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The Hormonal Regulation of Enzymes in Prenatal and Postnatal Rat Liver

EFFECTS OF ADENOSINE 3′,5′-(CYCLIC)-MONOPHOSPHATE

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1. The administration of glucagon, cAMP [adenosine 3′,5′-(cyclic)-monophosphate], BcAMP [6-*N*-2′-*O*-dibutyryladenosine 3′,5′-(cyclic)-monophosphate] or adrenaline to foetal rats during the last 2 days of gestation evoked the appearance of tyrosine aminotransferase and enhanced the accumulation of glucose 6-phosphatase in the liver. In foetuses 1–2 days younger only BcAMP was effective. After birth liver glucose 6-phosphatase no longer responds to glucagon or BcAMP. Tyrosine aminotransferase is still inducible by these agents in 2-day-old rats, but not in 50-day-old rats. After adrenalectomy of adults glucagon or BcAMP can enhance the induction of the enzyme by hydrocortisone. The results indicate that the ability to synthesize tyrosine aminotransferase and glucose 6-phosphatase when exposed to cAMP develops sooner than the ability to respond to glucagon with an increase in the concentration of cAMP; the responsiveness of enzymes to different hormones changes with age. A scheme illustrating the sequential development of competence in regulating the level of an enzyme is presented. 2. Actinomycin inhibited the effects of glucagon and BcAMP on liver tyrosine aminotransferase and glucose 6-phosphatase in foetal rats. Growth hormone, insulin and hydrocortisone did not enhance the formation of these enzymes. 3. The time-course of accumulation of glucose 6-phosphatase in the kidney is different from that in the liver. Hormones that increase the accumulation in foetal liver do not do so in the kidney of the same foetus or in the livers of postnatal rats.

Previous studies from our laboratory have shown that the administration of glucagon to foetal rats evokes the appearance of tyrosine aminotransferase (EC 2.6.1.5) and enhances the formation of glucose 6-phosphatase (EC 3.1.3.9) in foetal liver (Greengard & Dewey, 1967). In the present investigation adrenaline, growth hormone, insulin and thyroxine were tested for similar effects on enzymic differentiation in foetal liver and also kidney. An explanation was sought for the ability of glucagon to evoke tyrosine aminotransferase in livers of foetuses during, but not before, the last 2 days of gestation. cAMP,* as briefly reported (Greengard, 1969), appears to be involved in the process whereby glucagon and adrenaline promote the developmental formation of glucose 6-phosphatase and tyrosine aminotransferase and can evoke these enzymes earlier. After birth the same agents no longer regulate glucose 6-phosphatase; their influence on tyrosine aminotransferase also diminishes with age and is modified by the endocrine state of the adult animal.

* Abbreviations: cAMP, adenosine 3′,5′-(cyclic)-monophosphate; BcAMP, 6-*N*-2′-*O*-dibutyryladenosine 3′,5′-(cyclic)-monophosphate.

MATERIALS AND METHODS

Rats were of the Sprague–Dawley CD strain obtained from Charles River Breeding Laboratories, Wilmington, Mass., U.S.A. The technique of foetal injections and the enzyme assays were as described by Greengard & Dewey (1967, 1968). Enzyme activities are expressed in units (1 μmole of product formed/hr. at 25°)/g. wet wt. of tissue. The sources of substances were as follows: hydrocortisone acetate, Merck Sharp & Dohme, West Point, Pa., U.S.A.; growth hormone (0·5 USP unit/mg., Raben type), Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A.; thyroxine, cAMP and BcAMP, Calbiochem, Los Angeles, Calif., U.S.A.; glucagon and insulin (Iletin), Eli Lilly and Co., Indianapolis, Ind., U.S.A.; adrenaline (epinephrine–HCl), Parke Davis & Co., Detroit, Mich., U.S.A. Substances to be injected into foetal rats were dissolved or suspended in 0·1 ml. of 0·9% NaCl soln.; controls received the vehicle only. The doses of hydrocortisone acetate, cAMP, thyroxine, growth hormone, glucagon and actinomycin D were 0·125,

0·5, 0·003, 0·25, 0·05 and 0·01 mg./foetus respectively; the amount of insulin injected was 0·005 unit. The dose of BcAMP (or AMP) for foetuses above and below 25 mm. body length was 0·25 and 0·125 mg. respectively. Postnatal rats received intraperitoneally 2·5, 0·25 and 2·5 mg. of BcAMP, glucagon and hydrocortisone succinate respectively/100 g. body wt.

RESULTS

A series of agents were tested for an effect on the levels of liver tyrosine aminotransferase, glucose 6-phosphatase and NADPH dehydrogenase (EC 1.6.99.1) in foetal rats 1–2 days before term. Table 1 shows that 5 hr. after an injection of adrenaline, cAMP or BcAMP, foetal livers exhibited significant tyrosine aminotransferase activity, and that their glucose 6-phosphatase activity was doubled. BcAMP, which persists in the tissues longer (Pasternak, Sutherland & Henion, 1962), was more effective than cAMP. In contrast, AMP, insulin and growth hormone were without effect. The NADPH dehydrogenase activity that was raised by thyroxine was not raised by BcAMP.

The experiments of Table 2 tested whether uninhibited RNA synthesis is required for the induced rises in the activities of tyrosine aminotransferase and glucose 6-phosphatase in foetal liver. The results show that the administration of actinomycin D prevented the effects of glucagon and of BcAMP on tyrosine aminotransferase and glucose 6-phosphatase. This agent also inhibited the thyroxine-induced rise in the activity of glucose 6-phosphatase.

Table 3 compares the effect of glucagon, adrenaline and BcAMP on liver glucose 6-phosphatase in rats at different stages of development. In foetal rats 3–4 days before term, the basal level of 7·5 units, and the 12·3 units reached on an injection of glucagon, represent small increases over the substrate-free blank assay values of about 7 units. However, the effect of BcAMP at this age, resulting

Table 1. *Induced accumulation of enzymes in foetal rat liver 1–2 days before term*

The indicated substances were administered to individual foetuses intraperitoneally 5 hr. before assay unless otherwise indicated. Within each litter some foetuses served as controls (saline). Enzyme activities are expressed as means ± S.D. of the numbers of observations given in parentheses. —, Not determined.

	Enzyme activities (units/g. wet wt.)		
Substance injected	Glucose 6-phosphatase	Tyrosine aminotransferase	NADP dehydrogenase
Saline	38 ± 10 (11)	< 2 (30)	56 ± 10 (13)
Adrenaline	85 ± 9 (4)	11·8 ± 5 (4)	—
cAMP	79 ± 8 (4)	5·5 ± 3 (4)	—
BcAMP	95 ± 5 (4)	15·2 ± 6 (12)	45 ± 2 (6) (5 or 24 hr.)
BcAMP	40 ± 9 (4) (1 hr.)	< 2 (6) (1 hr.)	—
AMP	39 ± 3 (3)	< 2 (3)	—
Insulin	42 ± 9 (6)	< 2 (4)	—
Growth hormone	30 ± 6 (4)	< 2 (5)	—
Thyroxine	99 ± 11 (9)	< 2 (11)	110 ± 19 (18) (24 hr).

Table 2. *Effect of actinomycin D on induced prenatal enzyme formation in rat liver*

Within each of two pregnant rats (about 1 day before term) some foetuses were injected with saline, some with glucagon and some with actinomycin D plus glucagon. Four other litters were treated similarly, but instead of glucagon appropriate foetuses of two litters received BcAMP and those of the other two litters received thyroxine. The substances were injected 5 hr. before assay. Enzyme activities are expressed as means ± S.D. of the numbers of observations given in parentheses. —, Not determined.

	Enzyme activities (units/g. wet wt.)			
	Tyrosine aminotransferase		Glucose 6-phosphatase	
Substance injected	Without actinomycin	With actinomycin	Without actinomycin	With actinomycin
Saline	< 2 (10)	< 2 (6)	57 ± 7 (10)	49 ± 9 (6)
Glucagon	25 ± 3 (5)	2·8 ± 1 (5)	99 ± 8 (5)	53 ± 12 (5)
BcAMP	15 ± 4 (9)	< 2 (6)	95 ± 11 (7)	46 ± 9 (6)
Thyroxine	—	—	88 ± 6 (4)	46 ± 7 (7)

Table 3. *Effect of developmental age on the induced rise in glucose 6-phosphatase activity in rat liver*

The indicated substances were administered once. Each rat of the indicated age received one injection of the substance shown 5hr. before assay. The ranges of the crown–rump body length of foetuses for the experiments in the first three columns were 20–25, 26–36 and 37–40mm. respectively. Enzyme activities are expressed as means ± S.D. of the numbers of observations given in parentheses. —, Not determined. Significance of results: *$P<0.2$; †$P<0.01$.

	Glucose 6-phosphatase activity (units/g. wet. wt.)			
Time before (−) or after (+) birth (days)	−3–4	−2	−1	+2
Substance injected				
Saline	7·5±3 (5)*†	27±8 (6)	38±10 (9)	401±33 (6)
Glucagon	12·3±7 (6)*	50±7 (5)	84±4 (6)	399±52 (4)
Adrenaline	—	47±8 (8)	85±9 (4)	352±52 (4)
BcAMP	18·7±6 (7)†	45±9 (4)	95±5 (4)	330±20 (3)

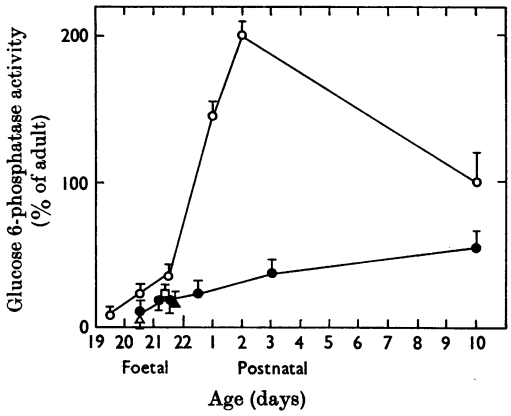


Fig. 1. Glucose 6-phosphatase in developing rat liver and kidney. Enzyme activities are expressed as percentages of those in adult males, 285 and 261 units/g. of liver and kidney respectively. The values are means of results with 10–30 individual livers (○); for postnatal kidneys (●) they are means of measurements of four or five pairs of kidneys. Each point for prenatal kidney represents a mean of assays of three pools of kidneys (six pairs each) from foetuses injected with saline (●) and their litter mates injected with thyroxine (△), glucagon (▲) or BcAMP (□) 5hr. before assay.

in 18·7 units of glucose 6-phosphatase, is highly significant. In the next two age groups (2 and 1 days before term) the basal level is higher and glucagon, BcAMP and adrenaline were equally effective in doubling it. After birth the glucose 6-phosphatase activity rises rapidly, reaching higher-than-adult values on the second postnatal day (see also Fig. 1). In these animals (or, in adults) glucagon, adrenaline and BcAMP caused no further rise in activity (Table 3).

Glucose 6-phosphatase begins to accumulate during late foetal life in kidney, but more slowly than in liver. Thyroxine, glucagon or BcAMP did not enhance its accumulation (Fig. 1). In agreement with an earlier report (Kretchmer, 1959) the activity of glucose 6-phosphatase in the kidney rises slowly after birth and it does not exhibit the precipitous postnatal rise seen in the liver.

The experiments of Table 4 illustrate the responsiveness of liver tyrosine aminotransferase to different agents as a function of age. At 3–4 days before term only BcAMP could evoke the appearance of the enzyme. This rise to 5–6 units is highly significant, since the basal activity as well as the blank values in this assay are less than ·2 units. At 2 days before term glucagon and BcAMP were equally effective, and at 1 day before term the highest activities were found in foetuses injected with glucagon. Adrenaline evoked less tyrosine aminotransferase activity at 2 days than 1 day before term.

In untreated rats tyrosine aminotransferase appears after birth, reaching higher-than-adult values on the first day (Sereni, Kenney & Kretchmer, 1959). On the second day the level is still high (see Table 4) and the ensuing slight fall is followed by a slow rise to the adult value (Franz & Knox, 1967). In 2–5-day-old rats glucagon or BcAMP can cause over sixfold rises in activity. In 50-day-old rats glucagon no longer has a significant effect and the small rise caused by BcAMP may be an indirect effect due to stimulation of the pituitary–adrenocortical axis. The last line in Table 4 illustrates the previously noted lack of effect of hydrocortisone in foetal rats (Sereni *et al.* 1959; Greengard & Dewey, 1967) and contrasts it with the ability of this hormone to induce tyrosine aminotransferase in postnatal rats, both young and adult.

Previous studies showed that in adrenalectomized

Table 4. *Effect of developmental age on the induced rise in tyrosine aminotransferase activity in rat liver*

Experimental details are similar to those indicated in Table 3. Enzyme activities are expressed as means ± S.D. of the numbers of observations given in parentheses. —, Not determined. Significance of results:* $P < 0.01$.

	Tyrosine aminotransferase activity (units/g. wet. wt.)					
Time before (−) or after (+) birth (days)	−3–4	−2	−1	+2	+5	+50
Substance injected						
None	< 2 (20)*	< 2 (24)	2 (30)	59 ± 15 (6)	32 ± 11 (4)	50 ± 9 (6)
Glucagon	< 2 (8)	9·6 ± 4 (6)	30·0 ± 5 (15)	334 ± 40 (4)	269 ± 37 (4)	66 ± 15 (6)
Adrenaline	—	4·4 ± 1 (5)	11·0 ± 5 (4)	—	—	—
BcAMP	5·9 ± 1·4 (10)*	11·4 ± 2 (6)	15·2 ± 6 (12)	305 ± 42 (3)	—	110 ± 15 (3)
Hydrocortisone	< 2 (9)	—	< 2 (14)	—	239 ± 25 (4)	175 ± 54 (6)

Table 5. *Effect of hydrocortisone, glucagon and BcAMP on tyrosine aminotransferase activity in adult rat liver*

The indicated substances, alone or in combination, were injected 5 hr. before assay. Enzyme activities are expressed as means ± S.D. of the numbers of observations given in parentheses.

	Tyrosine aminotransferase activity (units/g. wet wt.)	
Substance injected	Adrenalectomized rat	Intact rat
None	42 ± 3 (20)	60 ± 9 (6)
Hydrocortisone	220 ± 17 (7)	175 ± 54 (6)
Glucagon	107 ± 22 (6)	66 ± 15 (6)
BcAMP	97 ± 34 (5)	110 ± 15 (3)
Glucagon + hydrocortisone	419 ± 20 (5)	169 ± 39 (6)
BcAMP + hydrocortisone	369 ± 36 (4)	185 ± 21 (3)

adult rats the induction of tyrosine aminotransferase by hydrocortisone can be greatly enhanced by the simultaneous administration of glucagon (Greengard & Baker, 1966) or adrenaline (Reshef & Greengard, 1969). BcAMP in adrenalectomized adult rats raised the activity of tyrosine aminotransferase from 42 to 97 units (Table 5). The administration of BcAMP together with hydrocortisone resulted in 369 units of activity as opposed to 220 units obtained with hydrocortisone alone. This synergism between hydrocortisone and BcAMP is the same as with glucagon and is not seen in intact rats (Table 5).

DISCUSSION

A series of observations indicate that the effectiveness of agents that promote the developmental formation of an enzyme varies with age and with the metabolic state or nature of the organ. In foetal rats 3–4 days before term only BcAMP could evoke the formation of liver tyrosine aminotransferase and glucose 6-phosphatase whereas in the last 2 days of gestation these effects could also be obtained with glucagon and adrenaline. Agents that promoted the formation of glucose 6-phosphatase in foetal liver did not do so in kidneys of the same foetuses and did not raise the glucose 6-phosphatase activity in postnatal livers. Insulin, growth hormone and hydrocortisone did not enhance the prenatal formation of tyrosine aminotransferase and glucose 6-phosphatase, although the latter hormone is an effective inducer of both in postnatal rats (Knox & Auerbach, 1955; Weber, Singhal, Stamm, Fisher & Mentendiek, 1964). Explants of foetal liver cultured *in vitro* are different from both foetal and normal postnatal liver *in vivo* in that their tyrosine aminotransferase activity can be raised by glucagon, insulin and cAMP as well as by hydrocortisone (Wicks, 1968*a*,*b*). Adrenalectomy changes the adult rat so that an injection of glucagon or BcAMP enhances the induction of tyrosine aminotransferase by hydrocortisone (Table 5).

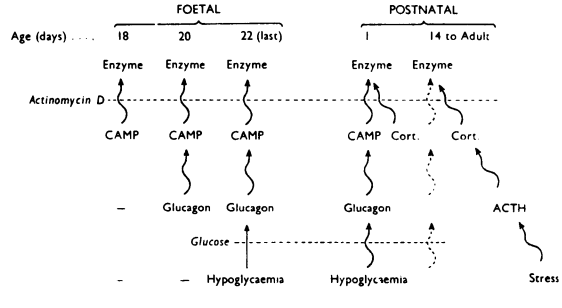
The developmental formation of liver glucose 6-phosphatase consists of distinct prenatal and neonatal phases, which are reproducible in foetal liver by the separate effects of either glucagon (and also adrenaline and cAMP) or thyroxine (Greengard & Dewey, 1968). The other two enzymes studied present a simpler picture. Tyrosine aminotransferase is evoked specifically by glucagon (or adrenaline or cAMP). NADPH dehydrogenase responds specifically to thyroxine. Thyroxine does not evoke tyrosine aminotransferase, and BcAMP (or glucagon) does not affect NADPH dehydrogenase. These results are consistent with the possibility that glucagon (or adrenaline) exerts its action on tyrosine aminotransferase and glucose 6-phosphatase through the mediation of cAMP. On the other hand, thyroxine does not exert its

action on NADPH dehydrogenase through cAMP (since BcAMP is without effect on this enzyme) and does not appear to raise the cellular concentration of cAMP (since it does not evoke tyrosine aminotransferase). Glucagon, cAMP and BcAMP do not affect the activity of tyrosine aminotransferase or glucose 6-phosphatase *in vitro*; the rises in enzyme activity induced by these agents *in vivo* require more than an hour and are inhibited by actinomycin D. Thus it is reasonable to assume that the actions of glucagon and BcAMP now studied do not consist of activations of existing enzyme molecules but increases in their amounts.

The appearance of an enzyme in the normal course of differentiation must be preceded by a series of events that permit the function of the corresponding gene; subsequent changes with age may eliminate the responsiveness of the enzyme to certain stimuli and enable it to respond to new regulators. Scheme 1, illustrating these sequential events, is based on experience with tyrosine aminotransferase, but is also relevant (except for the portion relating to adult animals) to a group of enzymes that include serine dehydratase, glucose 6-phosphatase and phosphoenolpyruvate carboxylase. The common features of these enzymes are that their upsurge is brought about by premature delivery (Dawkins, 1961; Holt & Oliver, 1968; Yeung & Oliver, 1968*a*), that the normal postnatal rise can be inhibited by the administration of glucose (Greengard & Dewey, 1967; Dawkins, 1963; Yeung & Oliver, 1968*b*) and that they can be prematurely induced in foetal liver by glucagon, adrenaline or cAMP (Greengard, 1969; Yeung & Oliver, 1968*b*).

In Scheme 1 the arrows do not imply mechanism, but indicate a competence to respond to the stimulus at the origin of the arrow with an increase in the amount of the substance at the point of the arrow. For the purposes of this scheme BcAMP is considered equivalent to cAMP. The competence to synthesize tyrosine aminotransferase under the influence of cAMP is present 4 days before birth since its injection evokes the enzyme at this age. During the next day or so the capacity develops to raise the concentration of cAMP significantly on exposure to glucagon or adrenaline. Thus the enzyme can be evoked by these hormones as well as by cAMP. On the last day before birth the organism is also competent to respond to hypoglycaemia by the secretion of these hormones, and therefore premature delivery at this time can evoke the enzyme. Finally, the normal newborn animal begins to synthesize tyrosine aminotransferase because hypoglycaemia can stimulate the secretion of the hormones that raise the concentration of cAMP, a compound that in turn can initiate the synthesis of the enzyme. The broken lines in Scheme 1 illustrate that glucose inhibits the chain of events if given at birth and that actinomycin D inhibits equally well the prematurely induced (Table 2) and normal postnatal (Greengard, Smith & Acs, 1963) accumulation of tyrosine aminotransferase.

Competence in the regulation of tyrosine aminotransferase continues to change after birth. Within a day, while the enzyme is still responsive to glucagon and cAMP, it becomes inducible by hydrocortisone. Two weeks later, when the pituitary–adrenocortical axis is re-established (Levine & Mullins, 1966), adrenocorticotrophic hormone or stress can also induce the tyrosine aminotransferase (Knox & Auerbach, 1955; Schapiro, Yuwiler & Geller, 1966). By this time the glucagon 'pathway' becomes ineffective (Scheme 1, broken arrows) in normal rats, but is still detectable in adrenalectomized rats (see Table 5). As indicated in Scheme 1, the mechanism by which glucocorticoids act is separate from the one involving cAMP. Actinomycin D does not distinguish between the two mechanisms: it inhibits both (Csányi, Greengard & Knox, 1967; see also Table 2). We have no detailed knowledge of the actual reactions implied by each arrow in Scheme 1. The scheme simply illustrates the resolution into sequential steps of the development of competence of the liver to synthesize an enzyme, and to regulate its activity by different physiological factors at different stages of development.



Scheme 1. Sequential development of competence for regulating an enzyme. ACTH, Adrenocorticotrophic hormone; Cort., glucocorticoids.

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