The effect of corticotropin-releasing hormone (CRH) on the adrenal medulla in hypophysectomized rats

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Summary. CRH occurs in the adrenal medulla of rats. We were interested to know whether CRH affects medullary chromaffin cells in the absence of ACTH. We investigated the morphological changes of the adrenal medulla in Sprague Dawley rats with light and electron microscopy in normal rats, hypophysectomized rats, and hypophysectomized rats following injections of CRH (10 μg = 3 nmol for three days). Chromaffin cells were characterized by immunohistochemistry (anti-tyrosine hydroxylase). At light microscopy level chromaffin cells of hypophysectomized rats were reduced in number. On electron microscopy the number of granules and cell organelles homogenously distributed, but with CRH microscopy in normal rats, hypophysectomized rats. We were therefore interested to know whether CRH affects chromaffin cells of the adrenal medulla in the absence of pituitary ACTH. As the HPA axis and the sympatho-adrenal system obviously interact within the adrenal gland, it seems of interest to analyze chromaffin cell structure in hypophysectomized rats. We therefore investigated the adrenal medulla morphology at light and electron microscopy level in normal rats, in hypophysectomized rats, and in hypophysectomized rats following application of high doses of CRH.

Key words: Corticotropin-releasing hormone (CRH), Hypophysectomy, Adrenal medulla, Rat (Sprague Dawley)

Introduction

The adrenal gland combines two endocrine tissues of diverse embryogenetic origin under a common capsule. Traditionally, a unilateral influence of the cortex on the medulla has been assumed. In this view, adrenocortical hormones are synthesized and released from the adrenal cortex, regulated by the pituitary adrenocorticotropic hormone (ACTH) and the hypothalamic corticotropin-releasing hormone (CRH). Evidence for the regulation of adrenocortical function based on intraglandular mechanisms, independent from the hypothalamus-pituitary-adrenal (HPA) axis, has emerged (Vinson et al., 1994). A new role for the adrenal medulla to influence adrenocortical function in a paracrine manner has been suggested (Hinson, 1990). Vice versa, adrenocortical steroids are known to be regulatory factors for medullary enzymes involved in catecholamine synthesis (Wurtman and Axelrod, 1966; Axelrod and Reisine, 1984). The intimate contact of chromaffin and cortical cells is suggestive for the formation of the cellular basis for intraadrenal interactions (Bornstein et al., 1994).

Hypophysectomy leads to adrenocortical atrophy (Deane, 1962). We have previously shown that in rats such atrophy could be reduced by daily intraperitoneal (i.p.) high-dose injections of CRH (10 μg = 3 nmol) given for three days starting at day 5 after the operation (Bornstein et al., 1990a). The morphological observations of this study indicated that CRH influences the adrenal cortex via extrapituitary mechanisms. Furthermore, CRH has been described to occur within the adrenal medulla itself (Hashimoto et al., 1984; Suda et al., 1986; Aguilera et al., 1987; Engeland et al., 1987; Mazzucchelli et al., 1994).

Based on these observations we were interested to know whether CRH affects chromaffin cells of the adrenal medulla in the absence of pituitary ACTH. As both the HPA axis and the sympatho-adrenal system obviously interact within the adrenal gland, it seems of interest to analyze chromaffin cell structure in hypophysectomized rats. We therefore investigated the adrenal medulla morphology at light and electron microscopy level in normal rats, in hypophysectomized rats, and in hypophysectomized rats following application of high doses of CRH.

Materials and methods

Five-week-old male Sprague-Dawley rats were used. Ten animals were hypophysectomized and kept for...
seven days under a normal diet with water ad libitum. Hypophysectomy was performed by Mollegard Ltd., Skensved, Denmark according to standard procedures. The completeness of the hypophysectomy operation was confirmed by the interruption of animal growth, as measured by cessation of weight gain at 1 week and physical inspection of the sella turcica. Of this group, six animals received daily i.p. injections of 10 μg CRH (3 nmol) in 200 μl normal saline for three days starting five days after the operation. Four animals received saline injections i.p. Four untreated, non-hypophysectomized animals were used as controls. Eight days after the operation, the animals were fixed by Nembutal anesthesia by perfusion through the left ventricle (2% paraformaldehyde, 2% glutaraldehyde in 0.1M phosphate, pH 7.3). The adrenal glands were removed, dissected, and fixed for a further 3 h in the above fixative. The tissue slices were postfixed for 90 min (2% OsO₄ in 0.1M cacodylate pH 7.3), dehydrated in ethanol and embedded in epoxy resin. Semithin sections (0.5 μm) were stained with toluidine blue. Ultrathin sections (70 nm) were stained with uranyl acetate and lead citrate and examined at 80 kV in a Philips EM 301.

For specific staining of chromaffin cells semi-thin sections of rat adrenals were immunostained with antityrosine-hydroxylase. Briefly, the sections were immunostained using the unlabelled peroxidase antiperoxidase (PAP) method with monoclonal mouse anti human tyrosine-hydroxylase antibody (Boehringer Mannheim, Germany). The sections were preincubated for 30 min with 5% normal rabbit serum in 0.1 mol/l tris-buffered saline solution (TBS), pH 7.4. This was followed by three washing steps in TBS and by incubation with the specific antibodies, diluted 1:10 in TBS with 5% normal rabbit serum, at 4 °C overnight. The sections were washed three times in TBS for 10 min and exposed to the second antisera (rabbit anti mouse) for 60 min at room temperature. After having been washed again three times in TBS, the sections were immersed in a rabbit PAP complex at the same dilutions as the primary antibody. Visualization was achieved by incubating the slides with 3-amino 9-ethyl carbamoyl (AEC) chromogen system (Imunotech, Hamburg, Germany) as described by the manufacturer. Slides were counterstained with hematoxylin, rinsed in running water, dehydrated and mounted with gelatin. For control, the specific antisera were replaced by nonimmune rabbit serum. Nonspecific staining was not observed.

Results

In animals hypophysectomized one week prior to fixation, the rat adrenal glands were atrophic as seen by light microscopy. The capsule was thickened and the cortex thin and dense compared to normal rats (Fig. 1a,b). In addition, the cortex and the medulla were separated by fibrous tissue located mainly in the zona reticularis; this was not present in control animals. In the medulla the number of chromaffin cells, which formed strings around a network of dilated veins, was reduced (Fig. 1b). Following the application of high-dose injections of CRH the atrophy of the adrenal cortex was reduced with an increased vascularization and a broadened width of the zona fasciculata. The medullary zone regained a more compact texture with an increase of chromaffin cells and less pronounced dilated veins. The fibrous tissue between cortex and medulla seen in untreated hypophysectomized rats was reduced (Fig. 1c).

Immunostaining revealed branches of chromaffin cells extending from the medulla into the cortical region. Clusters and single chromaffin cells, dispersed over the cortical region, even in subcapsular cell layers, could be identified, demonstrating the morphological interwovenness of both tissue types (Fig. 2a-d).

On electron microscopy, chromaffin cells of normal rats were identified by their characteristic presence of large populations of catecholamine-containing granules in all regions of the cytoplasm. Each granule exhibited a morphologic profile of a dense to moderate electron-dense interior with a narrow electron-lucent halo between the granular membrane and the dense core. Other organelles were scattered throughout the cytoplasm. Rough endoplasmic reticulum (RER) was occasionally observed in the form of short segments with preference for areas adjacent to the intercellular membrane. Following hypophysectomy, cortical cells were atrophic with reduced cytoplasm. There was an increase in liposomes. In chromaffin cells of hypophysectomized rats the granules were scattered inhomogenously in the cytoplasm. The granules were smaller and fewer in number. The electron-lucent halo between the granular membrane and the dense core was increased. Mitochondria and RER were reduced and found primarily in a juxtaglomerular location. The cytoplasm was reduced (Fig. 3a). After CRH treatment the cellular structure of cortical cells was restored with an increase of cytoplasm and mitochondria. Also, chromaffin cells regained a normal appearing number of catecholamine storing granules with a normal distribution of mitochondria and RER in the cytoplasm (Fig. 3b).

Discussion

This study shows that hypophysectomy affects chromaffin cell structure in the rat. This effect seems partially reversible by high-dose injections of CRH.

Recent studies revealed that the adrenal medulla releases, in addition to catecholamines, numerous neuropeptides, like vasoactive intestinal peptide (VIP), calcitonin gene-related peptide (CGRP), and CRH in response to stimulation of the peripheral end of the right splanchnic nerve (reviewed in Edwards and Jones, 1993). An intraadrenal CRH-ACTH system has been suggested based on observations like the identification of immunoreactive ACTH in adrenal glands (Suda et al., 1984), the release of ACTH (Jones and Edwards, 1990a) and of corticosteroids (Bornstein et al., 1990b) from the
Effect of CRH on the adrenal in hypophysectomized rats

adrenal in response to splanchnic nerve stimulation, and the release of ACTH in response to CRH administration (Jones and Edwards, 1990b). An influence on cortical function mediated by medullary CRH can also be assumed from reports that patients with Cushing's syndrome have been described where the syndrome could be the result of excessive CRH-like activity produced by endocrine and non-endocrine-tumours (Upton and Amatruda, 1971; Birkenhager et al., 1976; Hashimoto et al., 1980). Pheochromocytomas in particular may synthesize large quantities of CRH (Engeland et al., 1987) and cause

![Fig. 1. Light microscopy of rat adrenal gland in normal (a), hypophysectomized (b), and hypophysectomized rats treated with CRH (c). The hypophysectomized rats received injections of saline (b), or of 10 μg CRH (c) starting five days after the operation. The animals were killed eight days after the operation. a. The suprarenal medulla in normal rats appears bright in the centre of the gland and is comprised of chromaffin cells. The typical venous network with a wide central vein (arrow) is seen. b. In the hypophysectomized rat, medullary chromaffin tissue is reduced forming strings around dilated veins giving it a sponge like appearance. c. Following the application of high-dose injections of CRH the adrenal medulla regains a more compact texture. Semithin sections (0.5 μm) stained with toluidine blue. C: cortex; M: medulla; DV: dilated vein. x 50. Bar: 80 μm.]

![Fig. 2. Light microscopy of normal rat adrenal gland. a. Overview demonstrating the cortical and medullary region. x 50. Bar: 80 μm. b. Interwovenness of cortical and medullary tissue. x 100. Bar: 40 μm. c. Clusters and single chromaffin cells are found in the cortex at some places in the immediate subcapsular region (arrows). x 400. Bar: 10 μm. d. Medullary chromaffin cells surrounded by cortical cells. Original magnification: x 600. Bar: 5 μm. Semithin sections (0.5 μm) stained with toluidine blue. Chromaffin cells are immunostained with anti-tyrosine-hydroxylase. C: cortex; M: medulla.]

Effect of CRH on the adrenal in hypophysectomized rats

Fig. 3. a. Electron micrograph of chromaffin cells of hypophysectomized rats in direct contact with cortical cells of the zona glomerulosa. The cortical cells appear atrophic with large stores of lipid droplets. The chromaffin cells have a reduced number of granules and cell organelles. × 4,500. Bar: 1.5 μm. b. Electron micrograph of chromaffin cells of hypophysectomized rats treated with CRH surrounded by cortical cells. The cortical cells have regained a normal width of the cytoplasm with elongated mitochondrial cristae typical for zona glomerulosa cells (arrows). The chromaffin cells have regained an almost normal appearing structure with granules and cell organelles distributed homogenously within the cytoplasm. × 9,000. Bar: 1.0 μm. C: cortical cell; CV: dense-core granules; LD: lipid droplets; M: medullary chromaffin cell; MIT: mitochondria; N: nucleus; RER: rough endoplasmic reticulum.
Cushing's syndrome (Jessop et al., 1987). In addition, it has been shown that CRH is locally released from the adrenal medulla in response to hemorrhage (Bruhn et al., 1987) and to splanchnic nerve stimulation (Edwards and Jones, 1988).

Cell culture experiments suggest that local CRH is involved in preservation of chromaffin cell structure (Venihaki et al., 1997). The exact manner in which CRH influences the adrenal medulla remains to be elucidated. A direct influence might be assumed from reports that CRH and CRH receptors have been demonstrated in the adrenal medulla (Hashimoto et al., 1984; Suda et al., 1986; Aguilera et al., 1987; Engeland et al., 1987). A recent study showed that in hypophysectomized rats, whose adrenal atrophy had been reversed by ACTH infusion, subcutaneous infusions of alpha-helical-CRH or corticotropin-inhibiting peptide, competitive inhibitors of CRH and ACTH, evoked a further lowering of plasma cortisol concentrations and markedly enhanced adrenal atrophy (Markowska et al., 1993). These data suggest that an extrahypothalamic pituitary CRH/ACTH system may be involved in the maintenance of the growth and steroidogenic secretory activity of the rat adrenal cortex.

Application of CRH seemed to partially restore normal chromaffin cell structure in our study. Particularly chromaffin cells in the zona glomerulosa in hypophysectomized rats treated with CRH were well restored. A possible explanation for this phenomenon may be that the application of CRH in hypophysectomized animals may affect steroidogenesis in the adrenal cortex leading to the production of small amounts of cortisol which in turn may induce catecholamine synthesis. An intermingling of chromaffin and cortical cells occurs in the adrenal of various species including rats (Gallo-Payet et al., 1987; Singh and Mathew, 1987; Bornstein et al., 1991, 1994). The close morphological colocalization, as confirmed by our study, may suggest a paracrine interaction of chromaffin and cortical cells explaining observations of an extrapituitary CRH/ACTH system. The effect of CRH on chromaffin cells in hypophysectomized rats may provide further evidence for a coordinated function of these two intraadrenal systems.

In summary, our findings suggest a trophic effect of high-dose injections of CRH on chromaffin cells in the adrenal medulla of hypophysectomized rats. This observation may lend further support to the hypothesis that besides the traditional concept of the pituitary gland as a single master gland controlling adrenal cortical function, the adrenal gland may act as an integrated switchboard responding to and orchestrating inputs from two systems, the HPA axis on the one hand and the sympatoh-adrenergic system on the other.

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References


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Effect of CRH on the adrenal in hypophysectomized rats


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