrosine transaminase from membrane bound polysomes in a cell-free system from neonatal rat liver.

ACKNOWLEDGEMENTS

This investigation was supported by the Deutsche Forschungsgemeinschaft. The Stiftung Volkswagenwerk provided us with research equipment. Synthetic lysine-vasopressin and dexamethasone phosphate were generous gifts of Drs. Hammerschmidt and Meyer (Sandoz, Nürnberg) and of Dr. J.P. Marliac (Merck, Sharp + Dohme, Paris).

REFERENCES

1. Ray, P.D., Foster, D.O. and Lardy, H.A., J.Biol.Chem. 239, 3396 (1964)
2. Weber, G., Singhal, R.L., Stamm, N.B., Fisher, E.A. and Mentendiek, M.A., Advan.Enzyme Regul. 2, 1 (1964)
3. L'age, M., Ohly, B., Brod, H. and Seubert, W., Z.Physiol. Chem. 350, 143 (1969)
4. Barnett, C.A. and Wicks, W.D., J.Biol.Chem. 246, 7201 (1971)
5. Exton, J.H., Robison, G.A., Sutherland, E.W. and Park, C. R., J.Biol.Chem. 246, 6166 (1971)
6. Chase, L.R. and Aurbach, G.D., Science 159, 545 (1968)
7. Nagata, N. and Rasmussen, H., Proc.Nat.Acad.Sci.U.S. 65, 368 (1970)
8. Guder, W., Wiesner, W., Stukowski, B., and Wieland, O., Z.Physiol.Chem. 352, 1319 (1971)
9. Stumpf, B., Leimcke, H., Boie, A. and Seubert, W., in preparation
10. Krebs, H.A. and de Gasquet, P., Biochem.J. 90, 149 (1964)
11. Exton, J.H. and Park, C.R., J.Biol.Chem. 244, 1424 (1969)
12. Uete, T. and Ashmore, J., J.Biol.Chem. 238, 2906 (1963)
13. Seubert, W. and Huth, W., Biochem.Z. 343, 176 (1965)
14. Henning, H.V., Stumpf, B., Ohly, B. and Seubert, W., Biochem.Z. 344, 274 (1966)
15. Exton, J.H. and Park, C.R., J.Biol.Chem. 240, PC 955 (1965)
16. Wicks, W.D., J.Biol.Chem. 246, 217 (1971)
17. Chuah, C.C. and Oliver, I.T., Biochemistry 10, 2990 (1971)