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Effect of Sindbis Virus Infection on Hydrocortisone-Induced Hepatic Enzymes in Mice (40005)

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Interferon and interferon-inducing agents under *in vivo* and *in vitro* conditions have been found to cause effects which are apparently unrelated to interferon's well-known ability to inhibit viral replication. These include altered immune responses (1-3), inhibited replication of neoplastic cells (4), depressed rate of liver regeneration (5, 6), lethality for neonatal mice (7), and sensitization of adult mice (8, 9) and rats (10) to gram-negative bacterial endotoxin. Renton and Mannering (11) demonstrated that an injection of interferon-inducing agents in rats depressed hepatic cytochrome *P*-450 levels and the activities of cytochrome *P*-450-dependent monooxygenases. Other investigators suggest that interferon is also able to inhibit the hormonal induction of certain hepatic enzymes (12, 13).

We have shown that administration of bacterial endotoxin (14, 15) or double-stranded polyribonucleotide [poly(rI)·poly(rC)] (9) to mice results in inhibited hormonal induction of hepatic phosphoenolpyruvate carboxykinase (EC 4.1.1.32). Since endotoxin and poly(rI)·poly(rC) are both interferon inducers (16, 17), the possibility that viral infection also inhibits enzyme induction was investigated. Results reported in this communication show that this occurs in mice infected with Sindbis virus, an arbovirus that produces a nonlethal infection in adult mice (18).

Materials and methods. Pathogen-free, 5- to 7-week old, female Ha/ICR mice were used in these studies and were maintained under a 12-hr light-dark cycle. Unless otherwise stated, food (Wayne Lab Blox) and water were available at all times. Sindbis virus preparations (ATCC strain A339) were prepared and titrated on chick embryo fibroblasts and stored at −70°. Activities of hepatic phosphoenolpyruvate carboxykinase (19), tryptophan oxygenase (EC 1.13.11.11) (20), and tyrosine aminotransferase (EC 2.6.1.5) (21) were determined in whole liver homogenates as described previously. Inteferon titers in mouse serum were determined by the 50% plaque reduction assay on mouse L929 cells challenged with vesicular stomatitis virus. Commercial interferon and mock interferon were purchased from Bionetics Laboratory Products (Kensington, Md.). This was produced by infecting C243-3 cells with Newcastle disease virus (NDV) and contained 10^6 units of interferon/ml. Mock interferon, prepared from the same cells, had NDV added after harvest. The virus was inactivated by exposure to pH 2 for 5 days. Statistical significance was determined by the Wilcoxon rank sum test (22).

Results. Initial experiments demonstrated that mice infected intravenously with 1×10^9 plaque-forming units of Sindbis virus maintained a normal base level of phosphoenolpyruvate carboxykinase activity during the first 12 hr (see Table III). The same level of infection suppressed, however, the induction of the enzyme elicited by overnight fasting. These results are shown in Table I. The increase in enzyme activity that occurred in fasted mice was significantly reduced in infected mice. Enzyme induction was also significantly inhibited, in mice injected with an equal volume of Sindbis virus suspension that had been irradiated with ultraviolet light. Since less than 200 plaque-forming units remained following irradiation, these results suggest that viral inhibition of phosphoenolpyruvate carboxykinase (PEPCK) induction is not a direct result of an active viral infection.

The interval between Sindbis injection and the time when hormonal induction of

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phosphoenolpyruvate carboxykinase was inhibited was determined in the same manner as that described previously for poly(rI)·poly(rC) (9). At timed intervals following virus or saline injection, 1 mg of hydrocortisone acetate (Sigma) was injected subcutaneously, and enzyme activity was determined 4 hr later. Results are shown in Table II. Enzyme activity was as fully inducible in virus-infected mice as in normal mice for at least 4 hr. Six hours after virus infection, however, the enzyme was significantly less responsive to hormonal induction ($P \leq 0.001$) and the same was evident also at 8 hr. The total elapsed times were, respectively, 10 and 12 hr.

The titer of interferon was determined in pooled sera of mice 8 hr after an injection of either live virus or irradiated virus in amounts similar to those used to obtain the data in Table I. For live virus the titer was 1:160 and for irradiated virus it was 1:200. Since live virus inhibited the induction of the enzyme significantly more than killed virus, there is some doubt as to whether the factor responsible for this effect is interferon.

This doubt is strengthened by results obtained with a group of mice injected iv with 25,000 units of commercial interferon contained in 0.2 ml and with another group of animals given an equal volume of mock interferon. After 8 hr half the mice in each group were injected sc with 1 mg of hydrocortisone acetate and 4 hr later the livers of all animals were assayed for PEPCK activity. The control and induced values for mice given interferon were, respectively, 146 ± 5 for seven mice and 150 ± 9 for seven mice. Corresponding values for mice given mock interferon were, respectively, 152 ± 12 for seven mice and 154 ± 10 for seven mice. It would appear, therefore, that viruses present in both preparations were responsible for inhibiting PEPCK induction. This is borne out by the results obtained when the interferon and mock interferon preparations were centrifuged for 1 hr at 105,000*g* and the experiments just described were repeated with the supernatant fluids. The control and induced values for mice given an injection with the fluid from the interferon preparation were, respectively, 160 ± 13 for seven mice and 276 ± 16 for seven mice. Corresponding values for mice given injections from the mock interferon were, respectively, 158 ± 14 for

TABLE I. Hepatic Phosphoenolpyruvate Carboxykinase Activity in Fasted, Sindbis-Infected Mice[a]

Treatment	Number of mice	Enzyme activity[b] (mean ± SEM)	Significance vs fasted control
Fed controls	6	118 ± 15	$P \leq 0.01$
Fasted controls	6	191 ± 9	–
Fasted + 1 × 10^9 Sindbis PFU	6	144 ± 10	$P \leq 0.01$
Fasted + uv-irradiated Sindbis	6	163 ± 8	$P \leq 0.05$

[a] All injections were made iv in 0.1 ml at 5:00 PM when food was withdrawn. Enzyme activity was determined at 8:00 AM the following day.

[b] Micromoles of phosphoenolpyruvate formed in 6 min per gram (dry wt) of liver.

TABLE II. Hepatic Phosphoenolpyruvate Carboxykinase Activity in Normal Mice and in Mice at Different Times Postinfection with Sindbis Virus[a]

Time of hydrocortisone administration (hr after Sindbis infection)	Enzyme activity[b] (mean ± SEM)			
	Control mice		Mice infected with Sindbis virus	
	Uninduced	Induced[c]	Uninduced	Induced
0	144 ± 7 (8)[d]	249 ± 6 (14)	145 ± 6 (8)	249 ± 12 (4)
2	151 ± 10 (8)	249 ± 6 (14)	149 ± 7 (8)	276 ± 19 (14)
4	165 ± 11 (8)	262 ± 9 (14)	170 ± 12 (8)	268 ± 12 (14)
6	168 ± 12 (7)	292 ± 14 (8)	158 ± 10 (8)	166 ± 7 (7)
8	166 ± 9 (8)	272 ± 8 (7)	151 ± 6 (8)	152 ± 5 (14)

[a] Mice were infected iv with 10^9 plaque-forming units of Sindbis virus at time zero. Control mice were not infected.

[b] Micromoles of phosphoenolpyruvate formed in 6 min per gram (dry wt) of liver.

[c] Mice were injected sc with 1 mg of hydrocortisone acetate at the time postinfection indicated and they were sacrificed for enzyme assays 4 hr later.

[d] Numbers in parentheses represent number of mice used for the value shown.

seven mice and 281 ± 15 for seven mice. It is not likely, therefore, that interferon is responsible for blocking PEPCK induction in these experiments.

The effect of Sindbis virus infection on two other corticosteroid-inducible hepatic enzymes was investigated also. Results are shown in Table III. Tyrosine aminotransferase behaved in a manner similar to that of phosphoenolpyruvate carboxykinase. Although infection did not suppress enzyme activity in fed mice, enzyme induction by hydrocortisone was inhibited significantly in infected mice and in mice injected with virus particles which had been inactivated by exposure to ultraviolet light.

The effect of Sindbis on tryptophan oxygenase, however, differed from that of the other two enzymes. Although virus infection suppressed hormonal induction the base level activity of tryptophan oxygenase was significantly depressed in infected mice. Tryptophan oxygenase, unlike the other two enzymes, is apparently sensitive to some direct action of an active viral infection. This suggestion is supported by the observation that the enzyme was not only fully inducible, but was also superinducible in mice injected with inactivated virus particles. There is no explanation as to why this occurs.

Discussion. These results demonstrate that an injection of Sindbis virus inhibits hormonal induction of hepatic phosphoenolpyruvate carboxykinase, tyrosine aminotransferase, and tryptophan oxygenase. Bacterial endotoxin (14, 15, 20, 23) and poly(rI)·poly(rC) (9, 24) also inhibit corticosteroid induction of hepatic phosphoenolpyruvate carboxykinase and tryptophan oxygenase. The two toxic substances, however, induce tyrosine aminotransferase activity in mice rather than inhibit its induction (24, 25). Since the enzyme does not induce in endotoxin-treated, adrenalectomized mice (25), the difference between the action of the virus and these two substances is probably dependent on their ability to stimulate adrenal-cortical secretion. Our results suggest that virus injection, unlike endotoxin and poly(rI)·poly(rC), does not result in *in vivo* steroidogenesis.

We have previously shown that the inhibitory effect exerted by endotoxin on phosphoenolpyruvate carboxykinase is mediated by a factor produced by the poisoned host (26, 27) and suggest that inhibited hormonal induction of the three hepatic enzymes (with the possible exception of tryptophan oxygenase) in Sindbis-infected mice is mediated also.

While interferon may be responsible for the inhibitory effects in mice treated with each of these agents, proof will require more highly purified preparations and assay of the enzymes in isolated hepatocytes or a hepatoma cell line. It is important to note, however, that the time required for inhibition of phosphoenolpyruvate carboxykinase induction in Sindbis-infected mice (6 to 10

TABLE III. ACTIVITIES OF CORTICOSTEROID-INDUCIBLE HEPATIC ENZYMES IN SINDBIS VIRUS-INFECTED MICE[a]

Treatment	Phosphoenolpyruvate carboxykinase[b]	Tyrosine aminotransferase[c]	Tryptophan oxygenase[d]
Control	148 ± 17 (10)	6.6 ± 0.1 (7)	6.1 ± 0.7 (12)
12 hr after 1 × 10^9 Sindbis PFU	143 ± 10 (5)	6.0 ± 0.4 (7)	1.8 ± 1.3 (5)[e]
Control + 1 mg of hydrocortisone	242 ± 10 (6)	25.6 ± 0.7 (21)	23.3 ± 1.4 (12)
12 hr after 1 × 10^9 Sindbis PFU; 4 hr after 1 mg of hydrocortisone	196 ± 11 (6)[e]	16.7 ± 0.7 (14)[f]	13.0 ± 1.5 (12)[f]
12 hr after uv-Sindbis; 4 hr after 1 mg of hydrocortisone	183 ± 9 (7)[e]	19.9 ± 0.5 (7)[f]	32.1 ± 1.4 (12)[f]

[a] Sindbis virus and uv-inactivated Sindbis were injected intravenously in 0.1 ml of physiological saline at 12:00 midnight. Corticosteroid (1 mg of hydrocortisone acetate) was injected subcutaneously in 0.2 ml of saline 8 hr later at 8:00 AM. Enzyme activities were determined 4 hr later at 12:00 noon. Values are the mean ± SE, and numbers in parentheses represent the number of mice used for each determination.

[b] Micromoles of phosphoenolpyruvate formed in 6 min per gram (dry wt) of liver.

[c] Micrograms of *p*-hydroxyphenylpyruvic acid formed in 10 min per milligram (dry wt) of liver.

[d] Micromoles of kynurenine formed in 1 hr per gram (dry wt) of liver.

[e] Significantly different from corresponding control ($P \leq 0.01$).

[f] Significantly different from corresponding control ($P \leq 0.001$).

hr) coincides with the appearance of significant levels of interferon in the serum of virus-infected animals (16). In addition, interferon, at least in relatively high concentrations, is known to inhibit steroid induction of tyrosine aminotransferase in cultured hepatoma cells (12, 28). Our observations with commercially available interferon raise doubt as to whether the above referenced observations and our own in this report are, in fact, due to the action of interferon. This question will remain unsettled until further studies have been completed.

The metabolic significance of inhibited enzyme induction in Sindbis infection remains to be determined. It is pertinent to note, however, that seven fasted, Sindbis-infected mice were significantly more hypoglycemic after 15 hr than an equal number of fasted controls (64 ± 6 versus 87 ± 19 mg of glucose/dl of blood, respectively, $P < 0.05$). While it is premature to attribute the low blood sugar level to a suppressed activity of phosphoenolpyruvate carboxykinase, more careful investigation of carbohydrate metabolism in virus-infected mice may prove revealing.

Summary. Hormonal induction of three hepatic enzymes, phosphoenolpyruvate carboxykinase, tryptophan oxygenase, and tyrosine aminotransferase, was significantly inhibited in mice infected with Sindbis virus. Induction of phosphoenolpyruvate carboxykinase was suppressed whether it was elicited by overnight fasting or by administration of hydrocortisone. This enzyme responded normally to steroid induction 4 hr after virus administration, but after 6 hr, it was significantly less responsive. The timing of this effect corresponds with the appearance of interferon in the serum of virus-infected mice and mice injected with uv-irradiated virus, but results with commercially available interferon make it questionable whether interferon is the factor responsible.

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