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Effect of acute heat stress on rat adrenal glands: a morphological and stereological study

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Summary

The morphological and stereological structure of rat adrenal gland was analysed by light microscopy after an acute (60 min) exposure to high ambient temperature (38°C). A significant increase in plasma corticotrophin (ACTH) and serum corticosterone (CORT) concentrations was observed, confirming that acute heat exposure has a strong stressful effect. Under these conditions the adrenal gland mass and volume were decreased, probably as the consequence of adrenal cortex reduction, especially that of the zona fasciculata (ZF). Histological examination revealed that many ZF cells were deprived of lipid droplets. Fibrosis was observed in all parts of the adrenal gland, both cortex and medulla, of heat stressed animals. Mitotic figures were absent in cortical cells after heat exposure, but there were no differences in ZF and zona reticularis (ZR) small blood vessels compared to nonstressed controls.

Key words: heat stress, rat, adrenal gland, histology.

Introduction

A major neuroendocrine mechanism in a stress reaction is the activation of the hypothalamic–pituitary–adrenal (HPA) axis, resulting in a rapid increase in circulating corticotrophin (ACTH) and subsequent rise in glucocorticoids, which are critical for successful adaptation (Dallman et al., 1992). Thus, plasma levels of ACTH and glucocorticoids are a good indicator of stress response intensity, particularly in its acute phase (Pignatelli et al., 1998).

Bearing in mind that ACTH is the major stimulator of adrenal cortical function, investigation of their relationship is of great importance in both basal and stress conditions. Short-term ACTH treatment provokes a decrease in volume of the lipid-droplet compartments in rat zona glomeruloza (ZG) cells, and a rise in plasma and intracellular concentrations of corticosterone and aldosterone (Mazzocchi et al., 1986). ZG growth (i.e. the volume of the ZG and its parenchymal cells) is stimulated by angiotensin II, sodium deficiency, potassium loading, ACTH and prolactin, and inhibited by somatostatin. Rat ZG hyperplasia and mitotic activity were found to be induced by chronic ACTH and cAMP treatment (Lewinski and Szkudlinski, 1981; Payet and Lehoux, 1982). It was shown that ACTH itself, or stress-induced increased ACTH secretion, exert tropic (short-term) and trophic (long-term) effects on the adrenocortical zona fasciculata (ZF) and zona reticularis (ZR). Tropic effects involve an immediate increase in corticosteroid hormone secretion, which appears about 10 min after the beginning of stress and reaches the maximum 15–30 min later (Jaanus et al., 1970; Normand et al., 1982). The trophic effect of ACTH involves an increase in adrenal mass and in the steroidogenic capacity of adrenocortical cells (Nussdorfer, 1986).

Other results suggest that the HPA axis is activated in any type of stress (acute or chronic), but the adrenal gland response varies (Nussdorfer and Mazzocchi, 1983; Robba et al., 1985; Mazzocchi et al., 1986). Taking all this into consideration, as well as the fact that there are very few reports on morphological and quantitative analysis of adrenal glands after acute heat exposure, we decided to determine the effect of high ambient temperarture on rat adrenal gland morphology.

Materials and methods

Male Wistar rats *Rattus norvegicus* Berkenhaut 1769, weighing 200±20 g, were acclimated to 22±1°C, maintained under a 12 h:12 h light:dark regime, and fed a commercial rat food and water *ad libitum*. The rats were divided into two groups, each consisting of five animals. The first group were intact controls. The rats from the second group were exposed to an ambient temperature of 38°C for 60 min before sacrifice.

Animals were weighed and decapitated. The body mass of control rats was 203±9.79 g (mean ± S.E.M.), and 190.2±7.90 g for heat stressed rats. Animals were decapitated by guillotine (Harward-Apparatus, Holliston, MA, USA). The left adrenal gland was quickly excised, freed of fat tissue (4°C) and weighed. Blood was collected from the trunk, divided into two sets of tubes, and EDTA added to obtain plasma. Serum and

plasma were frozen for the ACTH and corticosterone (CORT) determination. Serum CORT was determined using a RIA kit (Biochemicals, Costa Mesa, CA, USA) and the values expressed as ng CORT ml^{-1} serum. Plasma ACTH concentration was determined by a chemiluminescence method using an IMMULITE automatic analyzer (DPC, Los Angeles, CA, USA). The values are expressed as pg ACTH ml^{-1} plasma. This protocol has been approved by the Canadian Council on Animal Care.

The total volume of the adrenal gland was determined according to Swinyard (1938). Relative adrenal mass was calculated as a % of body mass. Each left adrenal gland was fixed in Bouin's solution and embedded in paraffin, according to standard procedures. 5 μm thick sections were stained by the AZAN trichrome technique. Stereological analysis of every tenth section of adrenal gland and its components (capsule, cortex and medulla) was performed using Weibel's multipurpose test grid M_{42} by a point counting technique (Weibel et al., 1966).

The volume fractions of parenchyma cell nuclei, cytoplasm and connective tissue together with blood vessels (stroma) were estimated and the number of nuclear profiles of adrenocortical cells per unit area of section was counted at a magnification of 1000×. Ten fields of each adrenal zona (zona glomerulosa, ZG; zona fasciculata, ZF; zona reticularis, ZR) were counted in a single section from each adrenal gland. On the basis of earlier karyometric studies (Malendowiez et al., 1974), the shape coefficient β, which relates N_V to N_A and V_V and depends on the axial ratio of estimated nuclei, was assumed to be 1.382 for ZF and ZR, and 1.500 for ZG. The number of nuclei of adrenocortical cells per mm^3 was calculated according to the method of Weibel and Gomez (see Aherne and Dunnill, 1982). Since rat adrenocortical cells are mononucleic, the numerical density of nuclei corresponds to the number of cells per mm^3. The average volume V of cells and nuclei in each cortical zone was estimated from the formula: $V_{cell(nuclei)}=V_{Vcell(nuclei)}/N_{Vcell(nuclei)}$. The average diameter D of blood vessels in ZF and ZR was estimated from the formula: $D=6V_{Vblood\ vessels}/S_{Vblood\ vessels}$, where $S_v=2I/L_t$ (I, number of intersections on blood vessels; L_t, total length of test lines). The length of the blood vessels was calculated from the formula: $L=4/\pi\times V_{Vblood\ vessels}/D$.

The Student t-test was employed to determine statistically significant differences between means.

Results

A 60 min exposure of rats to heat (38°C) induced significant rises in plasma ACTH and serum CORT levels (Fig. 1).

The absolute and relative adrenal gland masses of heat-exposed rats were significantly decreased, as well as the absolute mass of adrenal cortex in comparison to controls (Table 1), probably as a consequence of the reduction of all cortical zones (ZG, ZF and ZR), especially that of ZF.

Histological investigation of adrenal gland revealed the existence of dark and light regions in the adrenal cortex of

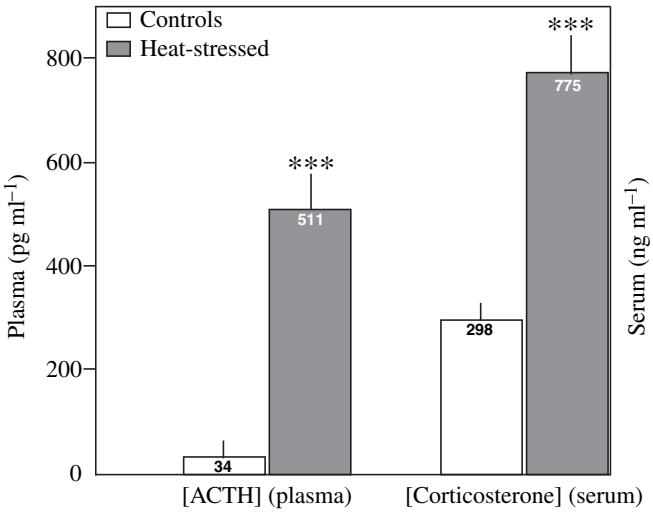

Fig. 1. Plasma ACTH and serum CORT levels in control and rats exposed to heat stress (60 min at 38°C). Values are means ± S.E.M., N=5 per group, ***P<0.001.

stressed rats (Fig. 2A). The cells in the light regions were filled with large lipid droplets (Fig. 2B), but those in the dark cortical regions were deprived of them. The whole ZG area was reduced and filled with enlarged cells and nuclei. Condensed nuclei were observed in ZR cells. Interstitial fibrosis was present in all parts of the cortical zones (Fig. 2C), as well as in the medulla. There were no mitoses visible in the adrenal glands of rats exposed to heat stress. In control rats, however, all components of the adrenal glands were clearly differentiated (Fig. 2D). ZG of control rats contained many nuclei, as compared to ZG cells in stressed rats, and many mitotic figures were present in the outer cortical zones (ZF in close proximity to ZG). The cytoplasm of ZF cells contained a moderate number of lipid droplets (Fig. 2E). All the nuclei in ZR of control animals cortex were large and light.

The volume densities of adrenal gland component and cortical zones are shown in Fig. 3. The volume density of medulla was significantly increased, and that of cortex decreased. Cortical ZG was significantly decreased, and that of ZR increased, in the rats exposed to heat stress. There were no differences in volume density of ZF between two groups of rats.

Fig. 4 illustrates that the absolute volume of adrenal glands and that of cortex, as well as the absolute volume of ZF was significantly reduced in comparison to controls.

The results of the stereological investigation of the cortical zones are shown in Table 2. The volumes of cells and nuclei in ZF and ZR were decreased and those in the ZG increased. There were no significant differences between the two groups of rats with respect to the volume of cells and their nuclei, except for ZR nuclei (P<0.05). The numerical density of cells showed a slight reduction in ZG, and augmentation in ZF and ZR. The mean diameter and lenght of blood vessels in ZF and ZR were similar in both groups of rats.

Table 1. *Absolute and relative masses of adrenal gland and all cortical zones in rat under control and heat stressed conditions*

Mass (mg)	Condition		*P* value
	Control	Stressed	
Adrenal gland			
Absolute mass	19.5±1.75	13.9±1.32	<0.05
Relative mass	9.6±0.69	7.3±0	<0.05
Capsule	1.1±0.19	0.8±0.15	NS
Cortex	17.5±1.64	12.0±1.09	<0.05
Zona glomerulosa	2.7±0.5	1.4±0.3	NS
Zona fasciculata	12.7±1.1	8.7±0.6	<0.05
Zona reticularis	2.1±0.2	1.9±0.3	NS
Medulla	0.9±0.05	1.1±0.10	NS

Values are means ± S.E.M. (*N*=5).
NS, not significant.
Relative mass, adrenal mass (mg) as % of body mass.

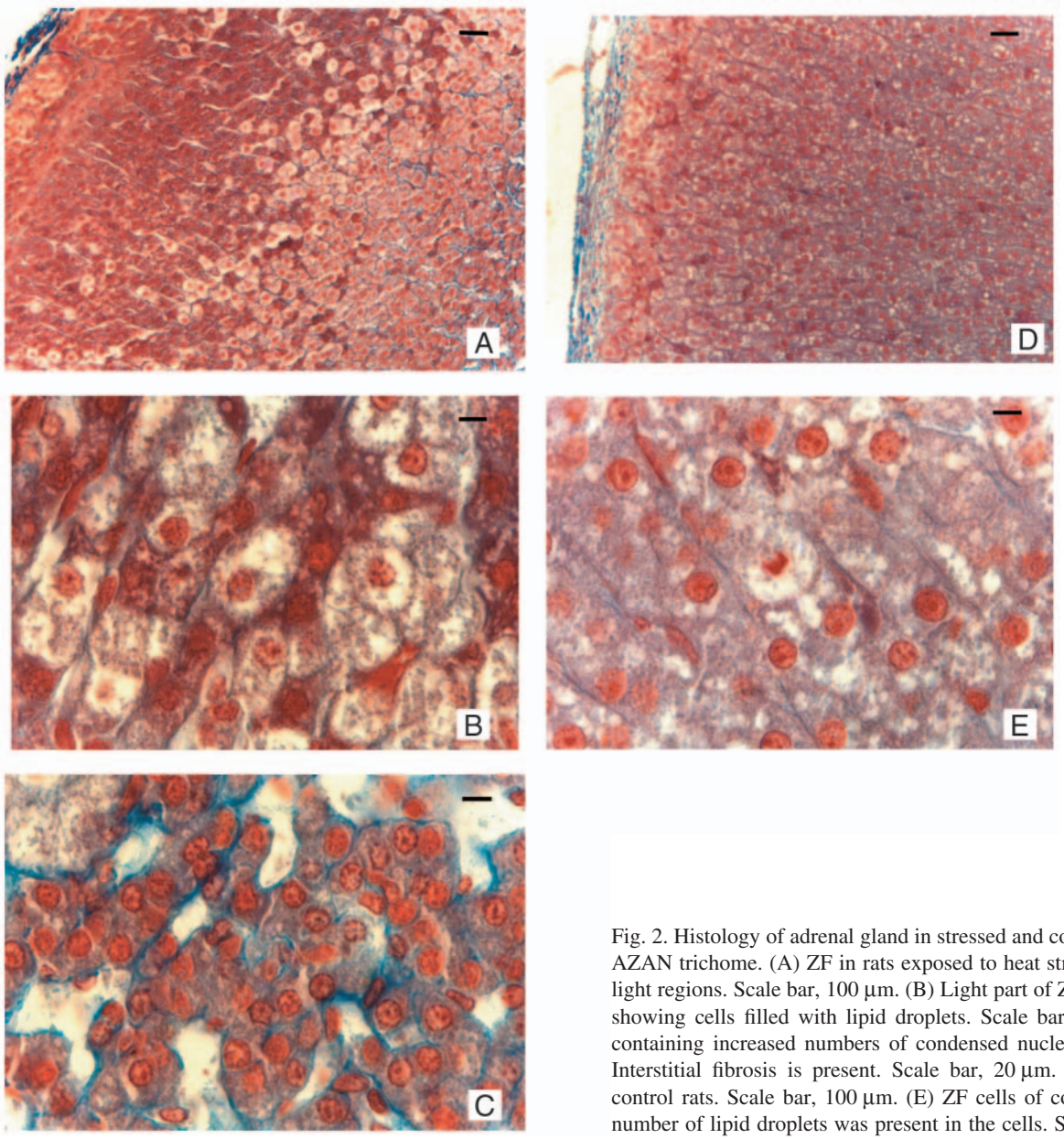

Fig. 2. Histology of adrenal gland in stressed and control rats, stained with AZAN trichome. (A) ZF in rats exposed to heat stress, showing dark and light regions. Scale bar, 100 µm. (B) Light part of ZF in heat stressed rats, showing cells filled with lipid droplets. Scale bar, 20 µm. (C) ZR cells containing increased numbers of condensed nuclei in heat stressed rats. Interstitial fibrosis is present. Scale bar, 20 µm. (D) Adrenal gland of control rats. Scale bar, 100 µm. (E) ZF cells of control rats; a moderate number of lipid droplets was present in the cells. Scale bar, 20 µm.

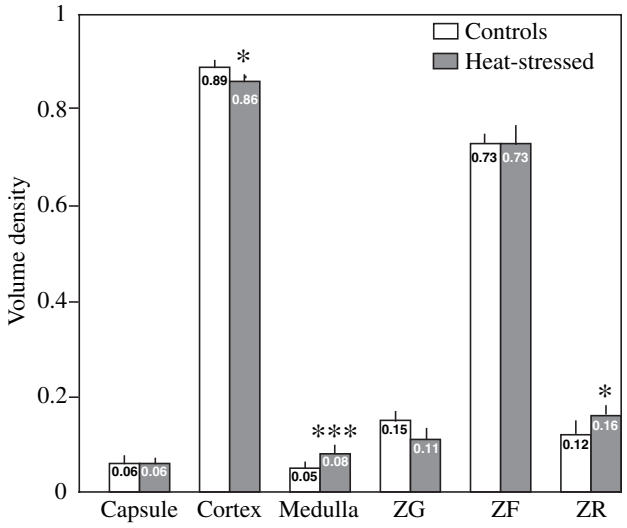

Fig. 3. Volume density of adrenal gland components and cortical zones in the control and heat stressed rats (60 min at 38°C). Values are means ± S.E.M., N=5 *P<0.05; ***P<0.001. ZG, zona glomerulosa; ZF, zona fasciculata; ZR, zona reticularis.

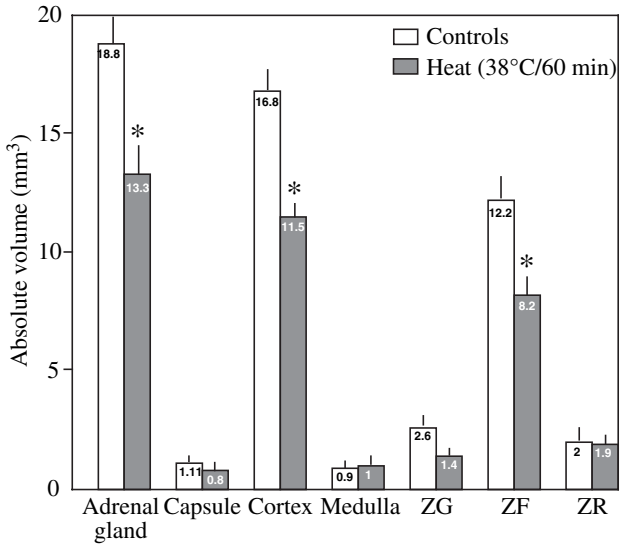

Fig. 4. Absolute volume of adrenal gland and adrenal gland components in the control and heat stressed rats (60 min at 38°C). Values are means ± S.E.M., N=5 *P<0.05. ZG, zona glomerulosa; ZF, zona fasciculata; ZR, zona reticularis.

Discussion

The results of the present study show that a 60 min heat (38°C) exposure of rats induced a significant rise in circulating ACTH and CORT levels which, according to Aguillera et al. (1996) and Piguatelli et al. (1996), demonstrate the intensity of the stress response, particularly in the acute phase. As the result of stimulation of the HPA axis, significant changes occurred in the adrenals.

High ambient temperature induced a significant reduction of absolute and relative adrenal mass due to the reduction of cortical mass, especially that of ZF. This was expected because glucocorticoid synthesis is performed mostly in the ZF and this is the largest part of the cortex.

Morphological and stereological studies revealed that heat exposure provoked a depletion of lipid droplets from the ZF cells and also a reduction of their volumes. The volume reduction was also observed in ZR cells, which represent the innermost layer adjacent to the medulla. Although the ZR cells were able to secrete glucocorticoids they primarily produced and secreted weak androgens. However, heat stress induced an increment of ZG cell volume. These cells are responsible for production of aldosterone (the salt-retaining hormone), whose physiological effects correspond to the hypothalamic hormone, vasopressin (AVP). This hormone, according to Gallo-Payet and Guillon (1998), can also stimulate cortisol secretion, but not that of corticosterone, which is the dominant glucocorticoid in rats. It was shown that under conditions of stress, both ACTH and AVP secretion was increased (Aguilera, 1996), and when ACTH was secreted in large amounts, such as in conditions of stress, it could also stimulate aldosterone secretion. Thus, under the heat stress, both ACTH and AVP stimulated enlargement of ZG cell volume, the site of aldosterone accumulation. Bearing in mind that, under heat stress conditions the animals were in hyperthermia, which is characterised by increased body temperature (39.9°C *vs* 37.2°C in controls, P<0.001) and accompanying salivation, resulting

Table 2. *Volume of cells and nuclei, numerical density of cells, mean diameter and length of blood vessels in the cortex*

	Condition		
	Control	Stressed	*P* value
Volume (μm^3)			
Cell			
ZG	739±26	807±107	NS
ZF	1712±279	1546±156	NS
ZR	844±98	635±56	NS
Nuclei			
ZG	179±17	207±20	NS
ZF	212±25	205±26	NS
ZR	156±14	115±11	<0.05
Numerical density per mm^3 (1×10^3)			
ZG	836±34	787±114	NS
ZF	420±52	428±47	NS
ZR	709±79	927±94	NS
Mean diameter of blood vessels (μm)			
ZF	7.6±1.10	6.8±0.32	NS
ZR	11.1±0.90	11.6±0.86	NS
Length of blood vessels (μm)			
ZF	0.0444±0.0046	0.0421±0.0044	NS
ZR	0.0406±0.0034	0.0390±0.0037	NS

Values are means ± S.E.M. (N=5).
NS, not significant.

in water and salt loss. The increased amount of aldosterone is necessary to re-establish osmotic homeostasis.

In the unstimulated adrenal gland, intracortical capillaries were constricted, and after operative stress, or following a 1 h period of ACTH perfusion, they become massively expanded (Pudney et al., 1981). In spite of the fact that the ACTH level in heat stressed rats was very high, the mean diameter and length of blood vessels in the ZF and ZR regions did not significantly change. Another type of acute stress is ethanol injection, which also caused a significant rise in ACTH and CORT levels (Rivier, 1996; Rivier and Lee, 1996), and induced the opposite morphological and stereological changes in adrenal glands (Milovanović et al., 2003) to those observed by us on heat stress. That is, ethanol provoked increased ZF and ZR and decreased ZG cell volumes with dilated small blood vessels and prominent hyperemia. These authors did not find any sign of fibrosis such as we found after heat stress. This could support the idea that adrenal gland responds differently to the various stressors (Pacak and Palkovits, 2001), despite the same reaction in the hypothalamic–pituitary part of the HPA axis.

There are reports suggesting that the outer part of the adrenal glands, particularly the ZG, as the source of new cortical cells, is the major site of mitosis and that most cell deaths occur within the inner part of the cortex, particularly in the ZR (Wright and Voncina, 1977; Stachowiak et al., 1990; Miyamoto et al., 2000). We also observed mitoses in ZG and ZF in control rats, but did not find any sign of mitoses in rat adrenal gland after heat stress. This phenomenon could be explained by the great sensitivity of mitotic cells to hyperthermia, in contrast to the interphase cells. Hyperthermia induces disruption of the mitotic spindle, a reappearance and an extension of the Golgi apparatus, an inactivation of microtubule nucleation, and a disorganization of the centrosome (Debec and Marcaillou, 1997). Regulation of the cell cycle during thermal stress involves many heat shock proteins. The 47 kDa heat shock protein (HSP 47) is a collagen-binding glucoprotein that is heat-inducible and sensitive to malignant transformation (Nagata et al., 1986; Nagata and Yamada, 1986). It is involved in collagen processing and/or secretion under normal conditions. Under stress conditions, HSP 47 synthesis is increased, which correlates with collagen synthesis in several cell lines (Nagata and Yamada, 1986; Takechi et al., 1992; Kudo et al., 1994). Schiaffonati et al. (1991) showed that after a 50 min heat exposure (41°C), there was an immediate increase in Hsp70 and Hsp90 levels in rat liver. Inaguma et al. (1995) suggested that induction of Hsp27 and Hsp70 in various tissues of heat stressed rats (20 min exposure, 42°C), including adrenal glands, is controlled by a physiological process(es) that is sensitive to ethanol and prazosin and is operative for a short time during the application of heat stress. These literature reports confirm and explain an interstitial fibrosis in all parts of the adrenal gland in rats after heat stress, as we found in the present study.

In conclusion, acute exposure to heat significantly increased the circulating ACTH and CORT concentrations in rats. A high ambient temperature reduced the absolute and relative mass of the adrenal cortex, especially that of ZF, which is partially depleted of lipid droplets. Fibrosis was present and mitotic figures were absent in all parts of adrenal glands of rats exposed to heat stress.

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