The Prematurely Evoked Synthesis of Liver Tryptophan Oxygenase

(hydrocortisone/RNA synthesis)

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ABSTRACT Livers of normal rats are devoid of catalytically or immunochemically reactive tryptophan oxygenase (EC 1.13.1.12) up to the 10th postnatal day; the enzyme reaches adult concentrations on about the twentieth day. Premature tryptophan oxygenase synthesis can be evoked in 4-day-old rats: if an injection of glucocorticoid is followed, a day later, by an injection of tryptophan, adult levels of tryptophan oxygenase activity and antigen content can be attained within 5 hr. The prematurely evoked tryptophan oxygenase is degraded in about 2 days, but the preparatory action of the glucocorticoid is long-lasting. Even 4 days later, an injection of tryptophan can evoke significant enzyme formation. Actinomycin D, injected together with or 12 hr before the tryptophan, is not inhibitory, but if injected with the glucocorticoid it prevents enzyme formation upon later injection of tryptophan. The observations suggest that appearance of the new enzyme in developing tissue results from the sequential action of more than one stimulus and that the potentiality for its transcription may develop long before its actual synthesis.

In recent years we succeeded in experimentally evoking the accumulations of several enzymes in rat liver at earlier stages of development than when they normally appear. It was thereby possible to identify some of the physiological factors that regulate enzymic differentiation (1). Thyroxine and glucagon evoked the formation of certain liver enzymes that normally emerge during late fetal and neonatal life, respectively, and glucocorticoids promoted the formation of some enzymes of the cluster that emerges during the late suckling period (2). Tryptophan oxygenase (L-tryptophan:oxygen oxidoreductase, EC 1.13.1.12) belongs to the latter cluster: as first shown by Nemeth (3), it is absent from rat liver until the third week of postnatal life. However, neither an injection of glucocorticoid (4) nor one of tryptophan (3) evoked the enzyme prematurely, although both factors raise the concentration of the enzyme in mature livers (5). The premature evocation of another enzyme in this cluster, glucokinase (ATP:D-glucose 6-phosphotransferase, EC 2.7.1.2), required prior treatment with hydrocortisone as well as an injection of the substrate, glucose (6). The present study shows that the evocation of tryptophan oxygenase also requires the sequential applications of hormone and substrate stimuli.

MATERIALS AND METHODS

Rats were of the NEDH inbred strain. L-Tryptophan, hydrocortisone, corticosterone, and actinomycin D were injected intraperitoneally, in amounts of 35, 2.5, 10, and 0.005 mg, respectively, per 100 g of body weight. Tryptophan oxygenase was assayed, after conversion to fully active holoenzyme, as described by Piras and Knox (7). Each preparation

was also assayed in the presence of excess antiserum from rabbits immunized with partially purified tryptophan oxygenase from rat liver. This antiserum inhibits tryptophan oxygenase; the low apparent activity in its presence, equivalent to an enzyme-free blank, was always subtracted from the activity obtained without added antiserum. The activities were then expressed in units (nmol/min at 25°C) per g liver, fresh weight. The immunochemical titration of tryptophan oxygenase in developing rat livers was carried out by the method of Knox *et al.* (8), in the presence of added amounts of adult liver supernatant with known amounts of tryptophan oxygenase. The antiserum was standardized against the adult liver preparation, with antigen units set equal to catalytic units.

RESULTS

The solid line in Fig. 1 illustrates the developmental formation of tryptophan oxygenase in normal rat liver through the third postnatal week. Up to the age of 10 days there was no significant rise in activity in either untreated rats or in those given an injection of tryptophan 5 hr before assay. At the age of 14 days, when there is already some tryptophan oxygenase in the liver, the same treatment with tryptophan resulted in a major rise in enzyme concentration. Rats adrenalectomized at the age of 11 days accumulated less tryptophan oxygenase, but adrenalectomy at the age of 14 days was without significant effect.

In animals less than 14 days old, tryptophan oxygenase could be evoked only if an injection of hydrocortisone or corticosterone (the natural glucocorticoid in rats) preceded that of tryptophan (Fig. 1). Hydrocortisone acetate was the most effective glucocorticoid: at the age of 4 days or later, adult concentrations of tryptophan oxygenase could be attained by the injection of tryptophan (5 hr before assay) 1 day after hydrocortisone acetate. Most of the enzyme was synthesized during the last 5 hr under the influence of tryptophan (as will be seen in Fig. 2 and Table 1, hydrocortisone alone caused little rise in activity or antigen content); this prematurely evoked enzyme does not persist in the liver, but disappears almost completely in 2 days (Fig. 1) unless tryptophan is injected repeatedly.

The consequences of varying the time interval between the administration of hydrocortisone and tryptophan are illustrated in Fig. 2. The simultaneous administration of the two agents was ineffective, while a 5-hr delay in giving tryptophan elicited some activity. Highest activity was obtained by the administration of tryptophan approximately 1 day after hydrocortisone. However, tryptophan raised the enzyme con-

FIG. 1. Developmental formation of tryptophan oxygenase. Tryptophan oxygenase was assayed in untreated rats (—●—) and in rats adrenalectomized at the age of 11 (■) and 14 days (□). Tryptophan was injected 5 hr before assay without prior treatment (×) or 24 hr after an injection of corticosterone (○). Rats injected with hydrocortisone acetate received tryptophan 24 hr later and were assayed 5, 24, and 48 hr thereafter (—▲—). Each point is a mean (vertical bars indicate 1 SE) of results with 4–8 rats.

centration even if administered 4 days after hydrocortisone; in rats of the same age (8 days) not given a prior injection of hydrocortisone, tryptophan evoked no tryptophan oxygenase activity. The effectiveness of tryptophan, even after a 4-day delay, is unlikely to be due to the persistence of the hormone in the body, since prior treatment with the highly soluble succinate salt of hydrocortisone also permitted tryptophan to be effective 4 days later.

2 or 3 days after an injection of hydrocortisone acetate, young rats show a 10–15% loss of body weight. The small amount of tryptophan oxygenases synthesized in these rats without tryptophan injections (see Fig. 2 and Table 1) is probably due to some endogenous release of tryptophan secondary to protein breakdown. This secondary action is apparently less when hydrocortisone succinate is used (Fig. 2).

The immunochemical estimation of tryptophan oxygenase involves activity measurements in the presence of various amounts of the inhibitory antiserum (8). We estimated the antigen content of preparations with low or undetectable catalytic activity by adding them to adult liver cytosol of known tryptophan oxygenase content. A value of zero in Table 1 indicates that the antigen titer of the latter was unaltered when mixed with 3 volumes of cytosol from developing livers. Values under 10 are of doubtful significance and only when the value is over 30 were the measurements reproducible within a 10–20% error. Table 1 shows that livers of 5- to 8-day-old untreated rats are devoid of tryptophan oxygenase antigen. In those injected with hydrocortisone acetate (24 hr or 4 days before assay) there may be a small amount of antigen, but in the 5 hr after an injection of tryptophan to such rats there is always a major increase in antigen content paralleling the increase in catalytic activity. Thus, in rats previously treated with hydrocortisone, tryptophan causes the

accumulation of new enzyme protein rather than the activation of preexisting antigen; without the hormone treatment, an injection of tryptophan is not followed by a rise in activity or antigen content.

RNA synthesis *de novo* appears to be necessary for the preparatory action of hydrocortisone but not during the actual

FIG. 2. Time course of the "preparatory" action of hydrocortisone. Each rat (4 days old) was injected with 0.25 mg of hydrocortisone acetate (HCA) or hydrocortisone succinate (HCS) at zero time. Some were assayed at the indicated times without further treatment (light bars). Others were also given 3.3 mg of tryptophan (dark bars) once, simultaneously with the hormone, or 5 hr, 1 day, and 4 days later; they were all assayed 5 hr after the tryptophan injection. Each bar shows the mean + 1 SE of results with 6–8 rats.

TABLE 1. *Immunochemical estimation of tryptophan oxygenase in developing liver*

Expt.	Injection	Tryptophan oxygenase (units/g liver)	
		Activity	Antigen
1	None	0	0
	Hydrocortisone	8.3	23
	Hydrocortisone and tryptophan	70	106
2	Tryptophan	0	0
	Hydrocortisone	26	12.5
	Hydrocortisone and tryptophan	97	95
3	None	7.3	0
	Hydrocortisone	16	26
	Hydrocortisone and tryptophan	56	76
4	Tryptophan	0	0
	Hydrocortisone	7.3	0
	Hydrocortisone and tryptophan	18	46

Each experiment utilized one litter (4 days old at the beginning of the experiment) divided into three groups; the three or four livers within each group were pooled. Tryptophan was always injected 5 hr before assay. In Expts. 1 and 2, hydrocortisone acetate was injected 29 hr before assay, i.e., 24 hr before tryptophan. In Expts. 3 and 4, hydrocortisone was injected 4 days before tryptophan.

formation of the enzyme (Table 2). If actinomycin D is given just before hydrocortisone, the subsequent injection of tryptophan 24 hr later evokes no tryptophan oxygenase. But if actinomycin D is given 21 or 25 hr after hydrocortisone (i.e., 3 hr before tryptophan or with the tryptophan), there is no significant inhibition. Partial inhibition is seen if actinomycin D is given 17 hr before tryptophan, which suggests that the RNA-dependent preparatory action of hydrocortisone requires some hours to be fully accomplished. This is consistent with the observation that tryptophan is not fully effective if administered too soon after the hydrocortisone (see Fig. 2).

DISCUSSION

In previous studies, neither adrenalectomy nor actinomycin D treatment at the age of 14 days inhibited the subsequent accumulation of tryptophan oxygenase in rat liver; it was concluded (9) that the developmental formation of tryptophan oxygenase was not under glucocorticoid control but was regulated by the "substrate-type" mechanism similar to that operating in the tryptophan induction of the enzyme in adult liver (10). The present experiments suggest that this interpretation is valid only for the later period during which the synthesis of the enzyme is already in progress. The preparation for the synthesis does in fact involve glucocorticoid action since adrenalectomy, performed not later than the eleventh postnatal day, inhibits the developmental formation of tryptophan oxygenase. Emergence of tryptophan oxygenase in normal rats does not coincide with the establishment of pituitary-adrenal activity around the time of birth, but with the secondary rise in this endocrine function which occurs around the tenth postnatal day, after a latency period of some 7 days (11). That glucocorticoid alone does not evoke tryptophan oxygenase prematurely further indicates that this hormone is a necessary, but not sufficient, cause for the appearance of the enzyme.

TABLE 2. *Effect of actinomycin D on the premature evocation of tryptophan oxygenase*

HC	Time of administration of		Tryptophan oxygenase activity (units/g liver)
	Actino. (hr before assay)	Trp	
29	—	5	67.7 ± 7
29	5	5	67.0 ± 5.2
29	8	5	60 ± 2.8
29	17	5	41.4 ± 3.2
29	29.5	5	7.7 ± 1.1

Each rat was injected, at the age of 4 days, with hydrocortisone acetate (HC), and 24 hr later, with L-tryptophan (Trp). When indicated, actinomycin D (actino.) was injected with tryptophan (second horizontal line), or shortly before hydrocortisone (last horizontal line), or at intermediate times. Each value is a mean (± SE) of results with 7–10 rats.

The synthesis of tryptophan oxygenase requires additional factors, apparently absent in the fetus and the neonate. In the premature induction experiments, tryptophan is seen to be the additional factor required. There is no evidence as yet that a rise in liver tryptophan concentration normally follows the restarted pituitary-adrenal activity after the tenth postnatal day so as to produce the natural emergence of tryptophan oxygenase. Broadening of the daily oscillations in tryptophan concentration could also have this effect.

The adult concentration of tryptophan oxygenase normally appears in the third postnatal week. The same level can be evoked experimentally as early as 4 days after birth by the proper sequence of factors which normally act only later. There is a crucial difference between the prematurely and the normally emerging enzyme. The concentration of the prematurely evoked enzyme subsides unless the administration of stimuli is continued, whereas the enzyme appearing at the usual time persists thereafter for the life of the animal. Single steps of enzymic differentiation do not appear to be in themselves irreversible.

The normal or induced developmental emergence of several enzymes has been inhibited by the administration of actinomycin D. Since this agent inhibits the synthesis of most RNA species and has a number of harmful side effects, such experiments do not prove that the appearance of the enzyme is the immediate consequence of the synthesis of its template RNA or that the triggering stimulus acted at the level of DNA → RNA. However, a negative result, a lack of inhibition by actinomycin D, is conclusive; it means that concomitant RNA synthesis is not required and the enzyme was synthesized on already available templates. Since there is a widespread desire to equate enzyme synthesis with messenger RNA synthesis, developmental rises in enzyme activity that were actinomycin-insensitive may have gone unreported because they were considered not to reflect enzyme synthesis *de novo*. With tryptophan oxygenase, which can be titrated with anti-serum, *de novo* synthesis is directly observable. In the premature-evocation experiments, the early actinomycin-sensitive step (effected by hydrocortisone) can be clearly separated from the period of actual enzyme synthesis (triggered by tryptophan), which is not sensitive to this inhibitor. The effect of hydrocortisone (which may be that of initiating template RNA synthesis) is long-lasting: even several days after

its administration it is possible to evoke tryptophan oxygenase synthesis with an injection of tryptophan. Such observations emphasize that, although the actual appearance of a new enzyme in the course of differentiation tells us that the corresponding gene has been "turned on", it does not tell us *when* this has happened. The initiation of template RNA synthesis does not necessarily result in enzyme accumulation; subsequent stimuli—cytoplasmic factors that are effective without concomitant RNA synthesis—may determine the time at which the tissue actually acquires the new enzyme.

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