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REGULATION OF HEPATIC TYROSINE AMINOTRANSFERASE IN THE FROG *RANA TEMPORARIA*

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Summary

The regulation of hepatic tyrosine aminotransferase (L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5) in the rat has been extensively studied but little is known about the enzyme from other sources. We have studied the regulation of this enzyme in the frog *Rana temporaria* and in this paper we report that:

1. Cortisone acetate, adrenocorticotropic hormone and α-methyl-*p*-tyrosine, an agent known to induce hepatic tyrosine aminotransferase in the rat via activation of the pituitary-adrenal axis, have no effect on the activity of the enzyme in the frog.
2. Dibutyryl-3′,5′-cyclic AMP induces the enzyme to about 2-fold.
3. Injection of tyrosine methyl ester and a protein-rich diet result in an increase in the enzyme activity. This increase is of the same order of magnitude as that caused by dibutyryl cyclic AMP.
4. Glucose significantly reduces tyrosine aminotransferase activity in frog liver.

These results suggest that cyclic AMP induces the enzyme via a mechanism independent of glucocorticoids. The frog offers a model for studies on the regulation of hepatic tyrosine aminotransferase in vivo without interference from secondary effects mediated by the adrenals.

Introduction

Hepatic tyrosine aminotransferase (L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5) of the rat and its regulation have been subjected to intensive study since the pioneering work of Lin and Knox [1] in which they showed that the enzyme is readily induced by hydrocortisone. In past years numerous other inducers of the enzyme have been reported, e.g. insulin [2], dibutyryl-

3′,5′-cyclic AMP [3] and glucagon [2,4], all of which have been shown to have a direct effect on the liver of the rat [5]. Adrenalin induces the enzyme in cell culture [3].

There are also many "secondary" inducers, the effects of which are mediated by the hormones mentioned above. Glucocorticoids mediate the induction by adrenocorticotropic hormone [6] and by some antiadrenergic drugs [7]. The adrenals are also partly responsible for the effects of tyrosine [8], reserpine and α-methyl-*p*-tyrosine [7]. Tyrosine causes an induction of 50–100% even in adrenalectomized rats [8] and in cell culture [9]; thus it also has a direct effect. α-Methyl-*p*-tyrosine, a well-known catecholamine depletor, induces the enzyme in adrenalectomized [7,10] but not in hypophysectomized rats [11]. Growth hormone is another secondary inducer [5]. Its mechanism of action is unknown.

It has been reported that no induction of the enzyme by glucocorticoids exists below the phylogenetic level of reptiles [12]. This study was undertaken to find out whether the enzyme of the frog is inducible by other effectors. Only dibutyryl cyclic AMP, a protein-rich diet and tyrosine induced hepatic tyrosine aminotransferase in the frog, while glucose was found to decrease the enzyme activity.

Materials and Methods

Animals and tissue preparations

The frogs used in these experiments (average weight 30 g) were provided by Porla Fisheries, Lohja, Finland. They were kept in large containers at +4° C and transferred to similar ones at +18° C for the time of the experiments. Sexes were used unsegregated as preliminary experiments showed that there are no sex differences in tyrosine aminotransferase activity.

The frogs were killed by decapitation and the blood was collected. The livers were removed immediately, rinsed in ice-cold isotonic NaCl solution and homogenized in 4 vol. of 100 mM potassium phosphate buffer, pH 6.8. The homogenates were centrifuged at 10 000 × *g* for 10 min and the resulting supernatant fractions were stored at −20° C for not more than 2 weeks. There was no loss of activity during the storage.

Assays and reagents

Tyrosine aminotransferase was assayed by the method of Diamondstone [13] as described by Granner and Tomkins [14]. The reaction was found to be linear at least 35 min at +37° C. At this temperature the reaction was 4.4 times faster than at +20° C which is near to the body temperature of the frogs during these experiments. Ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17) [15] and tyrosine [16] were assayed as described previously. Protein was estimated by the method of Lowry et al. [17]. Serum glucose was determined by the enzymatic assay kit of Ab Kabi (Sweden). The following drugs and reagents were used: N^6,O^2-dibutyryl adenosine 3′,5′-cyclic monophosphoric acid monosodium salt, 2-deoxyglucose, α-methyl-*p*-tyrosine methyl ester HCl and tyrosine (Sigma), bovine glucagon (Novo), synthetic adrenocorticotropins ACTH-18 (prep. no. C 41795-Ba) and ACTH-24 (tetracosactide; Syn-

acthen, kindly provided by Dr Juhani Jänne from Ciba-Geigy), porcine insulin (Medica, Finland), cortisone acetate (Merck, Sharp and Dohme), tyrosine methyl ester HCl (Fluka) and [1-^{14}C] ornithine (New England Nuclear Corp.).

Results

Firstly, the finding of Chan and Cohen [12] that glucocorticoids are not involved in the regulation of hepatic tyrosine aminotransferase of amphibians was confirmed by testing the effect of adrenocorticotropic hormone on the enzyme. ACTH-18 (two times 5 mg per kg) and ACTH-24 (two times 2.5 mg per kg) were given intraperitoneally 18 and 7 h before killing to six frogs kept at +18° C for 5 days; the control group (six animals) received two intraperitoneal injections of 0.9% saline. Hepatic tyrosine aminotransferase activities (nmol of *p*-hydroxyphenylpyruvate formed per mg of protein per min) were 23.6 (control), 22,0 (ACTH-18) and 20.7 (ACTH-24). Thus, no induction was observed; however, the hormones caused a clear-cut darkening of the skins of the animals.

The effects of a protein-rich diet and injection of the soluble methyl ester of tyrosine were investigated in further experiments (Table I). Feeding with hog liver was found to elevate hepatic tyrosine aminotransferase activity. Tyrosine caused an additive increase; it also raised blood glucose concentration by 110—200% which may be due to gluconeogenesis from this amino acid via the pathway that begins with transamination.

The results summarized in Table I suggest that tyrosine aminotransferase of the frog is closely connected with gluconeogenesis. It is also known that glucose prevents the induction of this hepatic enzyme by hydrocortisone in the rat (the "glucose effect") [18]. Thus it was interesting to see whether the sugar has any effect on hepatic tyrosine aminotransferase of the frog. The experiment performed to investigate this is summarized in Table II. The results show that glucose reduces the enzyme activity to less than half; serum tyrosine

TABLE I

EFFECT OF FEEDING AND INJECTION OF TYROSINE METHYL ESTER ON HEPATIC TYROSINE AMINOTRANSFERASE IN THE FROG

32 frogs were transferred from +4°C to the +18°C water bath. During the first 6 days 16 animals were fed by force with hog liver at 6 p.m. On the sixth day the frogs were given an intraperitoneal injection (100 µl) of either 0.9% saline or tyrosine methyl ester (20 mg) at 6 p.m. The injection was repeated at 4 a.m. and the animals were killed 5 h later. The blood samples from each group were pooled. Assays were performed as described in Materials and Methods. The activities of tyrosine aminotransferase are expressed as nmol of *p*-hydroxyphenylpyruvate formed per mg of protein per min. The concentrations of glucose and tyrosine are expressed as mmol per l.

	Hepatic tyrosine aminotransferase activity ± S.D.	Serum tyrosine	Serum glucose
Fasted; saline	24.6 ± 8.3	0.12	1.0
Fasted; tyrosine	33.4 ± 9.7*	1.83	2.2
Fed; saline	34.1 ± 11.0*	0.18	0.9
Fed; tyrosine	48.4 ± 21.4**	1.60	2.8

Different from control at * $P < 0.05$, ** $P < 0.01$.

TABLE II

EFFECT OF PERORAL GLUCOSE LOADING ON HEPATIC TYROSINE AMINOTRANSFERASE IN THE FROG

Two groups of eight frogs were transferred from +4 to +18°C on day 1. They were given 0.5 ml of water or glucose solution (200 mg/0.5 ml) through an intragastric feeding tube at 8 a.m. on day 3. The feeding was repeated at 12-h intervals with a dose of 160 mg of glucose in 0.4 ml of water. The frogs were killed at noon on day 7, 4 h after the last feeding. The units are defined in Table I.

	Hepatic tyrosine aminotransferase activity ± S.D.	Serum glucose	Serum tyrosine
Control	19.6 ± 4.4	1.4	0.07
Glucose	9.4 ± 4.1*	3.8	0.12

* Different from control at $P < 0.0025$.

concentration was elevated, possibly because of the decrease in the enzyme activity.

Dibutyryl cyclic AMP is known to induce hepatic tyrosine aminotransferase in the rat [3]. Therefore its possible role in the regulation of this enzyme in the frog was studied in further experiments (Table III). The compound was found to induce both tyrosine aminotransferase and ornithine decarboxylase, an enzyme that was assayed to ascertain that cyclic AMP had its typical effect. The nucleotide had a prominent hyperglycemic effect; serum tyrosine concentration was markedly lowered which may be due to the induction of the aminotransferase.

2-Deoxyglucose, an inhibitor of glucose-6-phosphate isomerase [19], is known to induce tyrosine aminotransferase in mammals [18]. However, as can be seen in Table III, it is ineffective as an inducer of hepatic tyrosine aminotransferase in the frog. Thus it seems that its effect on the enzyme in the rat depends mainly if not entirely on its ability to release glucocorticoids from the adrenals [20].

TABLE III

EFFECT OF DIBUTYRYL CYCLIC AMP AND 2-DEOXYGLUCOSE ON HEPATIC TYROSINE AMINOTRANSFERASE IN THE FROG

24 frogs were transferred from +4 to +18°C on day 1. On day 5 the animals were given two successive injections of dibutyryl cyclic AMP (4 mg), 2-deoxyglucose (20 mg) or 0.9% saline. The interval between the injections was 4 h and the frogs were killed 3 h after the last injection. The blood from groups of four animals was pooled; the values of serum tyrosine and glucose are the means of two such groups. The activities of tyrosine aminotransferase and the concentrations of glucose and tyrosine are expressed as in Table I. The activities of ornithine decarboxylade are expressed as pmol of CO_2 liberated per mg of protein per min.

	Hepatic tyrosine aminotransferase activity ± S.D.	Hepatic ornithine decarboxylase activity ± S.D.	Serum glucose	Serum tyrosine
Control	15.0 ± 2.9	<0.1	1.3	0.10
Dibutyryl cyclic AMP	28.2 ± 16.4*	8.4 ± 6.4	6.3	0.06
2-Deoxyglucose	14.8 ± 7.5	—	—	0.11

* Different from control at $P < 0.05$.

TABLE IV

EFFECT OF DIBUTYRYL CYCLIC AMP AND TYROSINE ON HEPATIC TYROSINE AMINOTRANSFERASE IN UNSTARVED FROGS

The frogs used in this experiment (average weight 10 g) were caught in summer and kept at room temperature at the water bath. The night following capture they were given two successive injections (at 1.00 a.m. and 5.30 a.m.) of 50 μl containing dibutyryl cyclic AMP (1 mg), neutralized tyrosine methyl ester · HCl (4 mg) or saline. The animals were killed at 9.00 a.m. The enzyme activities are expressed as in Table III. *n* = number of animals.

	n	Hepatic tyrosine aminotransferase activity ± S.D.	Hepatic ornithine decarboxylase activity ± S.D.
Control	8	10.2 ± 6.1	<0.1
Cyclic AMP	7	17.6 ± 4.2	11.2 ± 16.9
Tyrosine	8	16.6 ± 9.6	<0.1

The effect of α-methyl-*p*-tyrosine, another secondary inducer [11], was also studied. The soluble methyl ester of this catecholamine depletor was given intraperitoneally (two times 200 mg/kg) 19 and 6 h before killing; the control group received 0.9% saline. Hepatic tyrosine aminotransferase activities were 28.8 and 29.8 nmol of *p*-hydroxyphenylpyruvate formed per mg of protein per min in the control and in the treated group, respectively.

The possible involvement of pancreatic hormones in the regulation of the enzyme was also examined. Insulin (0.4 unit per animal), glucagon (100 μg per animal) and 0.9% saline were given intraperitoneally to groups of six animals in an injection of 100 μl, 6 h before killing. The enzyme activities were 26.0, 26.5 and 30.3 nmol of *p*-hydroxyphenylpyruvate formed per mg of protein per min in the control, insulin and glucagon groups, respectively. Insulin seemed to be ineffective; the small enhancement in tyrosine aminotransferase activity after administration of glucagon was statistically not significant. The slight elevation is probably due to activation of adenyl cyclase [21] and is in a way secondary in nature.

Some of the work described above was repeated with frogs caught during the summer season to exclude the effects of long starvation and "hibernation". The effects of dibutyryl cyclic AMP and tyrosine (Table IV) were found to be similar to those in Tables I and III, although in this particular experiment they were not statistically significant. Cortisone acetate (two times 1 mg per animal, 15 and 3 h before killing) was ineffective as was expected. The enzyme activities of both control and cortisone-treated groups were 10 nmol of *p*-hydroxyphenylpyruvate formed per mg of protein per min; both groups consisted of 10 animals.

Discussion

It has been reported previously [12] that hydrocortisone has no effect on hepatic tyrosine aminotransferase of amphibians. In this paper we have shown that cortisone acetate and adrenocorticotropic hormone are unable to enhance the enzyme activity. Thus it seems that glucocorticoids are not involved in the regulation of this enzyme in the frog. Neither insulin nor two secondary inducers, 2-deoxyglucose [13] and α-methyl-*p*-tyrosine had any effect on the

enzyme [11]. The slight rise in the activity after administration of glucagon is probably due to the activation of adenyl cyclase [21] and thus is a secondary effect. Only three substances had a clear-cut effect; dibutyryl cyclic AMP, glucose and tyrosine.

The induction of tyrosine aminotransferase by tyrosine was of the same order of magnitude as that reported for adrenalectomized rats [8] and hepatoma cell culture when the concentration of glucose in the medium is low [9]. Administration of tyrosine caused an elevation of about 150% in the blood glucose level of the animals; this may be due to accelerated gluconeogenesis from the amino acid. Administration of the sugar, on the other hand, reduced hepatic tyrosine aminotransferase activity. Of other agents tested, only dibutyryl cyclic AMP had an effect; this was of the same order of magnitude as that obtained with tyrosine. It has been proposed that the site of regulation of the enzyme that is tyrosine sensitive is close to that sensitive to cyclic AMP [22]. Thus, there seems to be some analogy between the regulation mechanisms of bacterial β-galactosidase [23—26] and hepatic tyrosine aminotransferase as suggested by Grossman et al. [22] for the aminotransferase of the rat. Such a mechanism has been questioned, however, by the finding that glucose can prevent the induction without altering the level of hepatic cyclic AMP [27].

In this paper we have shown that tyrosine aminotransferase activity in the frog is affected by tyrosine, glucose and dibutyryl cyclic AMP, while other compounds known to induce the corresponding enzyme in the rat are ineffective. The results also show that the induction of hepatic tyrosine aminotransferase by cyclic AMP is independent of glucocorticoid-mediated induction. The different time-courses of the two have given rise to the same suggestion [28].

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