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Morphology and functional responses of isolated inner adrenocortical cells of rats infused with interleukin-ß

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Summary. The effects of the prolonged infusion with interleukin-1ß (IL-1ß) (20 $pM.kg^{-1}.min^{-1}$) on the function and morphology of the isolated inner cells of the rat adrenal cortex were investigated. After 3 and 5 days of IL-1ß infusion, the level of circulating ACTH was below the control level, while the plasma concentration of corticosterone was strikingly elevated. After 5 days of infusion, isolated inner adrenocortical cells showed an enhanced basal and ACTH-stimulated corticosterone secretion, and showed a conspicuous hypertrophy. The acute exposure to IL-1ß 10^{-6} M did not affect the secretory activity of dispersed cell from either control or IL-1ß-infused rats. These findings indicate that the prolonged exposure to high levels of circulating IL-1ß, like those occurring during chronic inflammatory diseases, is able to enhance the growth and steroidogenic (glucocorticoid) capacity of the rat inner adrenocortical zones. Moreover, they suggest that the mechanism underlying this adreno-corticotrophic effect of IL-1ß does not involve either a stimulation of the hypophyseal ACTH release or a direct stimulatory effect of monokine on adrenocortical cells. It is suggested that IL-1ß may activate an intra-adrenal paracrine regulatory mechanism.

Key words: Interleukin-1ß, Adrenal cortex, Steroidogenesis, Rat, Isolated cells, Electron microscopy

Introduction

Interleukin-1ß (IL-1ß), a polypeptide monokine, which is released by activated macrophages and monocytes during immune responses (Dinarello, 1984, 1988), stimulated hypophyseal ACTH release and consequently enhances the level of circulating glucocorticoids (Sapolsky et al., 1987; Uehara et al., 1987; Malarkey and Zvara, 1989; Rivier et al., 1989; Suda et al., 1989, 1990; Tsagarakis et al., 1989; Naito et al., 1990; Andreis et al., 1991b). However, in rats chronically infused (3 days) with IL-1ß the plasma concentration of ACTH is decreased below the basal level, while that of corticosterone is elevated (Kasprzak et al., 1991).

To gain insight into the mechanism underlying this unexpected finding, it seemed worthwhile to investigate the morphology and functional responses of isolated inner adrenocortical cells obtained from rat chronically infused with IL-1ß.

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Materials and methods

Adult male rats of Wistar strain (about 300 g body weight) were divided into 2 groups (n = 16), one which was subcutaneously infused for 5 days (Alzet osmotic pumps; Alza, Palo Alto, CA) with IL-1ß (20 $pM.kg^{-1}.min^{-1}$; Calbiochem, La Jolla, CA). RIA assays (preliminary data not shown) indicate that this procedure creates a steady plasma concentration of IL-1ß of about 10^{-9}M, a figure of the same order of magnitude as that found in patients with sepsis ($10^{-10}/10^{-9}$M Dinarello et al., 1984). The other group of rats was infused with 0.9% NaC1 vehicle. After 3 days of infusion, blood samples (0.5 ml) were collected from the tail vein. Rats were decapitated between 10:00 and 11:00 a.m., their trunk blood was collected, and their adrenals were promptly removed, decapsulated and quartered. Dispersed inner adrenocortical cells were prepared by enzymatic disaggregation (Szalay, 1981), as previously detailed (Andreis et al., 1989).

Some of the isolated cells from control and IL-1ß-infused rats were suspended in Medium 199 (DIFCO Labs, Detroit, MI) and aliquots of cell suspensions (3 x 10^5 cells/ml) were incubated, in replicates of 6 each,

with ACTH 10^{-8} M (Sigma, St. Louis, Mo) or without ACTH. Other isolated cells were incubated, in replicates of 6 each, with IL-1ß 10^{-6} M or without IL-1ß. The incubation was carried out in a shaking bath at 37° C for 90 min, in an atmosphere of 95% O_2 and 5% CO_2. At the end of the experiment, the incubation tubes were spun at 4° C and corticosterone concentration in the supernates was assayed (see below).

Part of dispersed cells from both groups of rats were centrifuged at 200 g for 15 min, and the resultant loose pellets were processed for electron microscopy as described previously (Andreis et al., 1989). Thick and thin sections were cut with a Supernova LKB Ultrotome. Thick sections were photographed at x 1,000, and the volume of isolated cells was determined by calculating their mean diameter, as detailed earlier (Andreis et al., 1989). Thin sections were examined in a Hitachi H-300 electron microscope. The volume (V_v) and surface densities (S_v) of the various organelles were estimated on electron micrographs at x 24,000 and x 72,000, respectively, using conventional stereological techniques (Weibel, 1979). V_v and S_v were then transformed in absolute values per cell by multiplying them by the average cell volume. Each morphometric parameter was the mean of 6 separate measurements.

ACTH was extracted from plasma (Rees et al., 1971) and its concentration was determined by RIA: ACTH-RIA kit (Nichols, Los Angeles, CA; intra- and interassay variations, 6% and 8%). Corticosterone was extracted from plasma or supernates, and purified (Sippell et al., 1978); its concentration was measured by RIA: CORTX-RIA kit (Eurogenetix, Milan, Italy; Intra- and interassay variations, 7% and 9%).

The data were averaged per experimental group, and their statistical comparison was done by ANOVA, followed by the Multiple Range Test of Duncan.

Results

Prolonged (3- or 5 days) IL-1ß infusion caused a persistent 2-fold rise in the plasma corticosterone concentration, but a significant decrease (-12%/-19%) in the level of circulating ACTH (Fig. 1).

ACTH (10^{-8} M) evoked an 8-fold increase in the corticosterone production by isolated inner adrenocortical cells of control rats. IL-1ß infusion for 5 days caused a 2-fold enhancement in both basal and ACTH-stimulated corticosterone synthesis by dispersed cells (Fig. 2). Acute IL-1ß (10^{-6} M) exposure did not affect corticosterone secretion from either control or IL-1ß-infused rats (Fig. 3).

Isolated inner adrenocortical cells from control rats displayed the ultrastructural features of typical zona fasciculata-reticularis cells. They were round or ovoid elements (of about 13 µm in diameter) and contained ovoid or elongated mitochondria with vesicular or tubulo-vesicular cristae, a rather well-developed smooth endoplasmic reticulum (SER) and many lipid

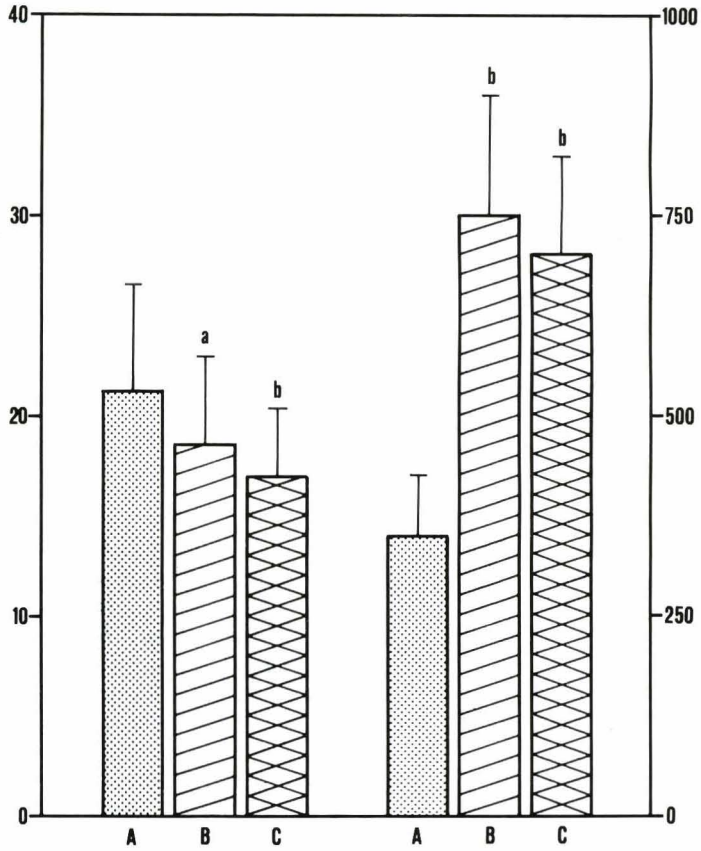

Fig. 1. Plasma concentrations of ACTH (left, $pM.l^{-1}$) and corticosterone (right, $nM.l^{-1}$) in control (A), 3-day IL-1ß infused (B) and 5-day IL-1ß-infused rats (C). Standard deviations are indicated. $^{a}p < 0.05$ and $^{b}p < 0.01$ versus control rats.

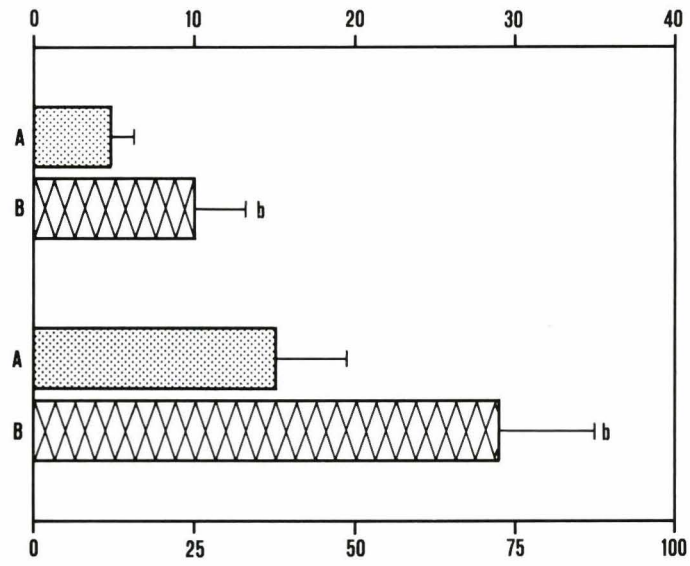

Fig. 2. Basal (top) and ACTH-stimulated (bottom) corticosterone secretion ($pM/10^5$ cells.h^{-1}) by isolated inner adrenocortical cells from control (A) and 5-day IL-1ß-infused rats (B). Standard deviations are indicated. $^{b}p < 0.01$ versus control rats.

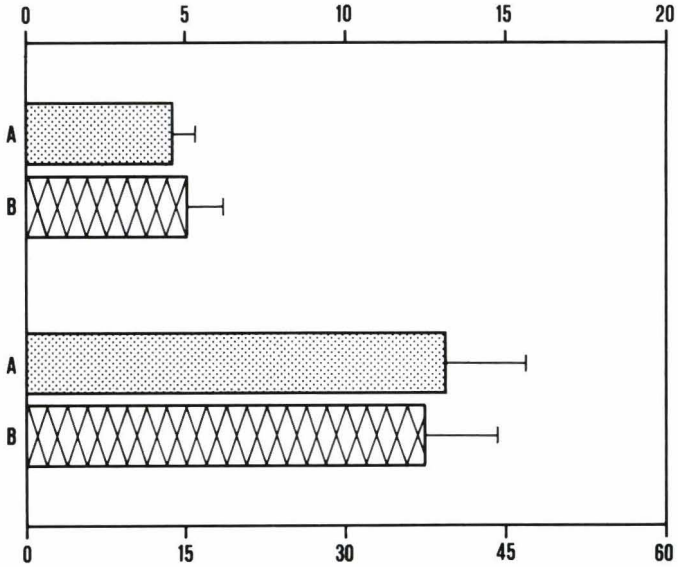

Fig. 3. Lack of effects of the acute IL-1ß exposure on corticosterone secretion (pM/10^5 cells.h^{-1}) by isolated inner adrenocortical cells from control (top) and 5-day IL-1ß-infused rats (bottom). A, control group; B, IL-1ß-exposed group. Standard deviations are indicated.

droplets (Fig. 4). IL-1ß infusion did not produce evident qualitative morphological changes, with the exception of an apparent lipid-droplet depletion (Fig. 5).

Morphometry showed that prolonged IL-1ß infusion induced a marked increase in the volume of isolated cells (42%) and their nuclei (29%) (Table 1). Stereology demonstrated that the IL-1ß-induced cell hypertrophy was associated with a significant increase in the volume of the mitochondrial compartment (41%) and in the surface area per cell of mitochondrial cristae (37%) and SER tubules (60%). Conversely, the volume of the lipid-droplet compartment was notably lowered (–37%) (Table 1).

Discussion

Our *in-vivo* results confirm that prolonged IL-1ß infusion causes a small but significant reduction in the level of circulating ACTH. Since acute administration and 24-h infusion of IL-1ß raise blood ACTH concentration (Kasprzak et al., 1991), this result may be ascribed to the exhaustion of hypophyseal corticotroph. In spite of this, the plasma concentration of corticosterone, the main glucocorticoid secreted by rat adrenals (for review, see Nussdorfer, 1986), is persistently increased. This agrees well with our *in-vitro* study, which shows that IL-1ß infusion enhances both basal and maximally ACTH-stimulated secretory activity of dispersed inner cells of rat adrenal cortices. The morphological counterpart of this IL-1ß-induced functional change is the hypertrophy of inner cells. This is mainly due to the increase in the volume of the mitochondrial compartment and to the proliferation of SER. These stereological data are in keeping with the elevated secretory capacity of dispersed cells, since the enzymes of steroid synthesis are located in both mitochondria and SER (for review, see Miller, 1988), and the changes in the surface area per cell of mitochondrial cristae and

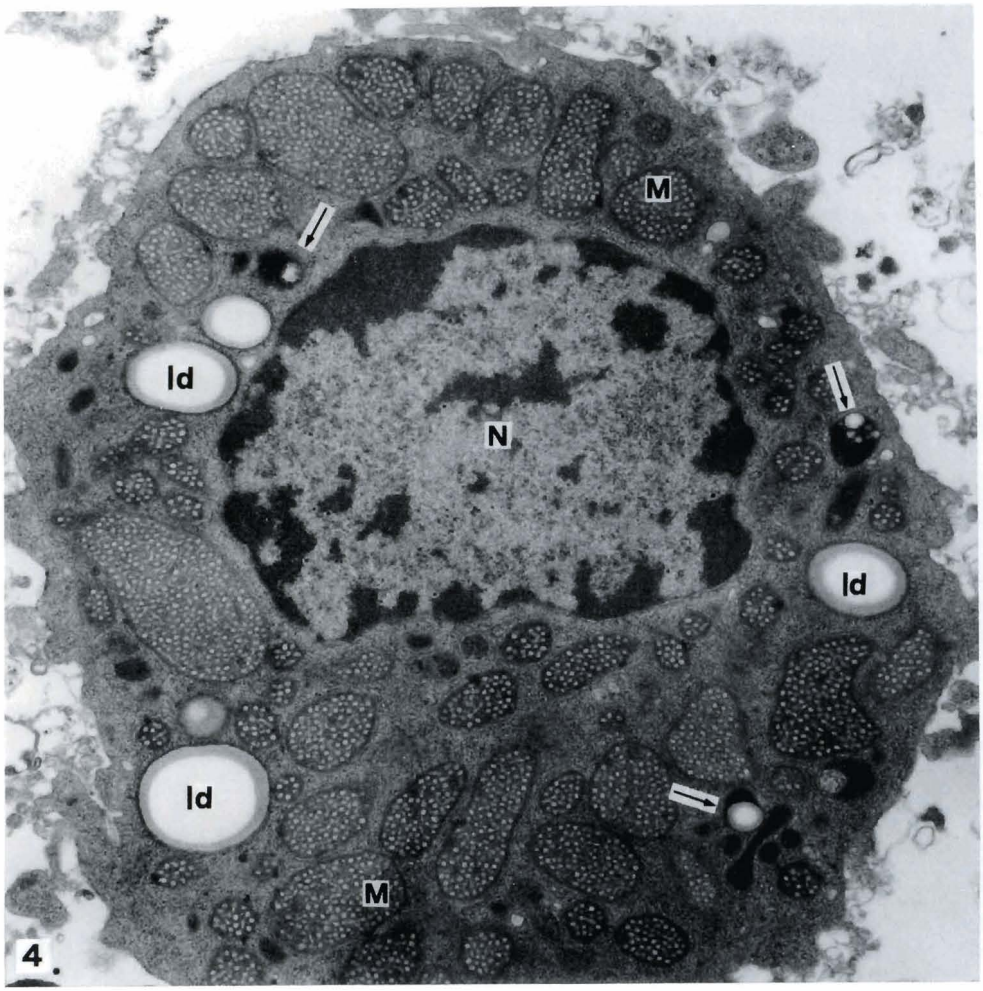

Fig. 4. Isolated inner adrenocortical cell of control rat. Mitochondria (M) show vesicular cristae, and lipid droplets (ld) are numerous. N = nucleus; arrows = dense bodies. × 16,500

Table 1. Effect of 5-day IL-1ß infusion on the morphometric parameters of isolated inner cells of the rat adrenal cortex.

	Control rats	IL-1ß-infused rats
Volume of cells (μm^3)	1318.5 ± 251.6	1984.7 ± 361.5[b]
Volume of nuclei (μm^3)	131.4 ± 18.6	169.2 ± 21.5[b]
Volume of mitochondrial compartment (μm^3/cell)	516.5 ± 150.1	733.4 ± 182.9[b]
Surface area of mitochondrial cristae (μm^2/cell)	10331.5 ± 1612.4	14154.2 ± 2512.5[b]
Surface area of SER (μm^2/cell)	6918.6 ± 1158.2	11086.6 ± 2019.3[b]
Volume of lipid-droplet compartment (μm^3/cell)	96.3 ± 25.1	61.7 ± 24.8[a]

Data are means ± S.D. (n = 6). [a]p < 0.05 and [b]p < 0.01 versus control rats.

SER tubules are closely coupled with corresponding changes in the activity of some of these enzymes (Nussdorfer and Mazzocchi, 1983). As previously suggested (Nussdorfer, 1986), an increase in membrane area is probably required to accommodate the newly synthesized enzymes of steroid synthesis. The nuclear hypertrophy, a finding frequently reported in stimulated adrenocortical cells (for review, see Nussdorfer, 1986), may be interpreted as the expression of enhanced nuclear transcription in inner adrenocortical cells; i.e. the mechanism whereby many stimulators, including ACTH, exert their adrenocorticotrophic effect (Simpson and Waterman, 1988; Miller, 1989). Lipid-droplet depletion can be the counterpart of the raised corticosterone production, since lipid droplets contain cholesterol and cholesterol

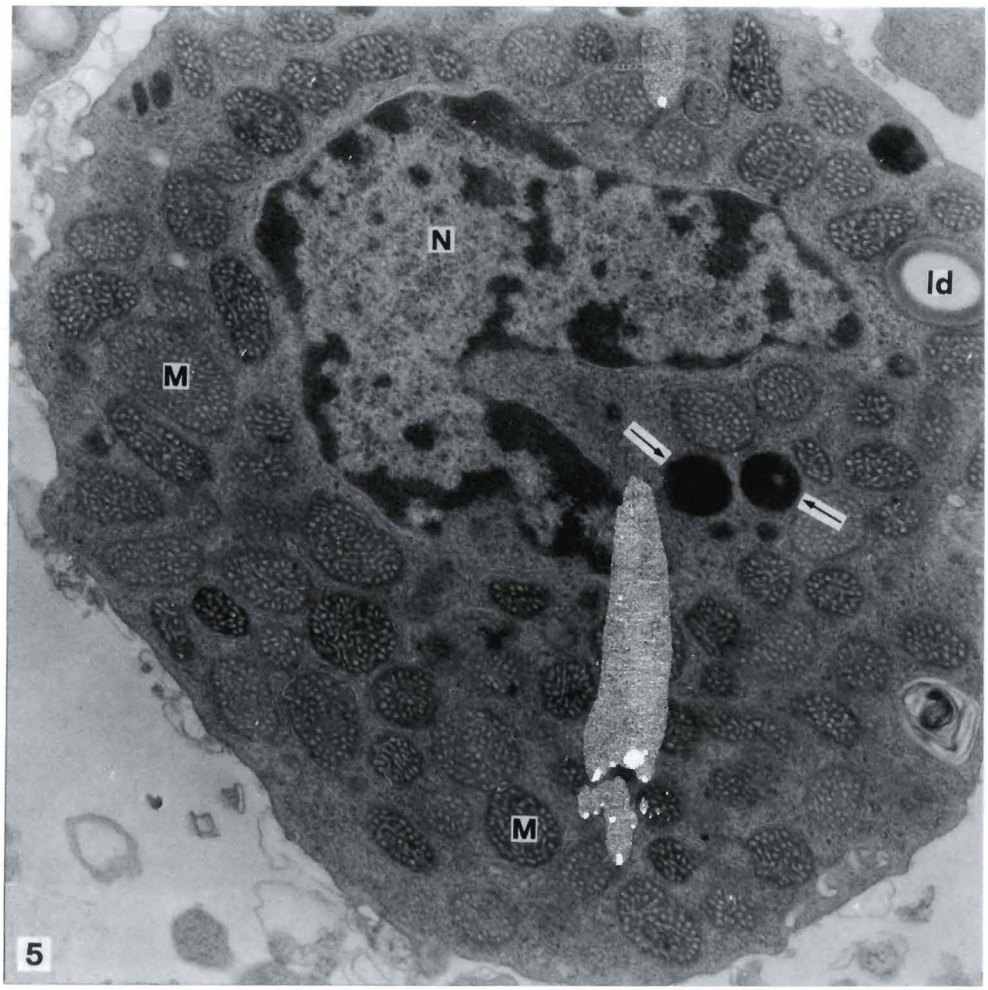

Fig. 5. Isolated inner adrenocortical cell of 5-day IL-1ß-infused rats. Note the evident lipid-droplet depletion. N = nucleus; M = mitochondria; ld = lipid droplet; arrows = dense bodies. × 14,500

esters (Moses et al., 1969; Nussdorfer, 1986), the main precursors of steroid hormones (Boyd et al., 1983).

In conclusion, our results indicate that prolonged IL-1ß administration enhances the growth and steroidogenic capacity of rat inner adrenocortical cells, independently of any stimulation of hypophyseal ACTH release. Only hypotheses can be advanced on the mechanism of such an adrenocorticotrophic effects of IL-1ß, since previous (Andreis et al., 1991b) and present findings seem to exclude the possibility of a direct stimulating effect of the monokine on rat adrenocortical cells. Paradoxically, these last results appear to be in contrast with those reported by Whitcomb et al. (1988) and Winter et al. (1990) in human and calf, respectively. Interspecific functional differences and the fact that these last authors employed cultured inner adrenocortical cells may easily explain this discrepancy.

There is recent evidence suggesting the existence of an intra-adrenal CHR/ACTH mechanism, which replicates that operating at the hypothalamo-hypophyseal level; such a mechanism would be located in the medulla and could act on the cortex in a paracrine manner (Fehm et al., 1988; Bornstein et al., 1990; Jones and Edwards, 1990a,b; Andreis et al., 1991a). There is indirect evidence that this intra-adrenal regulatory system may be involved in the acute secretory response of the adrenal cortex to IL-1ß (Andreis et al., 1991b). Thus, this local mechanism may ensure the elevated production of glucocorticoids during prolonged infective processes, when the continuous exposure to high levels of circulating IL's has exhausted hypophyseal corticotroph, the main target of these monokines.

This hypothesis is being tested in our laboratory, by employing bilaterally-adrenalectomized rats, which bear ACTH-responsive adrenocortical autotransplants always lacking medullary chromaffin cells (Belloni et al., 1990), and which therefore are deprived of such a hypothetical intra-adrenal regulatory mechanism.

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