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Stress Simulation and Iron Metabolism: Adrenaline Administration

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The effect of stress simulation on Wistar male rats through adrenaline administration (subcutaneous injection, 0.5 mg/kg) was investigated. Parameters measured were: plasma iron, copper and zinc levels; caeruloplasmin concentration and total ferroxidasic activity in plasma; iron, copper, zinc and manganese levels in liver and iron; copper and zinc levels in spleen. No significant short term effect of adrenaline administration on any of these parameters was observed.

Changes in trace-metal metabolism which may accompany acute stress have been quite recently reviewed (3). In the complex series of adaptive phenomena resulting in stress situation, an important aspect is the function of the hypothalamic-hypophyseal-suprarrenal system and it has been suggested a possible inhibitory catecholaminergic role (14, 23-25). The mineral element correlation with adenohypophyseal adrenal function during stress has been described for several metals (1, 7-9). The sympathicoadrenalmedullary secretion of catecholamines during stress is dependent on the kind of stressor. Under moderately stressful condition excretion rates increase 3 to 5 times in man (11) and even more during severe physical and mental stress (6). The strongest reaction to noise (21), mental work and electric shock (11) is shown by adrenaline excretion. Noradrenaline excretion is also affected but less strongly. Adrenaline in small or moderate doses gives rise to subjective emotional changes that resemble in some aspects to the symptoms of stress situations (10, 15) but noradrenaline evokes, if it does, much less intense responses (10).

A stress condition as immobilization (4) affects several plasmatic factors (iron, copper, zinc and total ferroxidasic activity) related to iron metabolism (2). The effect of ACTH administration as a way of simulating the quick surge of this hormone during immobilization has been studied (7) and ACTH appeared as an intermediate factor only for several of the changes described. Adrenaline administration has been previously used as a way

of simulating the surge of meduloadrenal hormone during stress (5), so it was interesting to study its effect on the same plasmatic parameters known to rapidly change under immobilization and that had been studied under ACTH administration. This is the purpose of this paper. In order to have a more complete description of related aspects of iron metabolism, parameters measured were extended to zinc, copper and iron in liver and spleen and manganese in liver as this metal has been shown to interfere with other metals storage in liver when present in high levels in diet (26).

Materials and Methods

Four groups of Wistar male rats of approximately the same age and weight (200 g) were used. Two groups received a subcutaneous injection of adrenaline (0.5 mg/kg) in a volume of 0.5 ml as a way of simulating the surge of meduloadrenal hormone during immobilization (5), control groups received the same volumes of saline solution.

Periods of time between injection and sacrifice were choosen in a short range where changes under immobilization have been described (2, 7). The groups received a subcutaneous injection either of adrenaline or saline solution 1 h 15 min or 4 h 30 min before the sacrifice. Blood was collected from the cava abdominal vein and liver and spleen were removed, washed thoroughly in saline solution to eliminate blood and kept at —25° C until trace metal analysis were carried on.

The parameters were measured by the following methods: plasma iron by RAMSAY method (19), plasma copper by the Boehringer (Manheim) Kit method, caeruloplasmin concentration in plasma according to RAVIN (20), plasma ferroxidasic activity as used by PLANAS and FRIEDEN (18), plasma zinc by an atomic absorption spectrometry method according to SPRAGUE and SLAVIN (22) and PARKER *et al.* (17) but with a few changes which made the technique more suitable for the samples. The method includes a dilution 1:2 in deionized water and the use of a bovin albumin standard solution to achieve the needed viscosity in the samples used to built up the standard curve. Zinc, copper, iron and manganese in the organs were determined by an atomic absorption spectrometry method using an acid digestion of dried samples with a mixture of nitric, sulphuric and percloric acid (1:1:1). Readings for all atomic absorption spectrometry methods were carried on in a Phillips Pye Unicam

Table I. *Effect of adrenaline administration on plasma in male Wistar rats.* Levels (mean ± standard deviation) of total ferroxidasic activity (TFA), caeruloplasmin (Cp), copper, iron and zinc in plasma after a subcutaneous injection of either 0.5 ml of saline solution or adrenaline (0.5 mg/kg of weight), 1 h 15 min and 4 h 30 min before the sacrifice. Number of animals per group, 6.

	TFA μM Fe/min/ml	Cp mg/100 ml	Cu μg/100 ml	Fe μg/100 ml	Zn μg/100 ml
Time: 1 h 15 min					
NaCl	306.52 ± 22.83	37.89 ± 3.10	132.50 ± 9.08	134.16 ± 21.33	146.70 ± 9.29
Adrenal.	311.24 ± 33.37	39.31 ± 7.62	123.12 ± 14.89	132.00 ± 33.83	139.19 ± 13.63
Time: 4 h 30 min					
NaCl	354.21 ± 35.28	40.23 ± 5.25	137.50 ± 13.96	135.83 ± 11.58	126.38 ± 9.88
Adrenal.	345.71 ± 18.77	42.57 ± 3.76	145.62 ± 16.50	117.50 ± 25.44	126.82 ± 13.86

SP-1900, with a special head for viscose samples when zinc was determined in plasma.

The significance of differences between means was assessed by a «t»-Student test program where the Behrens-Fisher modification of the «t» test is included in case the variances were not equal.

Results

Adrenaline has not evoked any important change in plasmatic factors related to iron metabolism as plasmatic iron levels, total ferroxidasic activity and caeruloplasmin concentration, plasma zinc and copper.

Adrenaline administration also failed to evoke any important change in trace metal levels in liver and spleen when 1 h 15 min or 4 h 30 min had elapsed between the injection and sacrifice. Metals analyzed were iron, copper and zinc in spleen and iron, copper, zinc and manganese in liver. All results are given as μg/g of dry weight.

Discussion

Immobilization alone (12, 13) or together with another stressor as a bath at 23° C (16) increases adrenaline and noradrenaline excretion. The stress-induced increase of catecholamine biosynthesis in

Table II. *Effect of adrenaline administration on liver in male Wistar rats.* Levels (mean ± standard deviation) of iron, zinc, copper and manganese are given as μg/g dry weight. The animals received a subcutaneous injection of either 0.5 ml of saline solution or adrenaline (0.5 mg/kg of weight), 1 h 15 min and 4 h 30 min before sacrifice. Number of animals per group, 6.

	Fe	Zn	Cu	Mn
Time: 1 h 15 min				
NaCl	551.1±123.7	130.6±11.35	16.71±4.47	4.96±0.69
Adrenal.	586.6± 55.9	133.1±23.58	15.18±0.92	5.42±0.86
Time: 4 h 30 min				
NaCl	664.6±62.89	130.1±14.19	15.00±0.90	5.49±0.41
Adrenal.	593.4±69.60	133.9± 9.26	14.37±1.27	5.27±0.59

Table III. *Effect of adrenaline administration on spleen in male Wistar rats.* Levels (mean ± standard deviation) of iron, zinc and copper in spleen after a subcutaneous injection of either 0.5 ml of saline solution or adrenaline (0.5 mg/kg of weight), 1 h 15 min and 4 h 30 min before sacrifice. Results are given in μg/g of dry weight. Number of animals per group, 6.

	Fe	Zn	Cu
Time: 1 h 15 min			
NaCl	1,573±471	90.84± 4.75	8.83±2.51
Adrenal.	1,383±528	91.26± 6.79	10.24±2.39
Time: 4 h 30 min			
NaCl	1,610±571	105.20±17.46	9.91±1.36
Adrenal	1,868±570	92.60±15.43	9.96±1.60

the adrenal medulla in immobilized rats is influenced by both endocrine (pituitari-adrenocortical) and neural regulation (13). Adrenaline subcutaneous injection has been reported as a suitable way of simulating the effect of medulosuprarrenal hormone during acute stress (5). Using a dose of 0.5 mg/kg of weight, it has not been detected any significant differences between groups treated with the hormone and groups receiving saline solution, in any of the parameters measured. An acute stress as immobilization affects very quickly plasma levels of iron, copper and zinc and the total ferroxidasic activity of plasma (2, 7). ACTH administration in a similar timing affects only iron, copper and ferroxidasic activity of plasma showing the same byphasic response of these parameters (7). Adrenaline doesn't seem to have similar effects on these parameters in these particular periods of time, so if this hormone has any effect on these plasmatic parameters they probably don't follow similar patterns than immobilization during the first hours. Independently of long term effects of both hormones ACTH and adrenaline, the quick byphasic response shown by plasma iron and copper and by total ferroxidasic activity of serum, mainly due to caeruloplasmin that contains copper, would be principally explained through ACTH surge (7). Significant plasma zinc diminution found in rats subjected to immobilization remains unexplained both by ACTH and adrenaline roles.

The effect of immobilization on trace metal levels in organs such as liver and spleen has not been reported, so it is not possible to relate results here described after adrenaline administration to stress effects. But as changes were described in plasma of immobilized rats (2) it could be inferred a possible relation of this plasma changes with other changes in the metabolism of these metals in other sites of the organism. ACTH has been shown to affect significantly iron, copper and zinc levels in liver and iron levels in spleen quickly after a subcutaneous injection of the hormone (1), but adrenaline administration has not evoked any similar change in any of the trace metal levels studied in liver or spleen.

Resumen

Se ha investigado en ratas macho Wistar el efecto de la simulación de stress mediante administración de adrenalina (inyección subcutánea, 0.5 mg/kg). Los parámetros analizados fueron: niveles plasmáticos de hierro, cobre y zinc, concentración de ceruloplasmina y actividad ferroxidásica total en plasma. Niveles de hierro, cobre, zinc y manganeso en hígado y de hierro, cobre y zinc en bazo. No se ha observado a corto plazo ningún efecto significativo de la administración de adrenalina sobre ninguno de los parámetros analizados.

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