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THE EFFECTS OF GLUCAGON AND INSULIN ON THE MIXED-FUNCTION OXIDASE SYSTEM

EVELYNE ROUER and JEAN-PAUL LEROUX

Faculté Necker-Enfants-Malades, INSERM U 75, 156, rue de Vaugirard, 75730 Paris cedex 15 FRANCE

We have previously reported (1-2) that insulin-dependent diabetes in mice, produced by streptozotocin, leads to both an increase of hepatic microsomal cytochrome P 450 and of some dependent monnoxygenase activities. Insulin treatment, which allows a retourn of the physiological parameters tc normal values, also corrects most of the modifications of the mixed-function oxidase system. Those data demonstrate that streptozotocin by itself is not responsible as an inducing agent, for the observed effects of diabetes on the mixed-function oxidase system. However they do not allow to determine whether the trigger event is a decreased insulin, or the elevated glucagon level which is always obser ved in insulin-dependent diabetes. In this purpose, the glucagon / insulin ratio in mice was modified either by starvation (72^h) or by a glucagon load (by repeated injections or continuous delivery by means of implanted osmotic minipumps Alzet) On the other hand we investigated the possibility of a regulation of cytochrome P 450 activity by phosphorylation since it was recently demonstrated (3)that the purified LM_2 cytochrome P 450 isozyme may undergo phosphorylation by a cAMP-dependent mechanism.

The glucagon-injected (twice daily, 0.5 mg/kg/day, 5 days) exhibited a slight decrease (20%) of hepatic microsomal cytochrome P 450 and small increase of the molecular activities of aniline hydroxylase, benzphetamine-N-demethylase and 7-ethoxycoumarin-O-deethylase. Only 4-nitroanisole-O-demethylase was substantially increased (2-fold). In glucagon-infused mice (35 µg/kg/day, 5 days) the same effects were observed but with a much greater amplitude : aniline hydroxylase and benzphetamine-N-demethylase were respectively 3 and 7 fold increased. Thus glucagon can modify the isozymic content of cytochrome P 450, with induction of specific forms since, in spite of a decreased overall cytochrom P 450 level, some monnoxygenase activities were strongly increased. Moreover, using implanted minipump which continuously deliver the hormone, we have demonstrated that glucagon may exert its effects at a low dose, in the range of human therapeutic use. However, the pattern of modifications of monooxygenase activities induced by glucagon-treatment is not similar to the one observed physiopathological hyperglucagonemic situations as starvation and diabetes (table1), in which moreover, the content in cytochrome P 450 is increased (100%). Thus, glucagon may not be the only factor involved in the modifications of the oxidase system.

Table 1 :	Cytochrome P 450 (a)	Aniline hydroxylase (b)	4-nitroanisole O-demethylase (b)	Benzphetamine N-demethylase (b)	7-Ethoxycoumarin O-deethylase (b)
control mice	1.00	0.89	7.62	3.97	2.76
glucagon-injected mice	0.80	1.28	13.01	5.00	3.59
control sham-operated mice	1.06	0.78	8.15	2.29	2.58
glucagon-infused mice	0.69	2.87	13.01	14.61	3.82
control mice	1.37	1.11	7.33	5.74	2.05
starved mice	2.60	1.97	6.81	6.29	4.27
control mice	1.01	1.38	8.64	3.90	3.61
STZ-diabetic mice	1.99	2.55	12.31	13.23	8.54

a) nmol x mg^{-1} microsomal proteins.
b) nmol of product formed x min^{-1} x $nmol^{-1}$ of cytochrome P 450.

mean of 3 to 6 animals

On the other hand, microsomes from either glucagon-infused or diabetic mice, preincubated with alkaline phosphatase (2 units / mg of microsomal proteins), lost a great part of their monooxygenase activities (about 65% of 7-ethoxycoumarin-O-deethylase decrease after 60 min of preincubation) while microsomes from either control starved mice lost only about 20% of their deethylase acitivity (Table 2).

Table 2 : 7-ethoxycoumarin-O-deethylase

	% of inhibition	
	30 min	60 min
control mice	12 ∓ 2	23 ∓ 2
STZ-diabetic	34 ∓ 6	59 ∓ 8
Glucagon-injected mice	62 ∓ 6	87 ∓ 7
Glucagon-infused mice	33 ∓ 7	66 ∓ 3

mean ∓ SEM of 3 to 6 individual determinations

At the end of the preincubation no change was observed in the spectrophotometric measurement of cytochrome P 450, and the activity of NADPH-cytochrome c reductase was poorly affected. Thus the decreased monooxygenase activities do not result from a general process of microsomal protein dephosphorylation, and the catalytic activities of some cytochrome P 450 isozymes may postulated to be modified by a phosphorylation-dephosphorylation process.

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