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PRELIMINARY COMMUNICATIONS

THE EFFECTS OF HORMONES ON LIVER FRUCTOSE BISPHOSPHATASE CONCENTRATION AND ACTIVITY: APPLICATION OF A NEW SPECIFIC RADIOIMMUNOASSAY

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The concentration, as well as the maximum assayable activity, of fructose bisphosphatase (FBPase) was measured in rabbit livers by the application of a specific radioimmunoassay technique. We obtained data in terms of nmoles/g wet weight of liver, units/g, units/nmole, nmoles/liver and units/liver in control rabbits given glucagon, glucocorticoids and thyroxine. Enzyme concentration (nmoles/g) increased following injection of all three hormones, but enzyme activity (units/g) did not increase proportionately. As a result, the average specific activity (units/nmole) decreased, which is best explained by hormonal induction of the enzyme without processing to give enzyme protein of normal specific activity. In no case of hormone administration, including glucagon, was the specific activity of the enzyme increased. Any increase in units/liver was always caused by an increase in enzyme mass rather than in catalytic activity.

MATERIALS AND METHODS

New Zealand rabbits (42; male and female) of approximately 1200 g were used. All rabbits were maintained on a conventional commercial rabbit chow until they were killed. Glucagon was obtained from the Eli Lilly Co., Chicago, IL, cortisone acetate from the Upjohn Co., Kalamazoo, MI, triamcinolone diacetate from Lederle, Chicago, IL, and levothyroxine from Flint Laboratories, Deerfield, IL. Radioiodination of FBPase was accomplished by indirect means using a modification of the method of Bolton and Hunter [1]. The N-hydroxysuccinimide ester was iodinated to a specific activity of approximately 5 mCi/μg. Radioimmunoassay of FBPase was as described by Mazzotta and Veneziale [2]. Maximal activity assays were carried out according to the method of Ulm *et al.* [3] and utilized an AMP removal system. Rabbits were anesthetized and then exsanguinated from severed carotid and jugular vessels. Livers were removed and weighed, and 20% homogenates (w/v) were prepared with 10 mM Tris-HCl, pH 7.6, containing 0.25 M sucrose, 0.1 mM Na_2EDTA and 1 mM dithiothreitol. A high speed supernatant fraction was prepared by centrifugation at 115,000 g for 60 min. For activity assays, 10 μl of this fraction was used; even the most active extracts did not exceed the capacity of this system to measure V_{max}. The high speed supernatant fraction was also analyzed for FBPase concentration by radioimmunoassay. Standard enzyme for the radioimmunoassay was prepared from the frozen livers of young rabbits according to the procedure of Ulm [3]. Purified enzyme was shown to be homogeneous by the criteria of sodium dodecyl sulfate gel electrophoresis; even 48 μg gave a single stained band. Standard enzyme concentration was assayed by a fluorescamine method [4,5]. Data are presented as the mean ± one standard deviation.

RESULTS AND DISCUSSION

Control rabbits maintained plasma glucose concentrations of 186 ± 27 mg/100 ml. Plasma levels were 287 ± 88 in glucagon-treated animals, 248 ± 104 in cortisone-treated animals and 300 ± 94 mg/100 ml in triamcinolone-treated rabbits.

Administration of glucagon resulted in an increase in the amount of liver FBPase from 157 to 336 nmoles (Table 1). Enzyme activity was also shown to increase over control animals but it failed to rise proportionately, increasing from 413 to only 581 units. The specific activity, defined as units/g divided by nmoles/g, therefore, decreased from a control value of 2.6 to 1.8. The same general effects were also noted when rabbits were injected with cortisone. In the case of triamcinolone and thyroxine the specific activity was again significantly decreased (from 2.6 to 1.5); variation in units/g after injection of these hormones made the increase in units/liver appear to be statistically insignificant. The effect of glucagon, glucocorticoids and thyroxine, which was to increase the mass of liver FBPase, was also clearly evident when the data were based on 1000 g body weight (Table 2).

Clark et al. [6] using rat hepatocytes provided evidence for stimulation of the carbon flux through the FBPase reaction by glucagon. Riou et al. [7] reported that isolated FBPase is subject to specific phosphorylation associated with an increase in enzyme activity. In contrast Mendicino et al. [8] showed that isolated swine kidney FBPase could be phosphorylated without a change in activity.

Glucagon has long been known to stimulate gluconeogenesis by effects on the pathway beyond phosphoenolpyruvate carboxykinase [9,10]. The hormone has been shown to cause inhibition of phosphofructokinase in isolated hepatocytes [11]. Whatever specific alterations of the enzymes occur in response to glucagon and its second messenger, cAMP, remain to be documented. The present study partly focuses on the effects of glucagon on both the concentration and activity of FBPase. The data provided by our approach indicate that glucagon induces the enzyme, but does not seem to activate it.

Zalitis and Pitot [12] have reported a half life of 45 hr for rat liver FBPase and have stated that, under a variety of hormonal conditions, there is little variation in its rate of synthesis and degradation. They concluded that the enzyme is a constitutive protein in liver. However, in the rabbit liver the situation appears to be different based on data presented in this paper and in Mazzotta and Veneziale [2].

This information helps to place into perspective the importance of measuring enzyme concentration independent of enzyme activity. Activity measurements do not necessarily reflect the intracellular enzyme protein concentration; there is not always a direct relationship between activity and concentration [13-16]. The use of a specific radioimmunoassay allowing for the direct measurement of enzyme concentration independent of activity measurements addresses this issue directly and provides a new investigative dimension not previously available.

FBPase also accumulates in liver after injection of glucocorticoids and thyroxine. Previous work [16], based strictly on activity measurements, supports the view that glucocorticoids induce the enzyme at the level of transcription or translation. It is unlikely that changes in the rate of degradation are entirely responsible for the elevated enzyme tissue concentrations. Induction of enzyme protein best explains the elevated concentrations we have demonstrated after hormone injection.

Table 1. Effects of glucagon, glucocorticoids and thyroxine on liver fructose bisphosphatase concentration and activity in rabbits*

Condition	Animal wt (g)	Liver wt (g)	μmoles / 1000 g wet wt liver	nmoles / liver	units / g liver	units / liver	units / nmoles enzyme	N
Control	1334 ± 173	60 ± 14	2.7 ± 0.9	157 ± 48	7.1 ± 1.7	413 ± 78	2.6 ± 0.5	10
Glucagon	1263 ± 241	64 ± 25	5.7 ± 2.0 P < 0.001	336 ± 115 P < 0.025	9.8 ± 2.8 P < 0.025	581 ± 188 P < 0.025	1.8 ± 0.3 P < 0.001	14
Cortisone	1280 ± 171	71 ± 19	4.4 ± 1.3 P < 0.01	306 ± 84 P < 0.001	7.5 ± 0.8 NS	530 ± 134 P < 0.05	1.8 ± 0.3 P < 0.01	6
Triamcinolone	1255 ± 172	68 ± 30	6.0 ± 3.2 P < 0.001	357 ± 132 P < 0.001	8.3 ± 2.9 NS	509 ± 150 NS	1.5 ± 0.3 P < 0.001	6
Thyroxine	1458 ± 90	74 ± 9	5.1 ± 1.8 P < 0.005	378 ± 125 P < 0.0002	7.2 ± 3.9	539 ± 288 NS	1.5 ± 0.8 P < 0.01	6

* Glucagon (100 μg) was injected every 6 hr for nine injections. Cortisone acetate (2.5 mg/injection), triamcinolone diacetate (4.0 mg), or thyroxine (20 μg) was injected intramuscularly on each of three successive mornings. The rabbits were killed within 1 hr after the final hormone injection.

NS = not significant.

Table 2. Hormonal effects on fructose bisphosphatase concentration and activity in terms of body weight*

Condition	nmoles / 1000 g body weight	units / 1000 g body weight
Control	120 ± 42	313 ± 64
Glucagon	264 ± 74 ($P < 0.001$)	454 ± 89 ($P < 0.001$)
Cortisone	240 ± 60 ($P < 0.001$)	413 ± 93 ($P < 0.05$)
Triamcinolone	288 ± 110 ($P < 0.001$)	404 ± 99 ($P < 0.05$)
Thyroxine	261 ± 88 ($P < 0.001$)	363 ± 186 (NS)

* Glucagon (100 µg) was injected every 6 hr for nine injections. Cortisone acetate (2.5 mg/injection), triamcinolone diacetate (4.0 mg), or thyroxine (20 µg) was injected intramuscularly on each of three successive mornings. The rabbits were killed within 1 hr after the final hormone injection.
NS = not significant.

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