Physiol. Res. 52: 67-72, 2003

Different Activation of ACTH and Corticosterone Release in Response to Various Stressors in Rats

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Received February 5, 2002
Accepted May 4, 2002

Summary

The aim of this study was to investigate the reaction of the hypothalamo-pituitary-adrenocortical (HPA) system to various stressors (fasting, crowding, cold and heat) by measuring blood ACTH and corticosterone (CORT) concentration as well as the cholesterol (CHOL) content in the adrenals. To examine the effects of stress termination, the rats were returned and kept under control conditions for the same period as that of stress duration (supposed recovery period). According to our results HPA system was activated by all the stressors applied. Heat seems to be the strongest stressor since the exposure of animals to a high ambient temperature resulted in the greatest rise of plasma ACTH concentration as well as CORT synthesis and secretion. These values remained elevated after the stress termination i.e. after the rats had been returned to room temperature. Fasting seems to be the weakest stressor given because it causes the smallest increase in blood ACTH and CORT concentrations. Moreover, in refed rats the HPA function was fully recovered. In conclusion, the various stressors applied seem to induce a different response of the HPA system as judged by quantitative changes in ACTH and CORT release.

Key words
ACTH • Cholesterol • Corticosterone • Stress

Introduction

It is well known that the hypothalamic-pituitary-adrenal (HPA) axis helps in maintaining basal and stress-related homeostasis of the nervous system as well as cardiovascular, immune and metabolic functions. The main regulation of circadian and stress-related activity of the HPA axis occurs at the level of parvicellular subdivision of the hypothalamic paraventricular nuclei (PVN) (Chrousos and Gold 1992). The majority of these neurons secrete CRH and vasopressin (VP), which synergistically stimulate ACTH secretion by the pituitary corticotrophic cells. ACTH then enters the systemic circulation, stimulating corticosterone (CORT) synthesis from the cholesterol (CHOL) and its release from the adrenal cortex, which in turn provides an inhibitory feedback signal to the system (Dallman *et al*. 1987).

According to the original stress concept, introduced by Hans Selye (1936) and Walter Cannon (1932), stress was thought to be a non-specific response to stressors always inducing the activation of adrenal glucocorticoid and catecholamine release. However, in recent years a large amount of accumulated data suggests that the reaction to stressors could be specific, depending on the type of stressor, duration of exposure etc. Studies, using a wide variety of stressors, have clearly indicated

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that the pattern of neuroendocrine response is dependent upon the stress stimulus applied (Pacák *et al.* 1995, Goldstein *et al.* 1996, Ježová and Škultétyová 1997). Substantial stressor specificity has been demonstrated in the activation of the HPA axis and sympathoadrenal system, known to be the main stress systems in both humans and experimental animals (Vigaš *et al.* 1984, Pacák *et al.* 1995).

The purpose of this study was to investigate the reaction of hypothalamo-pituitary-adrenocortical (HPA) system to various types of stressors (fasting as metabolic, crowding as psychosocial, cold and heat as environmental) by measuring blood ACTH and CORT concentrations as well as the adrenal cholesterol (CHOL) content. To examine the possible effects of stress termination on the HPA system function the animals were returned to the control conditions for the period equal to that of stress duration (supposed recovery period).

Methods

Male rats of the Wistar strain (*Rattus norvegicus*), 60-90 days old, weighing 180-220 g, were used for the experiments. The animals were acclimated to 22±1 ºC, kept at a 12:12 h light-dark cycle and given commercial rat food and tap water *ad libitum*. The animals were housed two per cage for 15 days before starting the experiment.

The rats were divided into 11 groups, each consisting of six animals. The first group represented intact controls. The second and third group consisted of rats completely deprived of food for 48 h. The rats of the second group were decapitated immediately after a 48 h fasting period and the third group was kept under conditions of free access to food during subsequent 48 h and then sacrificed. The rats of the fourth and fifth group were all housed in a single cage, 12 in all, each occupying 58.3 cm^2 for 3 h. In this way the animals were exposed to crowding stress, i.e. enforced movement restriction, which is characterized as a psychosocial stress. After stress termination, the fourth group was sacrificed immediately, whereas the rats of the fifth group were returned into cages in the original pairs (2 in each, 350 cm^2 per rat), and kept there over the subsequent 3 h and then sacrificed. The animals of the sixth and seventh group were carefully transferred in their home cages into the cold room and kept at 6 ºC for 3 h. The sixth group was decapitated immediately after cold exposure and the rats of the seventh group were brought back to room temperature (22±1 ºC) for the next 3 h and then killed. The rats from the remaining four groups were exposed to an ambient temperature of 38ºC for 20 and 60 min in a hot chamber. The eighth and tenth group were decapitated immediately after stress termination whereas the rats from the ninth and eleventh group were kept at room temperature for the subsequent 20 and 60 min and then sacrificed.

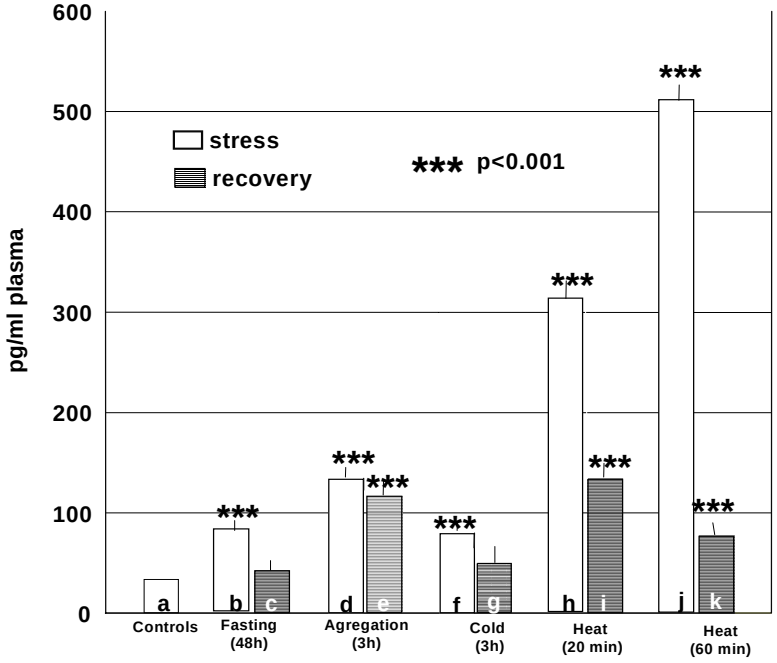

Fig. 1. *ACTH plasma levels during rats exposure to various stressors (**b** – fasting (48 h); **d** – crowding (3 h); **f** – cold (3 h); **h** – heat (20 min); **j** – heat (60 min). To examine the effects of stress termination the rats were returned to the control conditions (**c** – refeeding (48 h); **e** – 2 rats per cage (3 h); room temperature for 3 h (**g**), 20 min (**i**) and 60 min (**k**). The data are presented as means ± S.E.M. of six animals in pg/ml. Differences between the groups: b:a p<0.001, d:a p<0.001, f:a p<0.001, h:a p<0.001, j:a p<0.001, e:a p<0.001 i:a p<0.001, k:a, p<0.001, h:b p<0.001, h:d p<0.001, h:f p<0.001, j:b p<0.001, j:d p<0.001, j:f p<0.001, j:h p<0.001; b:c p<0.001, h:i p<0.001, j:k p<0.001.*

The rats were always killed by decapitation. Blood was collected from the trunk and divided into two sets of tubes. For plasma determinations EDTA was added to one of the sets. Serum and plasma were frozen for the CORT and ACTH determination. The adrenals were quickly excised, freed of fat tissue (+4 °C) and weighed. CHOL concentration in the adrenals was determined immediately after decapitation, according to the method of Zlatkis *et al.* (1953). The method consists of cholesterol reaction with ferric salts in concentrated sulfuric acid. The intensity of the formed violet color (560 nm) is proportional to the concentration of cholesterol. The values are expressed as μg CHOL per mg tissue. Serum CORT was determined by RIA kit (ICN Biochemicals, Costa Mesa, CA) and the values expressed as ng CORT/ ml serum. Plasma ACTH was determined by chemiluminescent method using IMMULITE automatic analyzer (DPC, Los Angeles, CA). The values are expressed as pg ACTH/ml plasma.

One-way ANOVA test was employed for the comparison of the experimental groups. The values are expressed as means ± S.E.M. of six animals and the level of significance was set at $p<0.05$.

Results

All applied stressors induced a significant increase in ACTH plasma levels (b:a $p<0.001$, d:a $p<0.001$, f:a $p<0.001$, h:a $p<0.001$, j:a $p<0.001$) as compared to the controls (Fig. 1). Exposure to 38 °C, for both 20 and 60 min, produced the largest increment in plasma ACTH levels in respect to other stressors (h:b $p<0.001$, h:d $p<0.001$, h:f $p<0.001$, j:b $p<0.001$, j:d $p<0.001$, j:f $p<0.001$). The release of ACTH was much greater after a 60 min exposure as compared to that of 20 min (j:h $p<0.001$). After heat stress termination, the rats were returned to room temperature, which resulted in a significant decrease of ACTH concentration as compared to that measured at the end of the stress exposure (h:i, j:k, $p<0.001$). However, ACTH concentration still did not reach the control level (i:a, k:a, $p<0.001$). The plasma ACTH concentration was also significantly elevated (d:a $p<0.001$) under the influence of crowding stress, being still significantly high after returning the rats to control conditions (e:a, $p<0.001$). Fasting and cold stressors were less potent in elevating plasma ACTH concentration (b:a, f:a, $p<0.001$), which returned to the control level after animals refeeding and keeping them at room temperature after cold exposure, respectively.

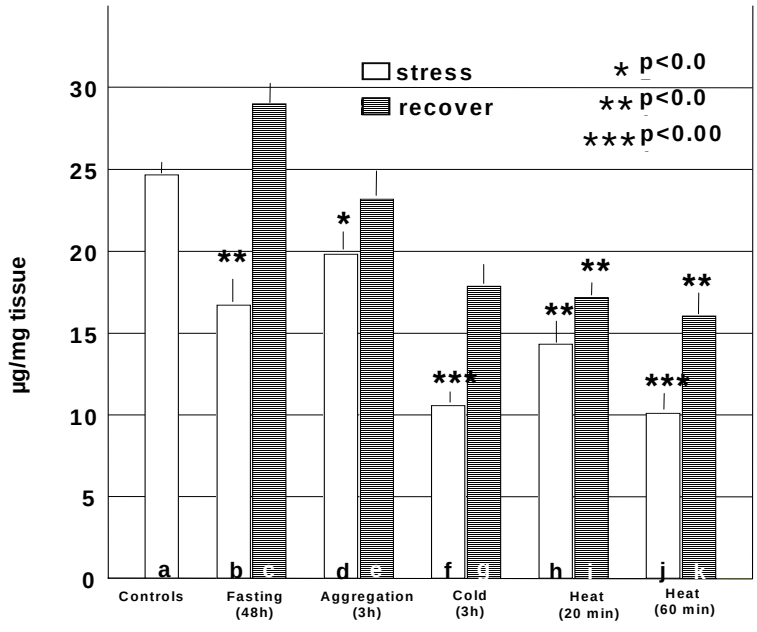

Fig. 2. *Cholesterol concentration in the adrenals during rats exposure to various stressors (**b** – fasting (48 h); **d** – crowding (3 h); **f** – cold (3 h); **h** – heat (20 min); **j** – heat (60 min). To examine the effects of stress termination the rats were returned to the control conditions (**c** – refeeding (48 h); **e** – 2 rats per cage (3 h); room temperature for 3 h (**g**), 20 min (**i**) and 60 min (**k**). The data are presented as means ± S.E.M. of six animals in μg/mg tissue. Differences between the groups: b:a $p<0.01$; d:a $p<0.05$; f:a $p<0.001$; h:a $p<0.01$; j:a $p<0.001$; i:a $p<0.01$; k:a $p<0.01$; f:d $p=0.01$; j:d $p<0.05$; b:c $p<0.05$; f:g $p<0.05$; j:k $p<0.05$.*

CHOL concentration in the adrenals was significantly decreased as a result of exposure of rats to various stressors (b:a $p<0.01$; d:a $p<0.05$; f:a $p<0.001$; h:a $p<0.01$; j:a $p<0.001$) which indicates an increased CORT synthesis (Fig. 2). The most intense CORT synthesis, as estimated by the larger decrement of CHOL, was observed after a 60-min heat and a 3-h cold exposure in respect to both the controls (f:a, j:a, $p<0.001$) and animals exposed to the crowding stress (f:d, $p=0.01$; j:d $p<0.05$). A 48 h refeeding of previously fasted rats and a 3 h recovery of restrained and cold stressed animals completely restored adrenal CORT synthesis, as estimated by changes in the CHOL concentration, which still remained low after a 20 and 60 min recovery from the heat stress (i:a, k:a, $p<0.01$).

Figure 3 illustrates that CORT concentration was markedly increased in the serum of all the stressed animals as compared to the controls (b:a, $p<0.05$; d:a $p<0.01$, f:a $p<0.001$, h:a $p<0.01$, j:a $p<0.001$), which confirms the intense glucocorticoid secretion in response to stressors. The exposure to extreme environmental temperatures (6 °C for 3 h and 38 °C for 60 min) produces a higher increment of CORT secretion in respect to controls. CORT secretion returned to the control level only after the rats were refed during 48 h.

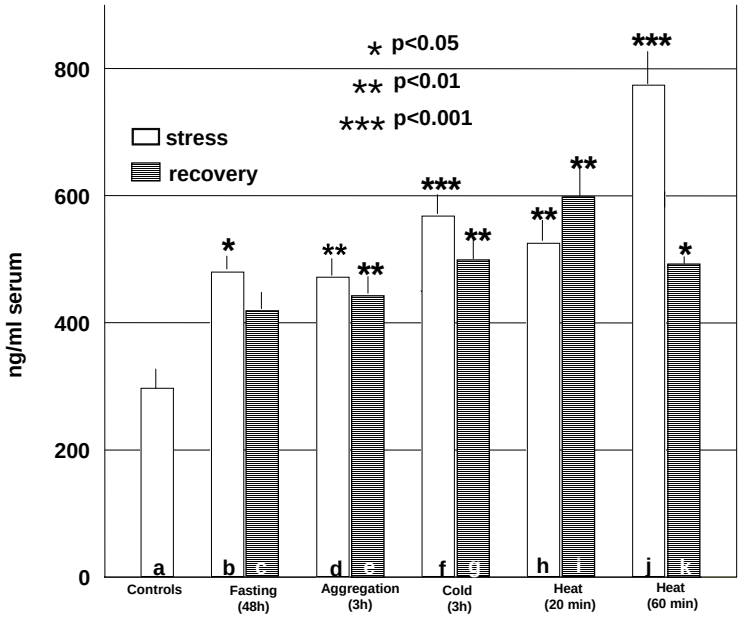

Fig. 3. *Corticosterone serum levels during rats exposure to various stressors (**b** – fasting (48 h); **d** – crowding (3 h); **f** – cold (3h); **h** – heat (20 min); **j** – heat (60 min). To examine the effects of stress termination the rats were returned to the control conditions (**c**- refeeding (48 h); **e** – 2 rats per cage (3 h); room temperature for 3 h (**g**), 20 min (**i**) and 60 min (**k**). The data are presented as means ± S.E.M. of six animals in ng/ml. Differences between the groups: b:a $p<0.05$; d:a $p<0.01$; f:a $p<0.001$; h:a $p<0.01$; j:a $p<0.001$; e:a $p<0.01$; g:a $p<0.01$; i:a $p<0.01$; k:a $p<0.05$; j:b $p<0.05$; j:d $p<0.05$; b:c $p<0.05$; j:k $p<0.05$.*

Discussion

Our results support previous findings that stressors activate the pituitary and adrenal cortex, which are functional parts of the HPA axis (Marti *et al.* 1994). Plasma ACTH concentration was elevated under the influence of all types of stressors applied, but quantitatively differently. The most intense ACTH increase was provoked by a 60 and 20 min heat exposure, as well as by crowding stress, being 15, 9, and 4 fold greater than the controls, respectively. These values remained enhanced after the animals were returned and maintained under the control conditions during a period equal to that of stress duration. On the other hand, fasting and cold stress were weaker stressors, as compared to those of heat and crowding, as they produced a 2.4 fold increment of ACTH concentration. This is in agreement with findings of Goldstein *et al.* (1996) who reported that the increment in plasma ACTH was larger under the influence of immobilization stress in respect to cold stress and insulin-induced hypoglycemia. The data of Van Oers *et al.* (1996) led to the conclusion that only 60 % of hypoglycemia-induced ACTH secretion is driven by CRH itself and the remaining 40 % is caused by CRH-independent mechanisms (probably also originating from the hypothalamus). De Goeij *et al.* (1992) also showed that during immobilization stress the CRH secretion rate is 9 fold higher than that observed during hypoglycemia. The pituitary response to acute stress is rapid and so is the return to the prestress level, except for CORT (Kant *et al.*

1989). The results of De Souza and Van Loon (1982) showed that the peak plasma ACTH response to a single restraint stress occurred at 2.5-5 min after the onset of the stress, returning to the basal concentration by 30 min. The plasma CORT concentration peaked at 15-30 min after the onset of restraint stress and returned to the control range by 60-90 min.

All applied stressors activated the adrenals and led to both intense CORT synthesis and secretion in a specific manner. The greatest increase of CORT synthesis was observed under the influence of environmental stressors, heat and cold, lasting for 60 min and 3 h, respectively, each producing a 2.3 fold decrement in the adrenal cholesterol concentration. CORT synthesis was followed by an intense CORT secretion under the influence of a 60 min heat and 3 h cold exposure. Crowding, as a psychological stressor, seems to be the weakest stressor as concerns the activation of CORT synthesis. This is in agreement with the findings of Briski (1996) who compared the effects of psychological and physical stressors on peripheral CORT concentrations. Plasma CORT levels were elevated in response to each stressor, being significantly greater in response to physical one. CORT synthesis and secretion remained elevated for 20 and 60 min after the termination of the heat stress. Otherwise, a 3-h recovery period of previously restrained and cold stressed rats completely restored adrenal CORT synthesis, but the hormone concentration in the circulation remained elevated probably due to its 90 min half-life.

Thus, the animal exposure to ambient temperature of 38 ºC appears to be the strongest stressor activating the HPA system. The HPA axis is triggered immediately after heat exposure, being further activated by a longer exposure, as judged by both ACTH and CORT release, remaining elevated after the stress termination when the animals had been returned to room temperature. With respect to the role of HPA system in transducing metabolic signals, fasting seems to be the weakest stressor given that its effect resulted in the smallest increase in the blood ACTH concentration as well as CORT synthesis and secretion and owing to the fact that in refed rats the HPA system function was fully recovered.

In conclusion, the stress-induced response of HPA system seems to be stressor-specific, probably having distinct central and peripheral pathways and mechanisms of regulation. This is in agreement with the results of Dobrakovova *et al.* (1987) who showed that after 1 min of handling, but not after 5 min of noise or exposure to blood, the plasma ACTH and CORT were elevated in the rat. Komesaroff and Funder (1994) also demonstrated that the ACTH and cortisol responses in the sheep were greater after insulin-induced hypoglycemia than those after audiovisual stress.

Our results are consistent with the concept of Goldstein *et al.* (1996) which suggests that each type of stressor has its own central neurochemical and peripheral neuroendocrine «signature», with quantitatively and qualitatively distinct mechanisms.

Acknowledgements

We are grateful to Mrs. Danka Filipovic for language editing of the manuscript. This paper was supported by the Serbian Ministry of Science, Technologies and Development, Grant No 1550.

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