INTERACTION OF ADRENAL STEROIDS AND GLUCAGON ON GLUCONEOGENESIS IN PERFUSED RAT LIVER

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Received August 29, 1967

A stimulatory effect of glucagon on gluconeogenesis in normal rats has been demonstrated by studies in vivo and in vitro (Kalant, 1956; Salter et al., 1957; Izzo and Glasser, 1961; Exton et al. 1966; Garcia et al. 1966; Sokal, 1966). However, livers from fasted adrenalectomized rats perfused with lactate or pyruvate fail to show this response (Exton et al., 1966; Eisenstein, 1967). This led us to investigate the possibility suggested by Friedmann and Wertheimer (1966) that glucocorticoids play a permissive role in the regulation of gluconeogenesis by glucagon. This paper presents data supporting this view. Injection of dexamethasone into adrenalectomized rats 30 minutes prior to sacrifice restored the response to glucagon in vitro almost to normal. In perfused livers from adrenalectomized rats, addition of dexamethasone to the perfusion medium caused a rapid rise in gluconeogenesis in the presence of glucagon but had no effect, except at high levels, in the absence of glucagon.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 95 to 140 g were used. Adrenalectomized rats were given 0.5% NaCl to drink and were used 5 to 10 days later. All animals were fasted for 18 to 22 hours before use.

The technique of liver perfusion and the methods used for the determination of glucose production and the conversion of C^{14} lactate to C^{14} glucose have been published in detail (Exton and Park, 1967). Livers were perfused at 37° with oxygenated Krebs-Henseleit bicarbonate buffer, pH 7.5, containing 3% bovine serum albumin and 22% bovine red blood cells. Dexamethasone phosphate was a commercial preparation of Merck Sharp and Dohme Inc., and glucagon was kindly supplied by Dr. O. K. Behrens of Eli Lilly Co.

RESULTS

The effect of glucagon on glucose synthesis from lactate was examined in livers from fasted normal and adrenalectomized rats. In these and all subsequent experiments, the livers were first perfused without substrate for one hour. As shown earlier (Exton and Park, 1966), the activity of the glucagon sensitive step in the gluconeogenic pathway falls to a low level during this time and a large response is seen when the hormone and substrate are subsequently added. Under these conditions, glucagon caused a 4-fold increase in glucose production in livers from normal rats as noted earlier, whereas livers from adrenalectomized rats did not respond at all to the hormone (Fig. 1).

These results indicated that the adrenal glands were required for a normal response of gluconeogenesis to glucagon. Since glucocorticoids were probably the adrenal factors involved in this response, the effects of dexamethasone administered in vivo and in vitro were examined. Subcutaneous injection of 80 μg of dexamethasone into fasted adrenalectomized rats 30 minutes prior to perfusion increased slightly the production of glucose from endogenous sources during the first hour (Fig. 2), and largely restored the stimulatory effect of glucagon on glucose production

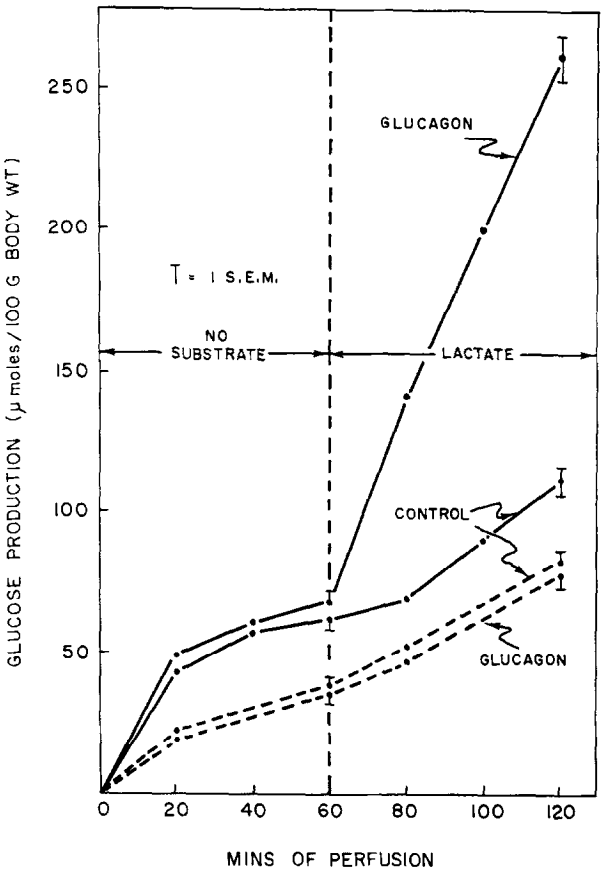

Fig. 1. The effect of adrenalectomy on the stimulation of gluconeogenesis from lactate by glucagon. Livers were perfused for 60 min. without substrate and then for 60 min. with 20 mM L-lactate. Where indicated, glucagon (0.1 μg/ml) was added at 60 min.
——— Livers from normal rats; ----- livers from adrenalectomized rats.

when lactate was added at 1 hour (Fig. 2). This effect was not due to increased glycogenolysis since glycogen could not be detected in livers from treated or untreated rats. The conclusion that it represented increased gluconeogenesis was supported by the finding that glucagon caused more than a 2-fold increase in the incorporation of ^{14}C from ^{14}C-lactate into glucose (Table 1).

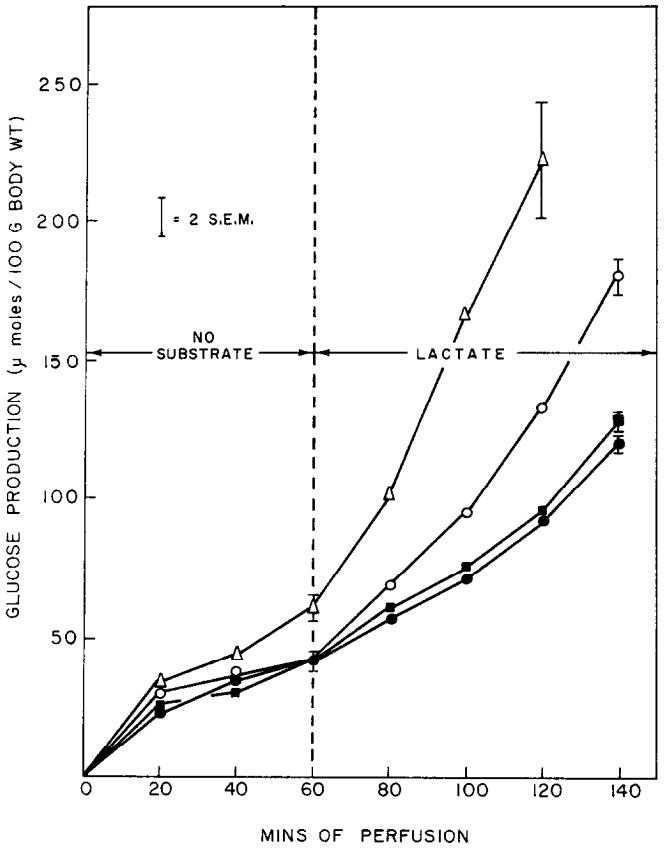

Fig. 2. The effect of dexamethasone injected in vivo or added in vitro on the effect of glucagon on lactate gluconeogenesis. Livers from adrenalectomized rats were perfused for 60 min. without substrate and then for 60 or 80 min. with 20 mM L-lactate. Glucagon and dexamethasone were added to the medium at 60 min. as noted. -Δ- Livers from rats injected with 80 μg of dexamethasone 30 min. prior to perfusion, glucagon (0.1 μg/ml). -O- Dexamethasone (2 μg/ml) and glucagon (0.1 μg/ml). -●-Dexamethasone (2 μg/ml). -■- Control.

To determine whether the effects of dexamethasone were exerted directly, livers from fasted adrenalectomized rats were perfused for 1 hour without substrate, then lactate and glucagon were added to the medium with and without 2 μg/ml of dexamethasone. As seen in Fig. 2 and Table I, the steroid rapidly increased glucose production and the incorporation of label from ^{14}C-lactate into glucose. In the absence of

Table I. Effects of dexamethasone on incorporation of ^{14}C from ^{14}C-lactate into glucose by perfused livers from adrenalectomized rats.

Experimental conditions were as in Fig. 2. In the experiments of series A, DL-lactate-2-^{14}C (20 mM, 0.45 μC) was added at 60 min and ^{14}C-glucose activity measured at 120 min; in those of series B to D, uniformly labeled L-lactate-^{14}C (20 mM, 0.5 μC) was added at 60 min. and ^{14}C-glucose measured at 140 min. Values are means ± S. E. M.

Experimental series	Hormones added	^{14}C-Glucose activity c pm/100g body wt x 10^{-3}	Significance
A*	None	123 ± 9 (4)	
	Glucagon	315 ± 25 (4)	$p < 0.0005$
B	Glucagon	266 ± 33 (5)	
	Glucagon, dexamethasone (2 μg/ml)	492 ± 27 (5)	$p < 0.0005$
C	None	232 ± 20 (5)	
	Dexamethasone (2 μg/ml)	265 ± 19 (7)	N. S.
D	None	292 ± 11 (7)	
	Dexamethasone (100 μg/ml)	345 ± 19 (7)	$p < 0.025$

*Livers from rats injected with 80 μg of dexamethasone

glucagon, 2 μg/ml of dexamethasone did not affect glucose production or ^{14}C-glucose synthesis whereas 100 μg/ml increased both processes to a small extent (Fig. 2 and Table I).

DISCUSSION

Although there is much evidence that glucocorticoids increase gluconeogenesis in intact animals, the initial site and mechanism of the effect remain unknown. Although several investigators have demonstrated direct effects of steroids on liver preparations (for review see Landau, 1965), the changes observed have been relatively small and slow. The present study has also shown that a high concentration of the

glucocorticoid, dexamethasone, can directly stimulate gluconeogenesis in liver. However, a finding of possibly greater physiological significance is the observation that adrenal deficiency abolishes the stimulatory effects of glucagon on gluconeogenesis from lactate and that addition of dexamethasone in vitro restores it.

The effect of glucagon on gluconeogenesis appears to be mediated by adenosine 3', 5'-monophosphate (cyclic AMP) the intracellular formation of which is greatly stimulated by glucagon. Exogenous cyclic AMP also stimulates gluconeogenesis apparently by activating a reaction in the pathway between pyruvate and P-pyruvate (Exton et al., 1966). The failure of glucagon to stimulate gluconeogenesis in adrenalectomized rats could therefore be due to an impairment of cyclic AMP formation or to excessively rapid breakdown of the hormone or cyclic AMP. Preliminary observations, however, have shown that glucagon produces approximately the same elevation in cyclic AMP in livers of adrenalectomized rats (control, 0.77; glucagon, 10.43 nmoles/g) as in normal livers (control 0.66; glucagon, 14.70 nmoles/g). An alternative explanation is that the reaction (or reactions) in gluconeogenesis activated by cyclic AMP becomes less sensitive to the action of the nucleotide as a result of adrenal insufficiency. This appears more likely on the basis of preliminary experiments in which livers from adrenalectomized rats failed to respond to exogenous cyclic AMP at a concentration which strongly activated gluconeogenesis in normal liver. The ability of dexamethasone to sensitize the liver to the action of cyclic AMP may be thought of as a permissive action of a glucocorticoid. It remains to be seen whether glucocorticoids have similar sensitizing effects on other systems activated by cyclic adenylate.

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