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STEROIDS AND THE MATURATION OF RAT TISSUES

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SUMMARY

In normal developing rats there are two periods when glucocorticoids exert important influences upon hepatic chemical differentiation: new enzymes appear in liver after the late fetal and also after the postnatal (2nd week) upsurge of plasma corticosterone. Cortisol, administered prior to the natural increase in endogenous corticosterone, induces these enzymes before the scheduled time. Such interference also causes premature losses in enzymes (in various organs) which appear to be more important to growth *per se* than to tissue-specific metabolic functions.

Current hypotheses about the mechanism of hormone action are inadequate to explain the fact that glucocorticoids, even in the same tissue, induce different enzymes at different stages of development. Several observations suggest that the competence of different synthetic systems to respond to a hormonal trigger develops in distinct steps, under the impact of additional, specific stimuli.

INTRODUCTION

During the last 5 or 6 years investigations in this laboratory have concentrated on identifying stimuli, in particular endocrine factors, which trigger the emergence of hepatic enzymes in the course of fetal and postnatal development [1–3]. Much of the data presented here is concerned with the role of glucocorticoids in the maturation of enzyme patterns in rat organs. The implication of the results to mechanisms which underlie the scheduled synthesis of specific proteins during differentiation will be discussed.

EXPERIMENTAL

The rats used in most of these experiments were of the albino inbred Kx or CDF strain. They were time-mated (involving a 24 h uncertainty); fetal body weights were used as additional criteria of conceptual age. Adult rats were 60- to 90-day-old males. All injections were intraperitoneal; fetal rats were injected through the uterine walls of the anesthetized, laparotomized dam. Cortisol (hydrocortisone acetate) was suspended in 0·9% NaCl; control rats received the vehicle.

Previously published methods [9, 11–15, 17–19] were used for assaying enzymes in freshly excised tissues, under conditions (optimal substrate concentrations, etc.) where the enzyme amount alone is limiting. Activities, units (μmol/min) per g tissues, are usually given as a percentage of those found in the cognate adult organ.

RESULTS AND DISCUSSION

In attempting to identify hormones responsible for stimulating the emergence of a given enzyme during normal development we use two simple criteria. One is that an increased secretion of that hormone should occur shortly before the "spontaneous" emergence of the enzyme in question. The other criterion is that the administration of the suspected hormone to the intact, fetal or postnatal animal should evoke the emergence of the appropriate enzyme *before* the scheduled time. Both criteria were satisfied in studies of the role of glucagon in the synthesis of hepatic enzymes of the neonatal cluster (see solid line 3 in Fig. 1): in developing rats the secretion of this hormone begins immediately after birth [4] and several enzymes which normally appear on the first neonatal day can be prematurely evoked in fetal rats, by injecting them with glucagon *in utero* [1, 2]. Investigations of the role of several other hormones (*e.g.* growth hormone, estrogen, etc.) is complicated by the fact that little quantitative information is available about their concentration, as a function of age, in the plasma of experimental animals. The fact that fetal rats begin to secrete glucocorticoids on about the 16th day of gestation [5], and that pituitary-adrenocortical activity rises again after the 10th postnatal day [6], has been known for some time. Recent quantitative information about circulating corticosterone in rats at various stages of development [8, 9] is illustrated by the broken line in Fig. 1. Unfortunately, the fetal [7] and the postnatal values [8] have been obtained in two different laboratories and their absolute values are not entirely comparable. It is clear, however, that increased corticosterone secretion precedes the emergence of both the late fetal and the late suckling cluster of enzymes (symbolized by solid lines 2 and 4 in Fig. 1). Thus, according to our first criterion, enzymes synthesized in response to endogenous glucocorticoid must be among those of the late fetal and late suckling cluster. Several enzymes within these two clusters fulfilled our second criterion: they were prematurely induced by cortisol administration (see example in Fig. 2). Alternate enzymes in these clusters responded to thyroxine

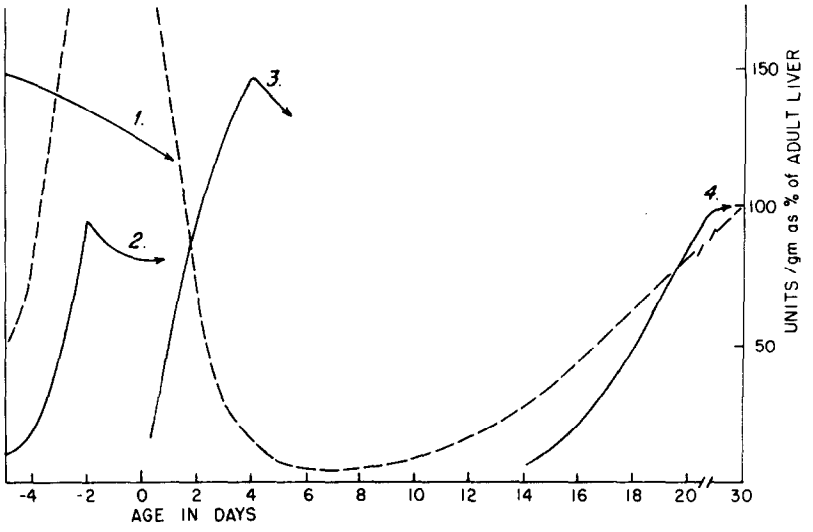

Fig. 1. Changes in plasma corticosterone levels and the evolving enzymic composition of rat liver. Values for plasma corticosterone concentration (broken line), as percent of that in 30-day-old rats, are from Cohen[7] for fetal rats and from Taylor and Howard[8] for postnatal rats. Each solid line (activity as percent of the adult value) stands for a cluster of enzymes; they symbolize decreasing concentrations (line 1) or emergence during late fetal (line 2), neonatal (line 3) or late suckling life (line 4).

alone [9]; for the synthesis of these, thyroid activity (which also undergoes a biphasic change [10]) rather than the coincident increase in adrenocortical secretion must be considered as the natural signal. Enzymes of the neonatal cluster conform to our criteria in a negative sense: the upsurge of these enzymes do not coincide with increased glucocorticoid (or thyroid hormone) secretion and none could so far be prematurely induced by the administration of cortisol (or thyroxine).

The role of glucocorticoids in the development of enzymes of the late suckling cluster is particularly well documented in the case of ornithine aminotransferase (E.C. 2.6.1.13, L-Ornithine: 2-oxo-acid aminotransferase): its normal rise is prevented by adrenalectomy on day 10 and an injection of cortisol could evoke it at least 1 week before the scheduled time (Fig. 2). And yet, there was no rise at all in the level of this enzyme before birth, even upon the administration of large doses of cortisol. The major, general question which emerges from such observations may be formulated as follows:

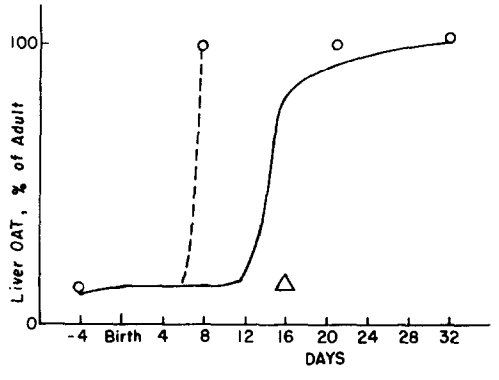

Fig. 2. Induced alterations in the developmental formation of hepatic ornithine aminotransferase. The solid line depicts the normal developmental formation of the enzyme. Open circles (and broken line) show the levels obtained 18 h after the administration of 0·25 mg cortisol per 10 g body weight. The triangle refers to rats adrenalectomized at the age of 10 days. For details and reproducibility of results see Ref. [11].

If indeed corticosterone triggers the synthesis of certain enzymes of the late suckling cluster, why did these enzymes not appear during fetal life in response to the first, even more extensive outpour of corticosterone? A further difficulty is presented by observations of reverse phenomena: there are enzymes whose responsiveness to cortisol is much *greater* in fetal than in mature liver. Clearly, age-dependent variations in the competence to respond to cortisol cannot be related to receptor availability unless we postulate the existence of many separate (and apparently enzyme-specific) receptors with independently waning and waxing concentrations. A more promising postulate is that inducibility of a given enzyme by cortisol is conditioned by additional specific regulators (hormones or other small molecules) of the synthetic machinery. For example, the hepatic enzyme, tyrosine aminotransferase (E.C. 2.6.1.5, L-Tyrosine: 2-oxoglutarate aminotransferase), is cortisol-inducible throughout postnatal life [12] but in the fetus, only glucagon can evoke it [1]. However, after the application of the natural developmental trigger, the capability to respond to cortisol appears already before birth [3]. In this instance, one hormone prepared the specific synthetic system to respond to another. Other examples of changing competence are exhibited by glucokinase (E.C. 2.7.1.2, ATP: D-glucose 6-phosphotransferase) and tryptophan oxygenase [E.C. 1.13.11.11, L-Tryptophan:oxygen 2,3-oxidoreductase (decyclizing)], two enzymes of the late suckling cluster (Fig. 3). The facts that: (a) adrenalectomy inhibited

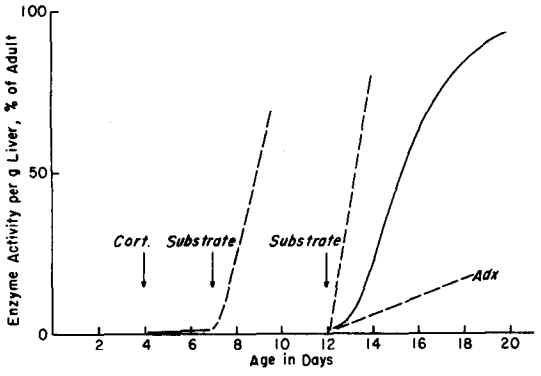

Fig. 3. Premature enzyme induction by sequential treatment with two stimuli. The solid line illustrates the similar developmental emergence of two enzymes, tryptophan oxygenase and glucokinase. Adrenalectomy on day 10 inhibits (see broken line with "Adx") and substrate (tryptophan and glucose, respectively) given on day 12 enhances their development. In younger rats cortisol alone (see first arrow) does not evoke these enzymes but after this pretreatment an injection (at the second arrow) of glucose or tryptophan will result in the precocious rise of glucokinase or tryptophan oxygenase, respectively. For detailed data see Refs. [13] and [14].

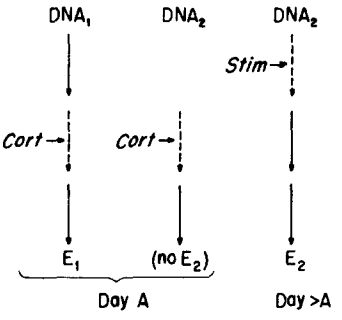

Fig. 4. A scheme to explain the age-dependent evocation of different enzymes by the same hormone. For details, see text.

the development of these enzymes; but (b) cortisol did not induce it prematurely, suggested that the glucocorticoid was a necessary but not a sufficient factor. Premature evocation required sequential treatment with cortisol and substrate (*i.e.* glucose and tryptophan, respectively). If one waits until the time (12th day) of the natural increase in glucocorticoid secretion both enzymes are inducible with substrate alone. Thus, in the normal course of development at least two changes must occur to promote the synthesis of these enzymes, an endocrine and a metabolic one. In general terms then, the capacity to express a given gene awaits not one but a series of signals, which promote the various reactions involved in the complete synthesis of a specific protein or a small group of specific proteins. In Fig. 4 the many reactions between DNA and the finished gene product are arbitrarily divided into three groups (see arrows). Let us assume that cortisol can promote one of the reactions within the indicated group. The synthesis of $Enzyme_1$ can thus be initiated on Day A by cortisol because all other necessary reactions are already operative (see solid arrows) at this age. However, the synthetic machinery for $Enzyme_2$ still lacks another potential (arrow absent) and thus only at a later age (Day > A), after the impact of another stimulus, could its appearance be triggered by cortisol. If Day A is about the 17th day of gestation, $Enzyme_1$ could exemplify members of the late fetal cluster whose synthesis starts immediately after the beginning of fetal corticosterone secretion. $Enzyme_2$, exemplifying appropriate members of the late suckling cluster, normally awaits the second, endogenous upsurge of corticosterone secretion, that beginning on the 10th postnatal day. Since administered cortisol can induce these enzymes already on the 2nd postnatal day, we know that the additional stimulus (Stim) acted at or shortly after term. This knowledge of "timing" should facilitate our efforts towards identifying the nature of this additional stimulus, which promotes the competence of certain synthetic systems to respond to the glucocorticoid.

It is well known that the same enzyme in different tissues of the adult organism is very often not inducible by the same hormone. This is, of course, also true for immature tissues. In fact, there has been little success in enhancing chemical differentiation in tissues other than liver and intestine. We found only two renal enzymes so far which responded to hormone treatment. Ornithine aminotransferase exhibited a premature upsurge in kidney in response to estradiol [11]. Cortisol induced aspartate aminotransferase (E.C. 2.6.1.1, L-Aspartate:2-oxoglutarate aminotransferase) in both liver [15] and kidney; however, the renal response required a much higher dose of cortisol and a much longer period. As shown in Table 1, in normal rat kidney much of the enzyme accumulates after the second postnatal week whereas in kidneys of rats given cortisol at birth a considerable increase could be seen by day 7. However, because

Table 1. The cortisol induction of renal aspartate aminotransferase

Time after cortisol administration	Enzyme activity (μmol/min/g kidney) 1 day	7 day	Adult
Control	10 ± 1·5 (4)	13·5 ± 4 (4)	33 ± 12·0 (3)
7		29·3 ± 3 (4)	39 ± 8·9 (4)

The soluble isozyme of aspartate aminotransferase was assayed as previously described [15] in kidneys of rats without (Control) and after the intraperitoneal injection of 1·25 mg cortisol per 10 g body weight. The results are means ± S.D. with the number of animals in parentheses.

Table 2. Cortisol-enhanced decreases in enzyme activity

Enzyme	Fetal tissue	Enzyme activity (% of adult) Control	Cortisol
Peptidyl proline hydroxylase	Liver	320	150
Peptidyl proline hydroxylase	Lung	270	125
Thymidine kinase	Liver	670	330
Phosphoserine phosphatase	Liver	311	205

Enzyme activities (units per g tissue) in fetuses of the 18th or 19th day of gestation are expressed as percent of activities found in the corresponding adult tissues. "Cortisol" refers to fetal rats (17th to 18th gestational day) which had received an intraperitoneal injection of 0·12 mg cortisol *in utero* 1 day before assay. For detailed studies on these three enzymes see Refs. [17, 18, 19], respectively.

of the large dose or cortisol required, these experiments do not prove that the "spontaneous" rise of renal aspartate aminotransferase during late suckling life is due to the endogenous increase in glucocorticoid secretion after the 12th postnatal day. It is possible that in kidney the postnatal accumulation of this enzyme is a delayed consequence of prenatal glucocorticoid action. Even so, there is a noteworthy, obvious change during postnatal life: in *adult* kidney aspartate aminotransferase did not respond to cortisol (Table 1). Thus, this renal enzyme like several hepatic enzymes (see Fig. 2 and Ref. [2]) which are responsive to cortisol during early postnatal life does not respond to the same dosage in the more mature animal.

In the experiments of Table 1, cortisol, given shortly after birth, at the ontogenetically least appropriate time (Fig. 1), inhibited the subsequent growth of the rats. In fetal rats, although growth was normal within the 1- or 2-day experimental period, cortisol exerted some negative effects on enzymes in several tissues. The group of enzymes of interest here is one whose concentrations decrease during late fetal or early postnatal life (symbolized by solid line 1 in Fig. 1). These are usually not organ specific and are probably necessary for rapid tissue growth. As seen in Table 2, the concentration of peptidyl proline hydroxylase in liver and lung of the normal fetus was about 3 times higher than that of the corresponding adult tissues, but in fetuses (of the same age) given an injection of cortisol one day before assay, the enzyme decreased to almost adult levels. The levels of thymidine kinase (E.C. 2.7.1.75, ATP: thymidine 5′-phosphotransferase) and phosphoserine phosphatase (E.C. 3.1.3.3, *O*-Phosphoserine phosphohydrolase) were also significantly higher (2- and 1·5-fold) in control than in cortisol-treated fetuses. Thus, the same cortisol treatment, which evoked the premature development of enzymes necessary for certain specialized functions, induced the premature loss of an alternate group of enzymes. This dual role of glucocorticoids has also been observed when studying the enzymic maturation of small intestine around the third postnatal week [16]. One must, therefore, conclude that both the initial secretion of corticosterone in the fetus and the second rise after day 10 trigger: (a) the synthesis of several enzymes with tissue-specific metabolic functions; and (b) the loss of enzymes that are more important to the growing than to the adult animal. Insight into the manner in which the two, apparently simultaneous actions are linked to each other would greatly deepen our understanding of the mechanism of differentiation and its regulation by glucocorticoids.

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