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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 were decreased. Following injections of CRH the medulla regained a more compact texture with cell organelles homogenously distributed, but with chromaffin granules still being reduced in number. Immunohistochemistry allowed the identification of chromaffin cells located within the adrenal cortex. In hypophysectomized rats these cells showed fewer signs of alterations compared to cells located within the medulla itself and had recovered better after treatment with CRH. In conclusion, CRH seems to exert a trophic effect on chromaffin cells in the absence of pituitary ACTH. This observation may provide further evidence for a close interaction of the two neuroendocrine stress systems.

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 adrenocorticotropin 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; Mazzocchi 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

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seven days under a normal diet with water ad libitum. Hypophysectomy was performed by Møllegard 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 under 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% OsO_4 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 anti-tyrosine-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 antiserum (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 carbaxol (AEC) chromogen system (Immunotech, 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 morphological 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

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

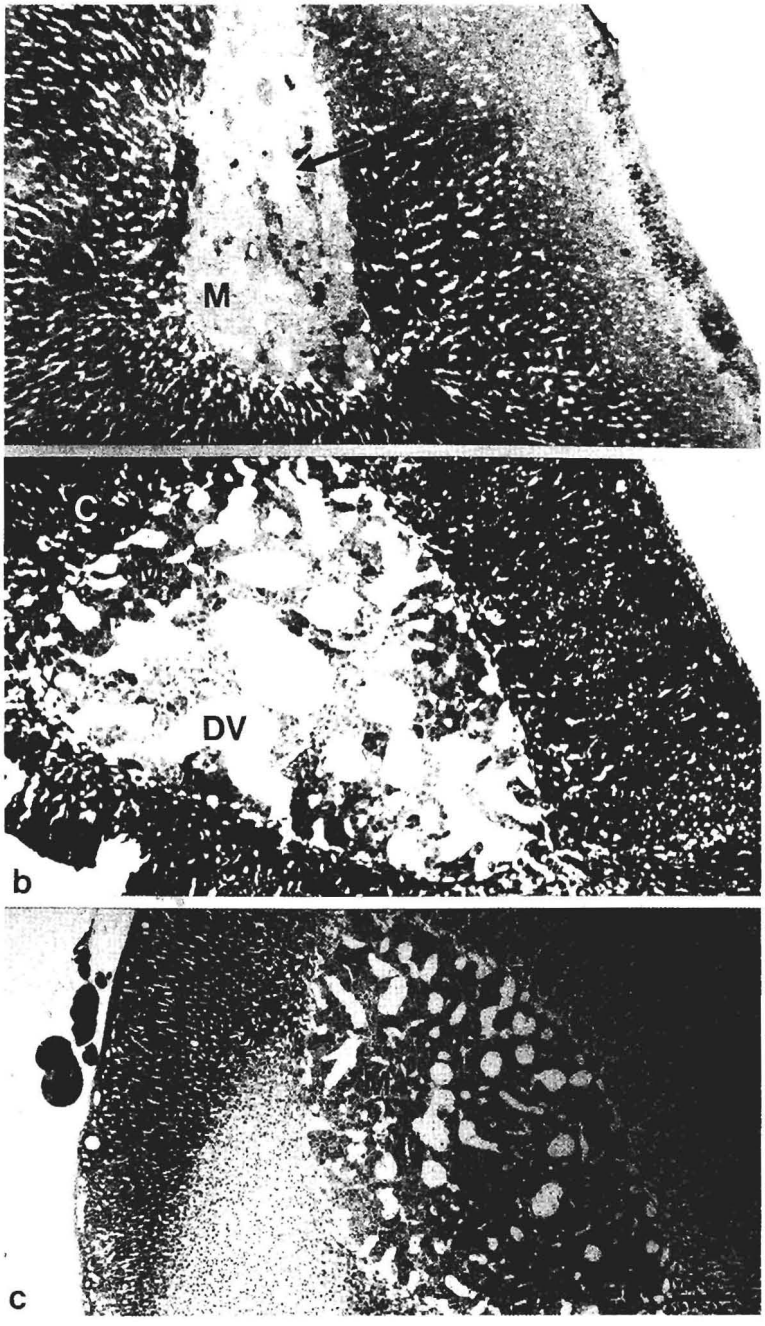
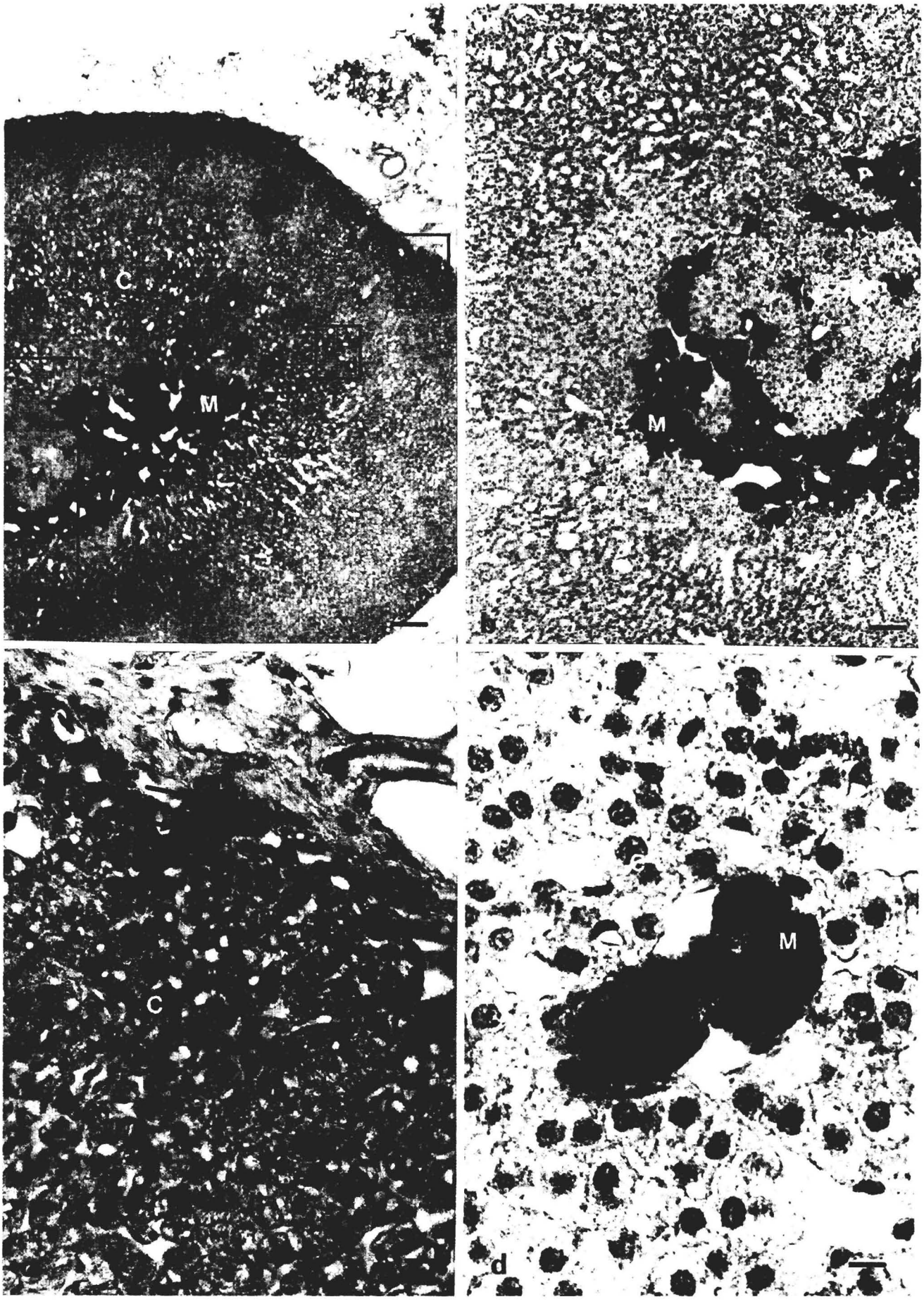


Fig. 1. Light microscopy of rat adrenal gland in normal (a), hypophysectomized (b), and hypophysectomized rats treated with CRH (c). The hypophysectomized rats received injections of saline (b), or of 10 μg CRH (c) starting five days after the operation. The animals were killed eight days after the operation. **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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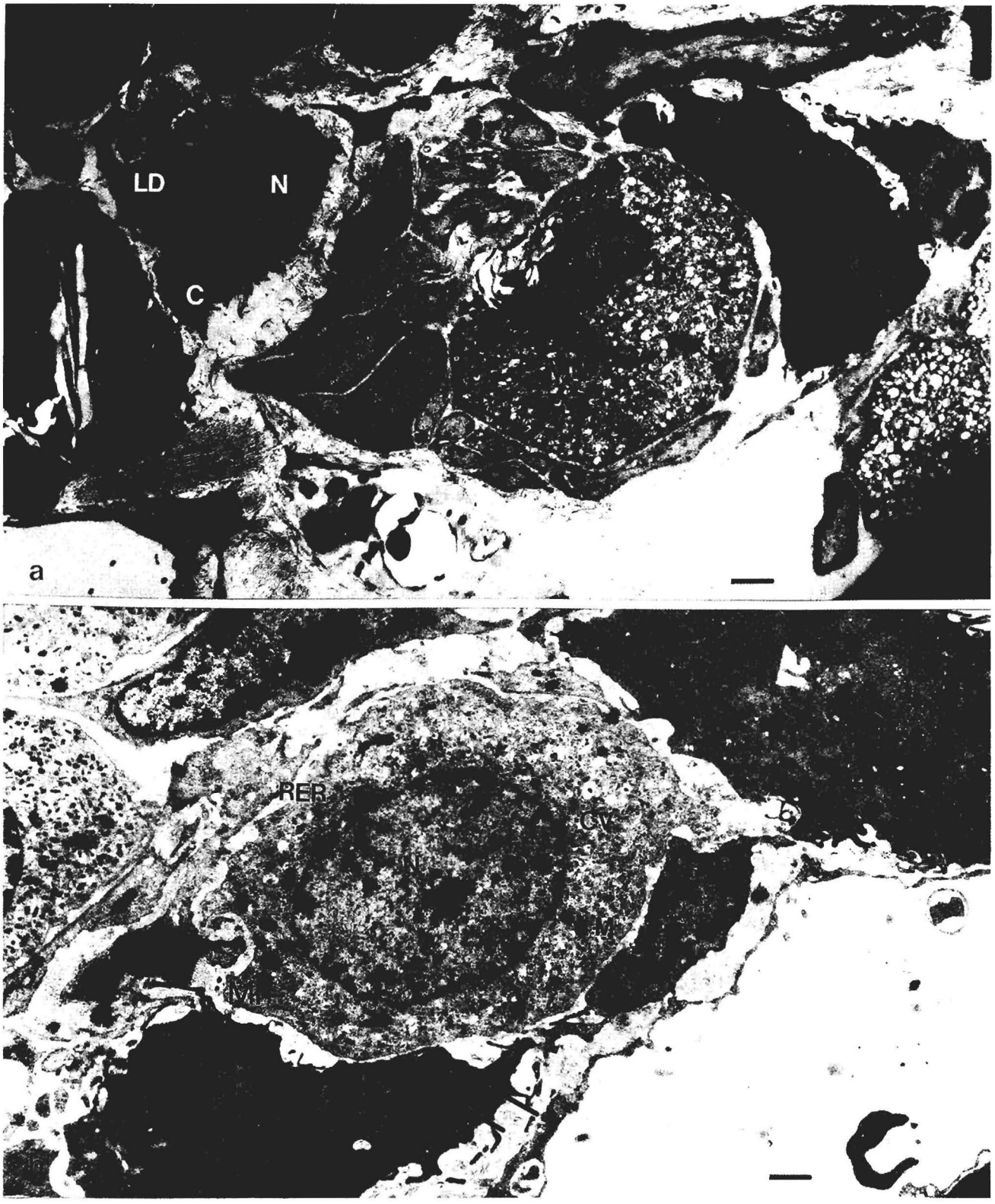

Fig. 3. a. Electron micrograph of chromaffin cells of hypophysectomized rats in direct contact with cortical cells of the zona glomerulosa. The cortical cells appear atrophic with large stores of lipid droplets. The chromaffin cells have a reduced number of granules and cell organelles. x 4,500. Bar: 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. x 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, subcutanous infusions of alpha-helical-CRH or corticotropin-inhibiting peptide, competitive inhibitors of CRH and ACTH, evoked a further lowering of plasma corticosterone 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 axis. 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 sympatho-adrenergic system on the other.

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References

Aguilera G., Millan M.A., Hauger R.L. and Catt K.J. (1987). Corticotropin-releasing factor receptors: Distribution and regulation in brain, pituitary, and peripheral tissues. Ann. NY Acad. Sci. 512, 48-66.

Axelrod J. and Reisine T.D. (1984). Stress hormones: their interaction and regulation. Science 224, 452-459.

Birkenhager J.C., Upton G.V., Seldenrath H.J., Krieger D.T. and Tashjian A.H. (1976). Medullary thyroid carcinoma: ectopic production of peptides with ACTH-like, corticotropin-releasing factor-like and prolactin production-stimulating activities. Acta Endocrinol. 83, 280-292.

Bornstein S.R., Ehrhart-Bornstein M., Scherbaum W.A. and Pfeiffer E.F. (1990a). Adrenocortical atrophy of hypophysectomized rats can be reduced by corticotropin-releasing hormone (CRH). Cell Tissue Res. 260, 161-166.

Bornstein S.R., Ehrhart-Bornstein M., Scherbaum W.A., Pfeiffer E.F. and Holst J.J. (1990b). Effects of splanchnic nerve stimulation on the adrenal cortex may be mediated by chromaffin cells in a paracrine manner. Endocrinology 127,900-906.

Bornstein S.R., Ehrhart-Bornstein M., Usadel H., Böckmann M. and Scherbaum W.A. (1991). Morphological evidence for a close interaction of chromaffin cells with cortical cells within the adrenal gland. Cell Tissue Res. 265, 1-9.

Bornstein S.R., González-Hernández J.A., Ehrhart-Bornstein M. and Adler G., Scherbaum W.A. (1994). Intimate contact of chromaffin and cortical cells within the human adrenal gland forms the cellular basis for important intraadrenal interactions. J. Clin. Endocrinol. Metab. 78, 225-232.

Bruhn T.O., Engeland W.C., Anthony E.L.P., Gann D.S. and Jackson I.M.D. (1987). Corticotropin-releasing-factor in the dog adrenal medulla is secreted in response to hemorrhage. Endocrinology 120, 25-33.

Deane H.W. (1962). The anatomy, chemistry, and physiology of adrenocortical tissue. In: Handbuch der experimentellen Pharmakologie. Vol 14. Part 1. Deane H.W. (ed). Springer. Berlin, Göttingen, Heidelberg. pp 1-185.

Edwards A.V. and Jones C.T. (1988). Secretion of corticotropin-releasing factor from the adrenal during splanchnic nerve stimulation in conscious calves. J. Physiol. 400, 89-100.

Edwards A.V. and Jones C.T. (1993). Autonomic control of adrenal function. J. Anat. 183, 291-307.

Engeland W.C., Anthony E.L.P., Gann D.S. and Jackson I.M.D. (1987). Corticotropin-releasing factor in the adrenal medulla. Ann. NY Acad. Sci. 512, 115-128.

Gallo-Payet N., Pothier P. and Isler H. (1987). On the presence of chromaffin cells in the adrenal cortex: their possible role in adrenocortical function. Biochem. Cell Biol. 65, 588-592.

Hashimoto K., Takahara J., Ogawa N., Yunoki S., Ofuji T., Arata A., Kandea S. and Terada K. (1980). Adrenocorticotropin, ß-lipotropin, ß-endorphin and corticotropin-releasing factor-like activity in an adrenocorticotropin-producing nephroblastoma. J. Clin. Endocrinol. Metab. 50, 461-465.

Hashimoto K., Murakami K., Hattor T., Nimi M., Fujino K. and Ota Z. (1984). Corticotropin-releasing factor (CRF)-like immunoreactivity in the adrenal medulla. Peptides 5, 707-712.

Hinson J.P. (1990). Paracrine control of adrenocortical function: A new role for the medulla? J. Endocrinol. 124, 7-9.

Jessop D.S., Cunnah D., Millar J.G.B., Neville E., Coates P., Doniach I.,

Besser G.M. and Rees L.H. (1987). A Phaeochromocytoma presenting with Cushing's syndrome associated with increase concentrations of circulating corticotropin-releasing factor. J. Endocrinol.113, 133-138.

Jones C.T. and Edwards A.V. (1990a). Release of adrenocorticotrophin from the adrenal gland in the conscious calf. J. Physiol. 426, 397-407.

Jones C.T. and Edwards A.V. (1990b). Adrenal responses to corticotropin-releasing factor in conscious hypophysectomized calves. J. Physiol. 430, 25-36.

Markowska A., Rebuffat P., Rocco S., Gottardo G., Mazzocchi G. and Nussdorfer G.G. (1993). Evidence that an extrahypothalamic pituitary corticotropin-releasing hormone (CRH)/adrenocorticotropin (ACTH) system controls adrenal growth and secretion in rats. Cell Tissue Res. 272, 439-445.

Mazzocchi G., Malendowicz L.K., Markowska A. and Nussdorfer G.G. (1994). Effect of hypophysectomy on corticotropin-releasing hormone and adrenocorticotropin immunoreactivities in the rat adrenal gland. Mol. Cell. Neurosci. 5, 345-349.

Singh D.N.P. and Mathew T.C. (1987). Adrenomedullary chromaffin cells of the rat. Acta Anat. 129, 329-332.

Suda T., Tomori N., Tozawa F., Demura H., Shizume K., Mouri T., Miura Y. and Sasano N. (1984). Immunoreactive corticotropin and corticotropin-releasing factor in human hypothalamus, adrenal, lung cancer, and phaeochromocytoma. J. Clin. Endocrinol. Metab. 58, 919-924.

Suda T., Tomori N., Yajima F., Odagiri E., Demura H. and Shizume K. (1986). Characterization of immunoreactive corticotropin and corticotropin-releasing factor in human adrenal and ovarian tumors. Acta Endocrinol. 111, 546-552.

Upton G.V. and Amatruda T.T. (1971). Evidence for the presence of tumor peptides with corticotropin-releasing-factor-like activity in the ectopic ACTH-syndrome. New Engl. J. Med. 285, 419-424.

Venihaki M., Gravanis A. and Margioris A.N. (1997). Comparative study between normal rat chromaffin and PC12 rat pheochromocytoma cells: production and effects of corticotropin-releasing hormone. Endocrinology 138, 698-704.

Vinson G.P., Hinson J.P. and Tóth I.E. (1994). The neuroendocrinology of the adrenal cortex. J. Neuroendocrinol. 6, 235-246.

Wurtman R.J. and Axelrod J. (1966). Control of enzymatic synthesis of adrenaline in the adrenal medulla by adrenal cortical steroids. J. Biol. Chem. 241, 2301-2305.

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