Effect of Cortisone on Aspartate and Alanine Aminotransferases in a Desert Lizard

Shaharuddin Aziz, S. N. Hasnain, and Barbara K. Zain

Department of Biochemistry, University of Karachi, Karachi

Z. Naturforsch. **33 c**, 70–72 (1978) ; received May 31/October 10, 1977

Cortisone, Aminotransferases, Hibernation, Lizard

1. Liver and serum aspartate aminotransferase (GOT) and alanine aminotransferase (GPT) activities were measured in a hibernating desert lizard, *Uromastix hardwickii*. The levels of both enzymes were found to be lower in hibernation than during the active period, particularly in the liver.

2. After intramuscular injection of 2 mg of cortisone acetate there was a rapid rise in the levels of these enzymes with a peak of 18 hours (GOT) and 12 hours (GPT).

3. The response of both enzymes to cortisone was much greater during the active period than during hibernation.

4. GOT showed a much more rapid and greater response to cortisone than GPT. This is in contrast to the response of rat liver where GPT is more responsive to this hormone.

5. These studies indicate that the transferase enzymes of this lizard differ from those of the rat in their sensitivity and time of response to cortisone.

In our laboratories we have carried out a number of biochemical investigations on a local hibernating desert lizard, *Uromastix hardwickii*. Significant differences have been observed in the levels of some of its blood constituents [1–4] and tissue enzymes [5–7] during the hibernating and active periods. However, the effects of hormones in these animals have not been reported. In the present investigation we have examined the effect of adrenal corticosteriod hormones on liver and plasma amino transferases and the blood non-protein nitrogen and liver free amino acids. All of these components increase in response to adrenal corticosteroids in the rat. The aim of the present study is to see if a similar effect prevails in the lizard and whether it is affected by the hibernating and active states of the lizard.

Materials and Methods

The lizards, *Uromastix hardwickii*, were collected from the fields in the outlying areas of Karachi and kept in wooden boxes in the laboratory. Animals weighing 150–250 g were kept in wire-netted cages without food or water for one week. 25 of these fasted animals were divided into 5 sets for the dosage study. Each set received 2, 4, 6, 8 or 10 mg cortisone acetate (British Drug House, England) intramuscularly in the hind limbs. This is in the range from 10–50 mg/kg. The cortisone was prepared in 0.6% saline, the 15 lizards serving as controls were similarly treated with saline. Since these studies were done in winter these control animals also were specimens for the assay of amino transferase levels in hibernation. The animals were sacrificed 24 hours after injection and samples collected as indicated below.

In the time sequence study 120 fasted animals were used. 1 mg/100 g body weight of cortisone was administered intramuscularly in the hind limb and the animals sacrificed at definite time intervals post-cortisone treatment. This dose was selected since the mean weight of the animals was about 200 g. Thus each animal received an average of 2 mg cortisone acetate. Since this study was done in summer the saline injected control animals indicated the value of aminotransferases in the active period. Blood was obtained by severing the blood vessels in the neck and the serum was collected after clotting was complete. From the liver 10% homogenates were prepared in 0.6% chilled saline, centrifuged at 6000 rpm for 1 hour and the supernatants used for assay of the enzymes.

Aspartate aminotransferase (EC 2.6.1.1), GOT and alanine aminotransferase (E.C 2.6.1.2), GPT were assayed by colorimetric methods [8] and the enzyme activities expressed as m.I.U./mg tissue or ml blood. The method of Harding and Maclean [9] was used for the estimation of liver free amino acids

Requests for reprints should be sent to Dr. S. N. Hasnain, Department of Biochemistry, University of Karachi, Karachi-32, Pakistan

after precipitation of liver proteins with pure absolute methanol. Plasma non-protein nitrogen was estimated by the Kjeldahl direct nesserlization method [10].

Results

The saline injected control groups in Tables I and II indicate the activities of GOT and GPT in the liver and serum during hibernation and activity respectively. GOT and GPT levels both in liver and serum were higher during the active state than in hibernation. The liver GOT was 5 times higher in activity (26.3 m.I.U. *vs* 5.36 m.I.U.) while the GPT was 6 times greater in activity than in hibernation (13.7 *vs* 2.28). The serum levels were elevated to a lesser degree: GOT 3.53 *vs* 2.97 and GPT 1.39 *vs* 0.83 m.I.U. GPT levels in liver as well as in serum were found to be less than half the GOT levels in both hibernation and activity.

The effect of the dose of cortisone is shown in Table I. A single dose of 2 mg cortisone acetate administered intramuscularly had little effect on liver GOT and serum GOT and GPT, while the liver GPT increased more than three fold. As the dose of cortisone was increased there was a rise in liver aminotransferase levels. A dose of 10 mg cortisone thus caused a 9 fold and 15 fold rise in liver GOT and GPT levels respectively. Serum enzyme levels showed inconsistent results. The serum GOT level increased by less than two times when animals were treated with 10 mg cortisone; however, serum GPT increased $2\frac{1}{2}$ fold with this dose. Lower doses did not show any significant rise in serum aminotransferase levels except a two fold increase in serum GPT after 4 mg cortisone.

Table II shows the time sequence on aminotransferase levels in liver and serum. These experiments were carried out during the active period. A two to four fold rise in enzyme levels in both liver and serum was observed in 2 hours. Liver GOT level showed a maximum level after 14 hours when about a 16 fold increase in activity was observed. Liver GPT level was increased about 9 fold in 6 hours and remained at about the same level up to 18 – 20 hours after which it declined.

Dose [mg]	Liver		Serum	
	GOT	GPT	GOT	GPT
0 (Control)	5.36 ± 0.70	2.28 ± 0.53	2.97 ± 0.23	0.83 ± 0.04
2.	5.52 ± 1.36	8.04 ± 0.04	3.19 ± 0.13	0.77 ± 0.07
4.	7.08 ± 1.20	8.48 ± 0.04	2.51 ± 0.08	1.70 ± 0.04
6.	43.54 ± 3.07	15.00 ± 0.02	1.47 ± 0.08	0.52 ± 0.04
8.	42.10 ± 1.06	12.20 ± 2.5	3.51 ± 0.09	1.05 ± 0.18
10.	49.40 ± 0.37	34.40 ± 4.07	4.89 ± 0.07	2.16 ± 0.025

Table I. Dose response to cortisone acetate of GOT and GPT activities in liver and serum of *Uromastix* during hibernating period. Animals were sacrificed 24 h after intramuscular injection of the hormone. Values given are mean ± S.E. of 5 (15 for control) animals.

Table II. Effect of time on the response of liver and blood to cortisone acetate during the active period. Fasted animal were given 2 mg cortisone acetate, control animals received saline. Values are mean ± S.E. of 5 animals (35 for control).

Time [h]	Liver		Serum		Plasma NPN [mg/100 ml]	Liver free amino acids [mg/100 gm]
	GOT [m.I.U.]	GPT	GOT [m.I.U.]	GPT		
Control	26.30 ± 1.62	13.70 ± 1.38	3.53 ± 0.14	1.39 ± 0.08	7.61 ± 0.14	23.09 ± 0.56
2.	87.00 ± 15.3	60.00 ± 5.0	6.26 ± 2.00	5.51 ± 1.28	9.00 ± 0.49	37.90 ± 6.08
4.	132.00 ± 21.5	78.00 ± 5.90	3.96 ± 1.49	9.76 ± 0.38	9.80 ± 0.38	37.40 ± 5.68
6.	252.00 ± 72.5	126.80 ± 27.00	7.60 ± 1.35	12.08 ± 1.74	10.00 ± 0.4	72.00 ± 6.06
8.	209.00 ± 23.8	120.00 ± 20.00	12.00 ± 2.54	14.00 ± 2.34	10.50 ± 0.37	74.00 ± 7.52
10.	357.00 ± 29.5	123.00 ± 25.90	14.40 ± 2.57	14.40 ± 2.48	12.10 ± 0.35	74.50 ± 4.37
12.	384.60 ± 37.5	128.00 ± 24.40	15.00 ± 2.55	18.00 ± 2.11	13.80 ± 0.36	64.00 ± 2.62
14.	420.00 ± 24.4	127.20 ± 25.30	18.30 ± 3.37	18.50 ± 2.88	13.50 ± 0.41	60.00 ± 5.92
16.	398.00 ± 16.30	108.00 ± 24.10	25.00 ± 6.06	17.00 ± 2.33	14.60 ± 0.78	56.00 ± 3.82
18.	245.00 ± 33.8	139.00 ± 23.40	20.90 ± 4.36	15.50 ± 3.40	16.00 ± 0.54	28.00 ± 2.37
20.	232.00 ± 49.00	102.00 ± 16.00	20.60 ± 2.61	8.65 ± 1.04	16.00 ± 0.11	30.00 ± 1.46
22.	260.00 ± 38.20	63.00 ± 7.86	16.00 ± 3.36	5.60 ± 1.45	16.50 ± 0.46	32.00 ± 2.64
24.	357.00 ± 36.70	49.00 ± 11.50	8.20 ± 1.83	6.30 ± 1.48	17.07 ± 0.11	16.00 ± 0.79

Enzyme activity in serum also showed a similar behaviour. Serum GOT reached a maximum 16 hours after cortisone treatment, while serum GPT required 14 hours for maximum activity.

A comparison of the two Tables (I and II) also shows the effect of cortisone on aminotransferase levels in *Uromastix* during active and hibernating periods. During hibernating period, administration of 2 mg cortisone acetate led to a significant rise in liver GPT and serum GOT levels, while liver GOT and serum GPT levels remains unchanged. On the other hand sacrifice of active animals 24 h after treatment with 2 mg cortisone acetate demonstrated significant increase in GOT and GPT levels both in liver and serum.

Table II also shows the effect of cortisone on liver free amino acids and plasma NPN. The plasma NPN increased gradually after cortisone administration with more than a 2 fold rise after 24 hours. No peak was evident in the time intervals studied. The liver free amino acids rapidly increased with a 3 fold increase in 6 hours. This level was maintained for four hours after which it gradually fell, returning to the basal level by 24 hours.

Discussion

The present investigations indicate a higher aminotransferase activity in the liver and serum of *Uromastix hardwickii* during the active period as compared to hibernation. Difference in enzyme levels in various tissues of *Uromastix* during active and hibernating periods have been previously reported [5 – 7] with some enzymes increasing in hibernation and others decreasing.

These studies show that cortisone also increases the aminotransferases in reptiles and this effect was more pronounced during the active period than in hibernation when the animals of both groups were sacrificed 24 hours following treatment with 2 mg cortisone acetate. This investigation moreover demonstrated a difference in the response of aminotransferase activity to cortisone treatment between a mammal and a reptile. Rat liver alanine aminotransferase has been shown to be increased to a greater extent as compared to aspartate aminotransferase activity [11, 12] however in the present studies, during the active period cortisone was found to raise the liver aspartate aminotransferase activity to a greater extent than the alanine aminotransferase activity. Perhaps this enzyme (GOT) has a more active role than GPT in reptiles than in mammals, since its basal level as well as its response to cortisone is greater.

The rise in plasma NPN and liver free amino acids is in accord with the protein catabolic action of corticosteroids. The increase in levels of transferases which reached a maximum after the liver amino acids and serum NPN, could be due to substrate induction. Other corticosteroid responsive enzymes as tyrosine amino transferase and tryptophone pyrrolase have been shown to respond to substrate, however, Rosen and Nichol [13] have reported evidence against substrate induction of alanine amino transferase in the rat. Nevertheless they observed that only those enzymes which respond rapidly to cortisone could be induced by substrate. In the rat GPT does not increase until a few days after cortisone treatment whereas in *Uromastix* the maximum response occurred in 14 hours. Since the lizard enzymes respond rapidly to cortisone, they may also be inducible.

From these studies we can conclude that the transferase enzymes in this lizard differ from those in the rat liver in their sensitivity to cortisone and in the time of response to the hormone.

Cortisone is not a natural compound in these animals. Therefore, the relevance of there effects for natural conditions is not clear.

[1] M. Zain-ul-Abedin and M. H. Qazi, Canad. J. Biochem. **43,** 831 (1965).
[2] M. Zain-ul-Abedin and B. Katorski, Canad. J. Physiol. Pharmacol. **45,** 115 (1967).
[3] M. Zain-ul-Abedin, Z. N. Behleem, and M. A. Rahman, Pakistan J. Biochem. **2,** 47 (1969).
[4] M. Zain-ul-Abedin, Z. N. Behleem, and B. K. Zain, J. Sci. (Karachi Univ.) **3,** 39 (1974).
[5] S. N. Hasnain and J. Ramwani, Z. Naturforsch. **27 b,** 698 (1972).
[6] F. Farzana and S. N. Hasnain, Z. Naturforsch. **27 b,** 977 (1972).
[7] S. Aziz, S. N. Hasnain, and M. Zain-ul-Abedin, Z. Naturforsch. **27 b,** 973 (1972).
[8] E. J. King, Practical Clinical Enzymology, p. 175, D. van Nostrand Co. Ltd., London 1965.
[9] V. J. Harding and R. M. Maclean, J. Biol. Chem. **24,** 503 (1912).
[10] H. Varley, Practical Clinical Biochemistry, E.L.B.S. 4th edition, chapter xi, p. 180, 1969.
[11] F. Rosen, N. R. Roberts, L. E. Budwick, and C. A. Nichol, Science **127,** 287 (1958).
[12] F. Rosen, N. R. Roberts, L. E. Budwick, and C. A. Nichol, Endocrinology **65,** 256 (1959).
[13] F. Rosen and C. A. Nichol, Advances in Enzyme Regulation, **vol. 2,** p. 115, Pergamon Press, Oxford 1964.