ROLE OF LIVER TRYPTOPHAN OXYGENASE ACTIVITY IN MICE ON OUTCOME OF ENDOTOXIN POISONING

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UDC 612.351.11.014.46:576.097.29

Injection of cortisone into mice simultaneously with typhoid endotoxin prevents the decrease in tryptophan oxygenase activity in the liver and prevents death of the animals. Tryptophan also maintained a normal level of activity of the enzyme but did not prevent death of the animals poisoned with endotoxin. The role of the level of tryptophan oxygenase activity in the pathogenesis of poisoning produced by endotoxin is discussed.

The importance of maintenance of a normal level of tryptophan oxygenase activity for the survival of animals exposed to the action of killed Salmonella typhimurium cells has been demonstrated experimentally [3, 4]. More recently, however, an absence of correlation has been found between the maintenance of a normal or raised level of activity of this enzyme and the survival rate of animals poisoned with endotoxin [8]. This conclusion has been based on the fact that α-methyltryptophan maintains tryptophan oxygenase activity above normal in mice poisoned with endotoxin, but does not at the time increase the rate of survival of the animals. On the other hand, 5-hydroxytryptophan lowers the enzyme activity without increasing the mortality of the animals poisoned with bacterial endotoxin.

The object of the present investigation was to determine the role of hormonal and substrate induction of tryptophan oxygenase in determining the outcome of typhoid toxicosis in mice, taking into consideration data in the literature indicating independence of hormonal and substrate induction of this enzyme [5, 6].

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino mice weighing 18-20 g. The animals were poisoned by intraperitoneal injection of typhoid endotoxin (strain $Ty_2$4446).

Tryptophan oxygenase activity was determined by the method of Knox and Auerbach [7] as modified by Berry and Smythe [3].

Cortisone acetate (Adrecon, Holland) was injected intramuscularly in doses of 0.01-50 mg/kg. L-tryptophan (Hungary) was injected intraperitoneally in doses of 0.5 mg/kg to 1 g/kg body weight. Cyproheptadine hydrochloride (Merck, USA), an antiserotonin compound, was injected intraperitoneally in a dose of 25 mg/kg. All substances were injected once only.

The chi-square method (survival rate of the mice) and constant formula method (level of tryptophan oxygenase activity [2]), were used for statistical analysis of the results.

EXPERIMENTAL RESULTS

The greatest decrease in tryptophan oxygenase activity occurred 17 h after injection of typhoid endotoxin into the mice (LD_{50}), and if large doses (LD_{100}) were given, the activity began to fall sooner (Table 1). These results fully confirmed those obtained by other workers [3,4] using different bacterial endotoxins.

Department of Infectious Pathology and Experimental Treatment of Infections, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR Kh. Kh. Planel'es.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 70, No. 12, pp. 34-36, December, 1970. Original article submitted March 19, 1970.

TABLE 1. Level of Tryptophan Oxygenase Activity in Liver of Intact Mice and Mice Poisoned with Endotoxin

Group of animals	Tryptophan oxygenase activity (in mmoles kynurenin/g fresh liver/h)			
	before injection of endotoxin	after injection of endotoxin		
		4 h	17 h	24 h
Control (intact mice)	7.9 ± 1.8	6.7 ± 0.8	7.0 ± 1.2	8.0 ± 1.3
Mice receiving endotoxin				
LD_{50}	–	7.0 ± 1.8	4.5 ± 0.5[2]	7.0 ± 1.0
LD_{100}	–	4.6 ± 0.8	3.8 ± 0.7[2]	–

[1]From 20 to 30 mice in each group.
[2]Difference from control statistically significant.

To study the role of hormonal and substrate induction of tryptophan oxygenase for survival of mice poisoned with endotoxin, the effect of different doses of cortisone and tryptophan on activity of this enzyme and the survival of the mice was investigated. If different doses of cortisone were used, a definite correlation was found between the inducing action of the hormone and its protective action. Cortisone (0.5 mg/kg) increased the enzyme activity in intact mice after 4 h to 8.7-20.9 mmole (initial value 5.6 mmole). In poisoned mice cortisone maintained the normal level of tryptophan oxygenase activity and prevented death of the animals in 100% of cases, compared with a mortality of 50-70% among the control group. Smaller doses of cortisone had no such effect. These experiments indicated a correlation between the inducing and protective actions of cortisone. This conclusion was confirmed by experiments using auranthin, an antibiotic belonging to the actinomycin group, the action of which is to reduce the increase in tryptophan oxygenase activity induced by cortisone [1]. Auranthin (0.25 mg/kg) lowered the tryptophan oxygenase activity to 5.9 mmoles initial level 7.1 mmoles) in intact mice and to 3.1 mmoles in poisoned mice. When endotoxin auranthin and cortisone were administered simultaneously to the mice, enzyme activity was reduced to 3.8 mmoles. Under these conditions cortisone did not prevent the decrease in enzyme activity, and 100% of the mice died, while the mortality among the control group was 50-70%. These experiments demonstrated the importance of maintenance in an intact state of the mechanism responsible for hormonal induction of tryptophan oxygenase for the survival of animals exposed to the toxic action of bacterial endotoxins.

Experiments using tryptophan showed that in intact mice tryptophan (250 mg/kg or more) increased the tryptophan oxygenase activity to 10-15 mmoles, and in animals poisoned with endotoxin to 9-13 mmoles (initial level 7.2 mmoles). Activity of the enzyme was increased after 2 h and it remained at the same level for 4-6 h after injection of tryptophan. Despite the higher tryptophan oxygenase activity, 50-70% of the mice died, the same proportion as among those not receiving tryptophan. These experiments showed that substrate induction, unlike hormonal induction, was not accompanied by an increase in the survival rate of animals poisoned with endotoxin.

Cortisone and tryptophan were found to differ in their action when administered to mice in the course of developing toxicosis. Cortisone did not prevent the decrease in enzyme activity, nor did it protect the animals from death, while tryptophan, which maintained a normal (or higher) level of activity, not only did not prevent death of the mice, but actually quickened its onset and increased the mortality rate among the mice (to 80-100%) compared with that among mice receiving endotoxin alone (40-60%). Mice receiving endotoxin in a dose of LD_{100}, followed 4 h later by tryptophan, died after 1-2 h despite a higher level of enzyme activity than initially. This toxic effect of tryptophan could be prevented by preliminary injection of cyproheptadine 4 h before endotoxin.

These results confirm the hypothesis put forward previously [8] to the effect that in animals poisoned with endotoxin the normal pathway of tryptophan metabolism is disturbed through the suppression of tryptophan oxygenase activity, and an alternative pathway proceeding to serotonin formation is activated; this pathway is responsible for the increased toxic manifestations.

In experiments using cortisone or tryptophan as inducers of tryptophan oxygenase, a correlation was thus established between the hormonal but not the substrate) induction of this enzyme and the survival rate

among mice poisoned with typhoid endotoxin. The raised level of tryptophan oxygenase as such does not play an important role in the survival of mice poisoned with endotoxin. Nevertheless, depression of its activity through the action of bacterial endotoxins is an important pathogenetic factor in the toxicosis, for it leads to disturbance of the normal pathway of tryptophan metabolism, and this may give rise, on the one hand, to a deficiency of NAD and NADP in the body, and on the other hand, to the formation of serotonin and of other toxic products of abnormal tryptophan metabolism. Both these effects create unfavorable conditions for the survival of animals poisoned with bacterial endotoxins.

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