Lack of Immediate Response of Liver Tyrosine-α-Ketoglutarate Transaminase to Exogenous Adrenaline in the Adrenalectomized Rat

In 1971, an increased activity was reported by us[1] of liver tyrosine-α-ketoglutarate transaminase (tyrosine aminotransferase, L-tyrosine: 2-oxo-glutarate aminotransferase; E.C. 2.6.1.5) (TAT) appearing in rats as soon as 90 min after exposing them to 6 min and 40 sec (400 rpm) in the Noble-Collip drum[2], and being very probably a consequence of enhanced production of enzyme protein. In bilaterally adrenalectomized animals, this response was substantially blunted or even absent[3]. Its existence in intact rats was explained by an action of glucocorticoids from the adrenals, which 1. are known as being strong inducers of the enzyme[4], and 2. were found to be increased in blood plasma of traumatized rats even before TAT activation[5,6], so that also the time sequence of both increases seems to testify in favour of a causal relationship between them. However, an induction[7] of liver TAT after an administration of catecholamines to intact rats has been described[8]. In our own experiments, the possibility of an influence of adrenaline, the main catecholamine of the rat adrenal, in adrenalectomized rats was investigated. In these animals, even a decrease of liver TAT activity after adrenaline administration was observed.

Materials and methods. Our experiments were performed on male, 'specific pathogen-free' rats (VELAZ, Prague), weighing about 240 g and kept at 24 °C and natural light conditions on a standard laboratory diet, but deprived of food 18 h before use. A sub-group of these animals was subjected to bilateral adrenalectomy, 5 days before use. To these animals, 0.9% saline was given instead of tap water and no preexperimental fasting was imposed upon them.

Both adrenaline dosage and timing schedule were the same as described by BARTHOLINI et al.[8]. The following groups of animals were used (see Table I): 1. Controls. 2. Injected with 0.1 ml/100 g b.w. of 0.9% saline at time 0 and killed 30 min later. 4. Injected with the same amount of saline at time 0 and also 60 min later, and killed 90 min after the first injection. Groups 3. and 5. injected with 10 μg/0.1ml/100 g b.w. of adrenaline (Adrenalin SPOFA, Prague) and otherwise treated as groups 2. and 4. The remaining 2 groups (Table II) consisted of adrenalectomized animals and were treated in the same manner as groups 4. and 5. from Table I. All injections were given by the i.p. route.

Killing of the animals was performed by decapitation, taking place in all groups, including controls, at the same time of the day, i.e., in the late morning hours, to avoid any influence of the well-known daily rhythm of TAT activity[9] on our results. In the plasma, corticosterone[10] and glucose (by test sets prepared by Boehringer and sons, Mannheim, Germany), and in the livers, glycogen content[11] and TAT activity[12] were determined. The latter was expressed in nmoles of product, *p*-hydroxyphenylpyruvic acid per min at 37 °C and per mg enzyme protein determined parallely[13].

Results. The control values of plasma corticosterone (Table I) were the same as were found in previous experiments[5,6]. Increases were found at 30 min after both saline and adrenaline, those after adrenaline being substantially higher. At 90 min, an increase was found after adrenaline only. Concerning liver TAT activity, increased levels were found, 90 min after adrnaline, however, only in intact animals. After adrenalectomy (Table II), the reverse, i.e., a decrease of enzyme level, was observed. Finally, in the adrenaline treated animals, a decrease of liver glycogen and a net hyperglycaemic response was observed at 30 min and at 30 and 90 min, respectively (Table I), the hyperglycaemic response taking place also after adrenalectomy (Table II).

Discussion. Our results are in contrast to these reported by WICKS[14] who succeeded in finding a direct stimulatory effect of adrenaline on the TAT activity of fetal rat liver in organ culture. The complex action of adrenaline in vivo may be an explanation for this discrepancy. So the decreased liver TAT activity of our adrenalectomized animals is very suggestive of an intervention of additional

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Table I. Liver tyrosine-α-ketoglutarate transaminase activity in nmoles of product/1 min/1 mg, glycogen in %, and plasma corticosterone (B) in μg/100 ml and glucose in mg/100 ml, of rats (means of 6 values per group ± S.E.)

Group	Control 1	Saline + 30 min 2	*P*	Adrenaline + 30 min 3	Saline + 90 min 4	*P*	Adrenaline + 90 min 5
B	15 ± 8	44 ± 7[c]	< 0.01	71 ± 3[a]	22 ± 4	< 0.001	54 ± 5[b]
TAT	39.5 ± 8.0	—	—	—	40.8 ± 4.2	< 0.05	73.3 ± 12[d]
Glycogen	0.61 ± 0.13	0.47 ± 0.10	< 0.01	0.13 ± 0.05[b]	0.49 ± 0.13	N.S.	0.42 ± 0.10
Glucose	86 ± 8	98 ± 3	< 0.001	199 ± 11[a]	95 ± 6	< 0.001	261 ± 10[a]

[a] Statistically significant against control with $P < 0.001$; [b] $p < 0.01$; [c] $p < 0.02$; [d] $p < 0.05$.

factors activated by the administered hormone. Perhaps an increased release of growth hormone took place under the influence of adrenaline. Growth hormone is known as a suppressor of liver TAT activity[15].

Our results in intact animals confirm the results by BARTHOLINI et al.[8]. These authors suggested, as one of the possibilities to explain the stimulatory effect of catecholamines on liver TAT activity, a mediation through glucocorticoid hormones, delivered under the influence of catecholamine administration secondarily. We are of the opinion that our results testify strongly in the sense of this suggestion, although a 100% proof is not easy to produce.

Our data concerning plasma glucose and liver glycogen are shown here merely as proofs for full activity of the applied adrenaline.

Table II. Liver tyrosine-α-ketoglutarate transaminase activity in nmoles of product/1 min/1 mg, and plasma glucose in mg/100 ml of adrenalectomized rats (means of 6 values per group ± S.E.)

	Saline ± 90 min	P	Adrenaline + 90 min
TAT	40.3 ± 3.5	< 0.05	27.0 ± 3.6
Glucose	117 ± 4	< 0.02	150 ± 11

Zusammenfassung. Es wird eine Steigerung der Tyrosin-α-Ketoglutarat-Transaminase-Aktivität in der Rattenleber durch Adrenalin bestätigt und eine Verminderung der Transaminase-Aktivität nach Adrenalektomie durch Adrenalin nachgewiesen.

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Effect of Age on the Activity and Citrate Inhibition of Malate Dehydrogenase of the Brain and Heart of Rats

The enzyme, malate dehydrogenase (MDH), is known to exist in two isoenzymic forms, cytoplasmic (cMDH) and mitochondrial (mMDH)[1-3]. The cytoplasmic malate dehydrogenase is essential for gluconeogenesis as it converts the malate to oxaloacetate and then to phosphoenolpyruvate in the cytoplasm[4]. The mitochondrial malate dehydrogenase is required for continuous operation of the Krebs cycle within the mitochondria. The two forms of MDH are NAD^+dependent and are also under the control of two separate genes[5,6]. The present investigation centers around the change in the isoenzymes of MDH of the brain and the heart of young and old rats and a possible control mechanism of the enzyme with citrate, one of the intermediates of the Krebs cycle.

Materials and methods. Animals. The female albino rats used were of Wistar strain taken from the rat colony maintained at 24 ± 2°C. They were fed a commercial rat diet (Anidiet 'A', Chelsea Chemical Laboratory, Poona, India) and were also given gram (*Cicer arietinum*) on alternate days and water ad libitum. The mature adult (22 weeks) and old (96 weeks) rats were killed at a fixed time on successive days.

Preparation of the tissues. The rats were killed by dislocation of the neck. Brain (cerebral hemispheres) and heart (ventricles) were removed immediately, washed in ice-cold sucrose solution (0.25 M) and weighed in a Unimatic CL-41 single pan balance. A 10% homogenate (w/v) of each tissue was prepared in cold 0.25 M sucrose using a Potter-Elvehjem homogenizer fitted with a teflon pestle. The homogenate was then centrifuged at 800 × g for 20 min in MSE high speed refrigerated centrifuge to remove the nuclei and cell membranes. The supernatant was further centrifuged at 10000 × g and the supernatant

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Activities (Units/g wet wt.) and percent inhibition by citrate (6.66 μM/ml) of cMDH and mMDH of the brain and heart of rats of young and old age

Tissue	Age (weeks)	Cytoplasmic malate dehydrogenase: Units/g wet wt. × 10^4	p	Inhibition (%)	p	Mitochondrial malate dehydrogenase: Units/g wet wt. × 10^3	p	Inhibition (%)	p
Brain	22	3.44	0.01	14.25	0.001	1.30	0.01	42.00	0.20 (NS)
	96	2.77		32.17		0.92		45.82	
Heart	22	2.30	0.05	31.50	0.20 (NS)	4.80	0.05	42.90	0.20 (NS)
	96	2.02		27.12		4.20		43.12	

Each value represents the mean of 4–5 animals. P values of 0.05 or lower were taken as significant signific. NS, notant.