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Stress Simulation: ACTH and Zinc Effect on Trace Metals in Liver and Spleen

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Effect of ACTH and zinc acetate subcutaneous injection on the trace metals in liver and spleen was investigated in male Wistar rats. Iron, copper, zinc and manganese in liver, and iron, copper and zinc in spleen were analyzed by AAS method. The iron, copper and zinc levels in liver, and iron levels in spleen showed a significant decrease at 1-1 h 30 min after administration of 30 UI of ACTH but values returned to normality or showed slightly higher levels at 4 h 30 min-7 h 45 min. Zinc acetate injection only elicited an increase in liver and spleen zinc levels. Results are discussed in relation with results given in previous papers.

Trace metal levels have been shown to change during stress and variations in plasmatic copper, iron and zinc; metals related to iron metabolism, have been reported in earlier studies (1, 2, 9). A possible role of ACTH as an intermediate factor (8) has been suggested in the byphasic response shown by plasmatic copper and iron as well as by serum total ferroxidasic activity in rats subjected to stress (1). Furthermore zinc is known to interfere iron metabolism by decreasing red cells half life due to the increase of their osmotic fragility, which accelerates iron turnover (16). A competitive inhibition of zinc on iron uptake by apoferritin at very low substrate has also been reported (11). Both, ACTH and zinc acetate administration have been used previously to simulate stress situation being reported several changes in plasmatic metal levels (8). Thus it was interesting to complete the study on the dynamic of these metals in organs playing an important role in their metabolism, after the same stress simulation. Iron, copper and zinc levels were measured in liver and spleen. Manganese was also measured in liver as this metal has been shown to interfere with iron hepatic storage when present in high levels in diet (18).

Materials and Methods

The experiments were performed using Wistar male rats of approximately the

same weight (200 g) and age, being kept in the laboratory for some weeks before treatment. Stress simulation was done by subcutaneous injection of ACTH or zinc acetate in a constant volume of 0.5 ml. Control animals received the same volume of saline solution.

Animals were sacrificed by exsanguination under chloroform anesthesia and liver and spleen removed, washed with saline solution to eliminate blood, and frozen to —25° C until the analysis was carried out. Zinc, copper, iron and manganase in liver, and zinc, copper and iron in spleen were determined by an atomic absorption spectrometry method (6, 10). The organs were dried 24 hours at 100° C and were then digested using a mixture of nitric, sulphuric and perchloric acid (1:1:1). In order to eliminate problems of different internal distribution of metals in the organs, complete liver and spleen were used. Standards and blank solutions were obtained following the same treatment as with the organs adding either deionized water or standard solutions of the metals. All reading were carried out in an atomic absorption spectrophotometer Phillips PYE Unicam SP-1900. The significance of differences between means was assessed by a «t»-Student test (15). When variances were found to be significantly different according to the Fisher test (15), the Behrens-Fisher modification of «t» test was applied (12). All results are given in μg/g dry weight.

Results

Results for ACTH effect on metal levels are summarized in table I (iron, zinc, copper and manganase levels in liver) and table II (iron, zinc and copper levels in spleen). Animals received either a subcutaneous injection of 30 UI of ACTH or the same volumen (0.5 ml) of saline solution, 1 h, 1 h 15 min, 4 h 30 min and 7 h 45 min before the sacrifice.

Results for zinc acetate effect on metal levels in spleen and liver are summarized in tables III and IV. In table III rats received a subcutaneous injection of either 2 mg Zn/animal, 4 mg Zn/a. or 0.5 ml of saline solution, 1 h 30 min before the sacrifice. In table IV animals received a s.c. injection either of 4 mg Zn/a. or 0.5 ml saline solution, 1 h 30 min and 4 h 30 min before the sacrifice.

Table I. *Liver changes after ACTH administration in male Wistar rats.* Levels of Fe, Zn, Cu and Mn are given as μg/g dry weight. The animals received a subcutaneous injection either of 30 UI of ACTH in a volume of 0.5 ml or the same volume of NaCl 0.9 %, at different times before sacrifice. Difference between means is statistically significant (as Student «t» test and Behrens-Fisher modification) with a significance level of 0.05 (*) or 0.01 (**) (mean ± standard deviation).

Treatment	Time	Fe	Zn	Cu	Mm	Anim.
NaCl	1 h	448.9± 74.5	129.2± 8.56	13.64±0.84	5.29±0.42	5
ACTH		293.7± 47.6 **	122.5±22.71	13.82±1.93	5.67±0.25	6
NaCl	1 h 15 min	570.6±124.4	127.8±13.83	14.40±0.95	6.07±0.96	6
ACTH		416.1±112.7 *	110.8± 4.41 *	12.95±1.07 *	5.55±0.45	6
NaCl	4 h 30 min	398.2± 67.8	128.8±13.37	12.76±1.35	5.62±0.36	6
ACTH		440.1±116.8	115.6±10.09	13.77±1.36	5.41±0.60	6
NaCl	7 h 45 min	393.2± 87.3	135.8±23.20	13.97±0.79	5.75±0.71	6
ACTH		459.0± 98.6	119.0±12.86	13.05±1.02	5.75±0.53	6

Table II. *Spleen changes after ACTH administration in male Wistar rats.*
Levels of Fe, Zn and Cu are given as μg/g dry weight. The animals received a subcutaneous injection either of 30 UI of ACTH in a volume of 0.5 ml or the same volume of NaCl 0.9 %, at different times before sacrifice. Difference between means is statistically significant (as Student «t» test and Behrens-Fisher modification) with a significance level of 0.05 (*). (Mean ± standard deviation.)

Treatment	Time	Fe	Zn	Cu	Anim.
NaCl	1 h	1,467± 909	96.77± 4.36	9.84±1.53	5
ACTH		654± 187	92.83± 4.70	10.18±1.02	6
NaCl	1 h 15 min	4,377±1,846	94.31± 6.36	11.10±2.76	6
ACTH		1,843±1,891 *	91.99± 4.70	9.55±1.13	6
NaCl	4 h 30 min	2,680±1,482	91.74± 4.98	11.85±1.61	6
ACTH		3,067±2,347	99.52±16.50	11.03±1.14	5
NaCl	7 h 45 min	1,765±2,255	93.33± 7.35	9.71±1.29	6
ACTH		2,301±1,861	94.57± 9.25	10.20±0.81	6

Table III. *Liver and spleen changes after different dosage of zinc administration in male Wistar rats.*
Levels of Fe, Zn, Cu and Mn are given as μg/g dry weight. The animals received a subcutaneous injection either of 2 mg Zn or 4 mg Zn in a volume of 0.5 ml or the same volume of NaCl 0.9 %, 1 h 30 min before sacrifice. (Mean ± standard deviation.)

mg Zn/a.	Fe	Zn	Cu	Mn	Anim.
		LIVER			
Na Cl	497± 104	123.1±15.49	11.52±0.79	5.73±0.37	6
2	538± 45	124.7±11.39	12.77±1.60	5.71±0.74	6
4	476± 123	121.7±13.14	11.88±2.35	5.69±0.74	5
		SPLEEN			
Na Cl	3,096±2,926	98.13±17.83	13.23±2.30		6
2	4,040±1,284	98.95±27.88	13.62±3.07		6
4	2,740±1,075	121.60±48.20	13.47±3.10		5

Table IV. *Liver and spleen changes after zinc administration in male Wistar rats.*
Levels of Fe, Zn, Cu and Mn are given as μg/g dry weight. The animals received a subcutaneous injection either of 4 mg Zn/a. in a volume of 0.5 ml or the same volume of NaCl 0.9 %, 1 h 30 min and 4 h 30 min before sacrifice. Difference between means is statistically significant (as Student «t» test and Behrens-Fisher modification) with a significance level of 0.01 (**). (Mean ± standard deviation.)

Treatment	Time	Fe	Zn	Cu	Mn	Anim.
			LIVER			
NaCl	1 h 30 min	493± 86	129.7±20.2	11.92±0.89	5.48±0.67	12
Zn Ac.		469± 83	127.8±12.1	12.32±1.66	5.27±0.69	11
NaCl	4 h 30 min	633± 145	112.5±14.0	13.20±1.69	5.96±1.17	12
Zn Ac.		541± 129	132.3±12.5 **	12.70±1.70	5.81±1.34	12
			SPLEEN			
NaCl	1 h 30 min	3,817±2,469	95.6±12.7	12.46±1.90		12
Zn Ac.		2,897± 909	111.9±32.2	13.13±3.03		11
NaCl	4 h 30 min	4,142±1,775	91.6± 7.9	13.03±1.25		12
Zn Ac.		2,841±1,856	104.7±11.6 **	12.62±2.35		12

Discussion

Stress simulation through ACTH administration appears to affect iron and other trace metal metabolism in a more important way than through zinc administration. Significant changes in iron, zinc and copper levels in liver, and in iron levels in spleen have been observed after ACTH subcutaneous injection, but only significant changes in zinc levels in both organs after treatment with zinc were detected. In this case, the increase in liver and spleen zinc levels is less spectacular than the one described in plasma (8) after zinc administration. This would be in accordance with the low variability of this metal reported in spleen, liver and kidney compared with pancreas (19). Variations in zinc concentration in serum and/or the diet only affect very slightly zinc levels in liver and spleen (19) being necessary a very high level of zinc in the diet for the metal to accumulate in liver (5). It has been suggested that the zinc in the viscera could be accumulated in such a way as to make it unavailable for the exchange with ^{65}Zn, for example zinc located deep within particular enzyme molecules (13), but in experiments on ^{65}Zn turnover the specific activity in spleen and liver follows the one in serum so closely that it indicates that little of the zinc in these organs should be bound in this manner (19).

Stress simulation through ACTH administration affected iron, zinc and copper levels in liver and only iron levels in spleen. Zinc and copper concentration in liver showed a slight but significant decrease immediately after the ACTH subcutaneous injection. As both copper and zinc can be accumulated in metallothionein in liver (4, 5), perhaps ACTH could affect in some way their storage and facilitate the liberation of both metals from the protein, appearing then a similar decrease of their levels in liver. It is difficult to relate the dynamics in both liver and serum. It has been reported a quick diminution of plasmatic copper but not of zinc in the same experimental design (8). The fact that zinc changes in liver are not reflected in plasma, could be explained by the quick and probably passive equilibrium that exists between zinc in blood and organs (3). Both metals could be mobilized to other tissues because *a*) hepatic copper levels decrease cannot be explained by an increased biliary excretion caused by glucocorticoids (greatly liberated after ACTH injection) being their role merely permissive, at least in adult rats (7). *b*) zinc is important in nucleic acid and protein synthesis, and in carbohydrate metabolism, having a role in tissue reparation (17).

The important iron decrease both in liver and spleen after ACTH administration coincides in the time with increased plasmatic levels of iron reported previously in the same experimental design (8), which suggests a quick exit of iron from liver and spleen to other organs through plasma, as for example to the erytropoietic tissues which could be related to the stimulatory effect of corticosteroids on eritropoiesis facilitating iron passage to erytropoietic organs described in rats (14).

Of all metals analyzed, the one that has been less affected by both treatments has been the manganese, which agrees once more with the stability of this metal in the body.

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Resumen

Se ha determinado por espectrofotometría de absorción atómica el efecto de la inyección subcutánea de 30 UI de ACTH y acetato de

zinc sobre los oligoelementos en hígado y bazo de ratas Wistar machos. Los niveles de hierro, cobre y zinc en hígado y los de hierro en bazo mostraron un descenso significativo entre la 1 h y 1 h 30 min después de la administración de ACTH, volviendo los valores a la normalidad o mostrando niveles ligeramente superiores entre las 4 h 30 min y las 7 h 45 min. La inyección de acetato de zinc provocó incremento tan sólo de los niveles de zinc en hígado y bazo. Los resultados se discuten en relación con los indicados en publicaciones anteriores

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