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The Role of Catecholamines and Glucagon on Serum and Liver Metallothionein Response to Restraint Stress

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The role of glucagon and catecholamines on serum and liver metallothionein (MT) concentrations in basal and stress conditions has been studied. Glucagon showed no effect on serum MT either in basal (unstressed) or stress (20 hours of restraint) conditions. In contrast, glucagon administration increased both unstressed and stressed levels of liver MT. No effect of the $\alpha+\beta$-catecholamine blocker labetalol on serum MT levels was observed in unstressed rats. However, the administration of labetalol abolished the increase in serum MT levels caused by stress. These data suggest that catecholamines might be involved in serum MT regulation during stress, while they might not be important in the maintenance of basal serum MT levels. Finally, no significant effect of adrenergic blockade was found on basal and stress levels of liver MT, in agreement with previous data from this laboratory.

Key words: Catecholamines, Glucagon, Metallothionein, Stress.

Metallothionein (MT) is present in several tissues, but especially in the liver (17). Although the its actual physiological function remains to be established, it appears that it may be related to Zn and Cu metabolism (2, 4, 17). Liver MT induction by a wide range of systemic and neurogenic stresses is now well established (15-17, 19, 22), but the hormonal systems involved are at present unclear. Glucocorticoids (1, 8, 9), catecholamines (1, 3) and glucagon (6, 8) have been claimed to be involved in MT regulation. However, data from our laboratory do not give support to a role of these hormones on liver MT induction by stress.

Circulating levels of MT, the source of which appears to be, at least in part, the liver (5, 7), have also been measured (11). Serum MT levels are increased by Cd, Zn or endotoxin administration (12, 21). A mild stressor such as restraint in tubes was (16) or was not (14, 15) able to increase serum MT levels, whereas a strong stressor such as immobilization in wood-boards consistently increases serum MT levels (unpublished data). Few

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data regarding the regulation of serum MT are available. It has recently been described that glucocorticoids might exert a positive effect on MT release during stress (submitted), but no data on the role of other hormonal systems have been presented.

Therefore, the aims of the present work were to study the role of catecholamines and glucagon on serum MT; and to further characterize the role of those hormonal systems on MT regulation during stress.

Materials and Methods

Adult male Sprague-Dawley rats were maintained under standard conditions (lights on from 07.00 to 19.00 hours, temperature 22 ± 1° C, food and water *ad libitum*, for at least one week before starting the experiments. The animals were randomly assigned to the different experimental groups.

Experiment 1. — Rats were i.p. injected with saline, the β-catecholamine blocker propranolol at a dose 2 mg/kg, or the α+β-catecholamine blocker labetalol (in its hydrochloride salt) at a dose of 5 mg/kg. One half of the animals were then subjected to 20 h of restraint stress in plastic tubes provided with several holes and killed. The remaining rats were returned to their home cages without food and water available and killed 20 h later.

Experiment 2. — Rats were s.c. injected with saline, a long-lasting glucagon preparation (20) at a dose of 0.25 mg/kg, the glucagon vehicle, or a long-lasting insulin preparation at a dose of 2IU/100 g bw. One half of the animals were then returned to their home cages and killed 20 h later. The remaining rats were subjected to 20 h of restraint stress and killed. Vehicle-treatment was not studied in stressed rats.

The procedure for serum and liver metallothionein obtention has previously been described (16).

Serum and liver MT were analyzed by a highly specific and sensitive radioimmunoassay as previously described (10, 11). Serum and liver cytosolic Zn levels were measured by atomic absorption spectrophotometry. Results were analyzed with the Student «t» test. Data were subjected to logarithmic transformation where necessary, to achieve homogeneity of variances.

Results

Twenty hours of restraint stress plus starvation significantly increased serum MT levels (fig. 1). The administration of the β-adrenergic blocker propranolol was without effect. However, the administration of the labetalol abolished the increase in serum MT caused by stress ($p < 0.05$).

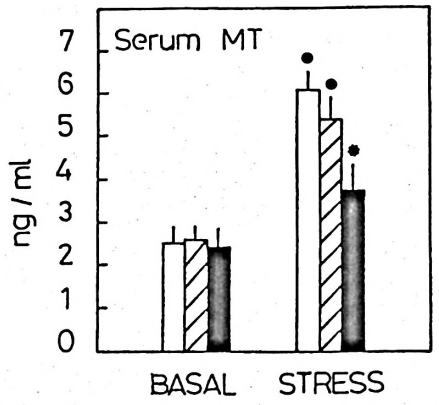

Fig. 1. *Effect of adrenergic blockade on serum metallothionein response to stress.*
Means ± SE are represented (n = 6). Rats were injected with saline (□), (▨) propranolol (2 mg/kg) or labetalol (5 mg/kg) (■). Some rats were returned to their home cages without food and water available (basal) and the remaining rats were subjected to restraint stress (stress). All of the animals were killed 20 hours later. • $p < 0.05$ vs basal values. * $p < 0.05$ vs saline stressed rats.

Table I. *Effect of adrenergic blockade on liver metallothionein (MT) and cytosolic Zn levels and on serum Zn levels in basal and stress conditions.*
Means ± SE are represented (n= 6). Rats were treated with the different drugs and returned to their home cages without food and water available (starvation) or subjected to restraint stress (stress + starvation). They were killed 20 h later. * p at least < 0.05 vs. the respective basal value.

	Liver MT (μg/g)	Liver cytosolic Zn (μg/g)	Serum Zn (μg/ml)
STARVATION			
Saline	5.22 ± 0.53	24.6 ± 0.8	1.71 ± 0.04
Propranolol	4.16 ± 0.25	23.9 ± 0.7	1.66 ± 0.05
Labetalol	4.37 ± 0.69	23.6 ± 0.4	1.63 ± 0.04
STRESS + STARVATION			
Saline	10.72 ± 1.53*	28.6 ± 1.0*	1.27 ± 0.05*
Propranolol	16.40 ± 2.13*	28.8 ± 0.6*	1.02 ± 0.05*
Labetalol	9.57 ± 1.04*	27.3 ± 1.4*	1.34 ± 0.13*

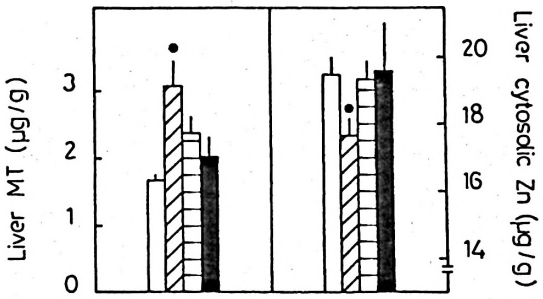

Fig. 2. *Effect of glucagon and insulin administration on liver metallothionein and cytosolic Zn levels.* Means ± SE are represented (n = 6). Rats were treated with saline (□), glucagon (0.25 mg/kg) (▨), the vehicle of glucagon (⊟) or insulin (2 U/100 g bw) (■). Rats were returned to their home cages and killed 20 h later. * p < 0.05 vs saline treated rats.

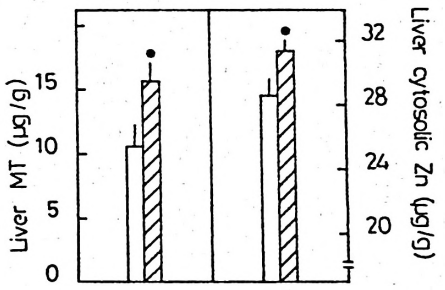

Fig. 3. *Effect of glucagon administration on liver metallothionein and cytosolic Zn levels during stress.* Means ± SE are represented (n = 6). Rats were treated with saline (□) or (▨) glucagon (0.25 mg/kg). Rats were then subjected to restraint stress for 20 hours and killed. * p < 0.05 vs saline-treated rats.

Neither of the two blockers modified serum MT levels in starved rats.

As expected, restraint stress significantly increased liver MT levels as compared to starved rats. Liver MT concentration was not modified by the adrenergic blockade. Liver cytosolic changes fully correlated to MT changes (table I).

Glucagon as well as insulin administration did not modify significantly serum MT levels in control rats (table II). When administered to stressed rats, glucagon tended to reduce the effect of stress, although no statistical significance was obtained. Stressed animals treated with insulin died within few hours after onset of stress.

Glucagon administration to control rats significantly ($p < 0.05$) increased liver MT levels (fig. 2). Surprisingly, glucagon caused a decrease of liver cytosolic Zn levels ($p < 0.05$). The effect of

Table II. *Effect of glucagon administration on serum metallothionein (MT) and Zn levels in basal and stress conditions.*

Means ± SE are represented (n = 6). Rats were treated with the different drugs and returned to their home cages (basal) or subjected to restraint stress (stress). The animals were killed 20 h later.

	Serum (MT) (ng/ml)	Serum Zn (μg/ml)
BASAL		
Saline	2.81 ± 0.15	1.63 ± 0.06
Glucagon	3.04 ± 0.22	1.67 ± 0.05
Vehicle	2.59 ± 0.19	1.77 ± 0.03
Insulin	2.61 ± 0.14	1.65 ± 0.05
STRESS		
Saline	6.15 ± 0.36	1.27 ± 0.05
Glucagon	4.62 ± 0.62	1.37 ± 0.05

glucagon on liver MT levels was more marked when administered to stressed rats (fig. 3), since it was found an increase of 5 μg MT/g in stressed rats, and an increase of approximately 1 μg/g in control rats.

Serum Zn levels were significantly decreased by stress, but no significant effect of the adrenergic blockade (table I) or glucagon administration (table II) was found.

Discussion

Restraint stress significantly increased serum MT levels. Since the α+β-adrenergic blockade with labetalol abolished the rise in serum MT caused by stress, catecholamines appear to have some role on the regulation of serum MT levels during stress. In contrast, no effect was observed in unstressed rats, suggesting that catecholamines would not be important in the maintenance of basal levels of serum MT. Similar results have been obtained for glucocorticoids (submitted).

The level(s) at which catecholamines may act during stress to control serum MT is unclear. Serum MT levels are the consequence of two different processes: (i) MT release from the tissues (likely the liver, 5, 7), and (ii) MT clearance from the blood. Therefore, catecholamines might act at any of the two processes. Since it was found that liver MT levels were not affected by adrenergic blockade, it appears likely that catecholamines could regulate serum MT levels mainly through MT clearance from the blood. However, further work is needed to demonstrate it.

In unstressed rats, glucagon administration did not increase serum MT levels. In addition, despite the 50% increase of liver MT levels caused by this hormone in stressed rats, no further increase was found in serum MT levels. This supports the idea that serum and liver MT levels are not necessarily correlated, which is what it has been usually found in our laboratory (14-16).

As expected, liver MT levels were increased by restraint stress (14-16). Adrenergic blockade did not show a significant effect. This was in disagreement with the previously attributed role for catecholamines in liver MT induction by stress (1) and in agreement with data from our laboratory (submitted). The reasons for these discrepancies are not known, although conflictive data on the role of catecholamines on other physiological variables, for instance gastric ulceration caused by stress, have been reported (13, 18).

In accordance with previous reports (8), glucagon significantly increased liver MT levels, even using a much lower dose than in the previous report. The effect of glucagon was more marked during stress. It has been demonstrated that a sinergic effect on liver MT levels is present after dexamethasone and glucagon administration (8). Since corticosterone release is greatly enhanced by stress (14, 15), it could be argued that the potentiation of the effect of glucagon during stress was

due to corticosterone. However, this assumption is not clear, in view of the finding that corticosterone has an inhibitory role on liver MT concentration (submitted).

The present results suggest that catecholamines may mediate at least in part the increase in serum MT levels during stress; and, the positive effect of glucagon on liver MT concentration is greater during stress than in basal conditions, suggesting that a sinergic interaction occurs between this hormone and some mechanism activated during stress.

Resumen

Se estudia el papel del glucagón y de las catecolaminas en la regulación de los niveles de metalotioneína (MT) sérica y hepática en situación basal y durante el estrés de inmovilización. El glucagón no muestra efecto significativo sobre la MT sérica ni en situación basal ni durante el estrés (20 h), pero incrementa los niveles hepáticos de MT en ambas situaciones. El labetalol no muestra efecto significativo sobre los niveles séricos de MT en los animales controles, aunque elimina el incremento de los niveles séricos de MT en los animales sometidos a estrés. Estos resultados sugieren que las catecolaminas puedan estar implicadas en la regulación de la MT sérica, aunque no parecen tener importancia en el mantenimiento de los niveles séricos basales de esta proteína. Finalmente, se confirma que el labetol no tiene efecto significativo del bloqueo adrenérgico sobre la MT hepática, ni en situación basal ni durante el estrés de inmovilización.

Palabras clave: Catecolaminas, Glucagón, Metalotioneína, Estrés.

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