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Paracrine Control of Adrenal Cortical Function by Medullary Chromaffin Cells

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I. Introduction

The intimate morphological interrelationships between cortex and medulla and the peculiar vascular arrangement in the mammalian adrenal gland are well known to possess a great functional relevance. In the adrenals, blood flows centripetally from the outer subcapsular zona glomerulosa, through the capillary network of zonae fasciculata and reticularis to the medulla, where it drains into the medullary veins (for review, see Vinson et al., 1992). Thus, in the adrenal medulla, blood-borne steroid hormones can reach a concentration sufficient to induce and stimulate the activity of the enzymes involved in the epinephrine synthesis (for review, see Cryer, 1992).

Today, a large mass of findings has been accumulated suggesting that, in the adrenal glands, medullary chromaffin cells also exert a control on the function of the cortex. The morphological counterpart of this paracrine control is two-fold: (*a*) many nerve fibers originate from medullary neurons and reach the cortex; and (*b*) islets or rays of medullary tissue are strictly intermingled with the cortical tissue. The nerve fibers and the secretory products of chromaffin cells, locally released inside the cortex, may modulate steroid secretion by acting either (*a*) directly on the adrenocortical cells, or (*b*) on the gland vasculature by modifying cortical blood-flow rate, which is known to influence steroid-hormone and especially glucocorticoid release. Moreover, there is the possibility that adrenal medulla may control the function of the cortex by regulating the local release of endothelins and/or the activity of renin-angiotensin system (RAS)[a] located in the zona glomerulosa.

The main secretory products of medullary chromaffin cells are epinephrine, norepinephrine and dopamine. Epinephrine, norepinephrine and adrenergic fibers stimulate steroid secretion, whereas dopamine exerts an opposite effect. Medullary chromaffin cells also secrete serotonin, a potent stimulator of steroid secretion. There is also evidence that adrenal medulla, at least in the rat, contains a corticotropin-releasing hormone (CRH)-adrenocorticotropin (ACTH) system, duplicating the hypothalamo-pituitary one, that controls in a paracrine manner the secretion and growth of the cortex. In addition to catecholamines, serotonin and CRH-ACTH, adrenal chromaffin cells store and release numerous regulatory peptides, many of which have been found to affect the function of the cortex both in vivo and in vitro (Tóth and Hinson, 1995).

Some excellent review articles on the paracrine control of the mammalian adrenal cortex by adrenal medulla have already been published (Vinson et al., 1994; Ehrhart-Bornstein et al., 1995). However, they were mainly concerned with the role played by the nerve fibers of medullary origin. In the following sections of this survey, I will summarize and discuss the morphological and functional background of the cortico-medullary interactions in the adrenal gland. Furthermore, I shall review the evidence concerning the involvement of medullary monoamines (catecholamines and serotonin) in the regulation of the cortex secretion, the functional significance of the intramedullary CRH-ACTH system, the possible role played by the adrenomedullary regulatory peptides in the fine-tuning of adrenocortical function, the control of the release of intramedullary regulatory molecules and, finally, the possible pathophysiological significance of the paracrine control of the cortex by adrenal medulla.

Abbreviations: α-CRH, α-helical corticotropin-releasing hormone; ACTH, adrenocorticotropic hormone (adrenocorticotropin); ANG-II, angiotensin II; ANP, atrial natriuretic peptide; AVP, arginine-vasopressin; BNP, brain (B-type) natriuretic peptide; $[(Ca^{2+})]_i$, intracellular (cytosolic) calcium concentration; cAMP, cyclic adenosine 3′, 5′-monophosphate; cGMP, cyclic guanosine 3′,5′-monophosphate; CGRP, calcitonin gene-related peptide; CIP, corticotropin-inhibiting peptide; CNP, C-type natriuretic peptide; CRH, corticotropin-releasing hormone; $D_{1,2}$: dopamine-receptor subtypes; DALA, D-Ala^2-Met-enkephalin; GABA, γ-aminobutyric acid; GRP, gastrin-releasing peptide; HPA, hypothalamo-pituitary-adrenal; ir, immunoreactivity; mRNA, messenger ribonucleic acid; $NK_{1,2,3}$, tachykinin (neurokinin)-receptor subtypes; PACAP, pituitary adenylate cyclase-activating polypeptide; POMC, pro-opiomelanocortin; PYX-1, -2, neuropeptide Y-receptor antagonists; RAS, renin-angiotensin system; RIA, radioimmunoassay; RT-PCR, reverse transcription polymerase-chain reaction; SRIH, somatotropin release-inhibiting hormone; TRH, thyrotropin-releasing hormone; VIP, vasoactive intestinal peptide; V_1, V_2: arginine vasopressin-receptor subtypes; Y_1, Y_2: neuropeptide Y-receptor subtypes; 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4: serotonin (5-hydroxytryptamine)-receptor subtypes.

II. The Morphological and Functional Background of the Cortico-Medullary Paracrine Interactions

A. Innervation of the Cortex by Medullary Neurons

Much evidence indicates that the mammalian adrenal cortex, and especially zona glomerulosa, is richly innervated. In addition to adrenergic, dopaminergic (Kleitmann and Holzwarth, 1985; Oomori et al., 1991, 1994; Charlton et al., 1992; Vizi et al., 1992, 1993) and cholinergic fibers (Charlton et al., 1991), several peptidergic fibers have been identified. Nerve fibers of this last group release at their terminal ends a number of peptides that all are also contained in adrenal medulla and are able to affect the function of the cortex (see Section V).

According to Vinson et al. (1994), who accurately reviewed this topic, nerve fibers reaching the cortex may have a two-fold origin. A group of fibers originates from neurons located outside the adrenal gland and reaches it by following blood vessels or splanchnic nerves. A second group, which probably includes all the peptidergic fibers, has its cell body in the adrenal medulla. Vizi et al. (1992) demonstrated the presence in the rat zona glomerulosa, in addition to the classic nerve endings lying in apposition to local vessels, of numerous varicose axon terminals located in close proximity of parenchymal cells. Morphometry showed that about 20% of the nerve fibers in the zona glomerulosa end free, without forming obvious synaptic contacts. Neurochemical evidence indicated that catecholamines (norepinephrine and dopamine) are released into the vicinity of zona glomerulosa cells in response to axonal firing (Vizi et al., 1992, 1993). These features, which are reminiscent of the hypothalamo-pituitary neurosecretory process, strongly suggest the possibility of a paracrine non-synaptic modulatory role of catecholamines and other neuropeptides on zona glomerulosa cells.

B. Interlacement of Cortical and Medullary Tissues

More than 20 years ago, Palacios and Lafarga (1975) reported the occasional presence of islets of chromaffin cells in the zona glomerulosa of adult rats. Unfortunately, they attributed this finding to an error in the adrenal organogenesis and therefore considered the finding devoid of functional significance.

As the number of investigations suggesting the possibility of a paracrine control of the adrenal cortex by medullary chromaffin cells grew, this rather common morphological observation (figs. 1 and 2) acquired more relevance. Gallo-Payet et al. (1987), by performing an accurate serial-sectioning study of rat adrenals, reported the constant presence of rays of medullary tissue transversing the cortex and reaching the capsule. Bornstein et al. (1991), by immunostaining chromaffin cells for synaptophysin and chromogranin A and adrenocortical cells for 17α-hydroxylase, confirmed the existence

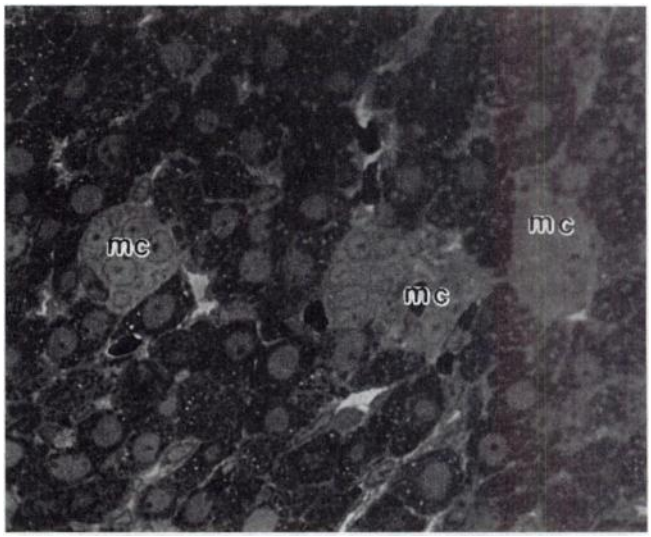

FIG. 1. Light micrograph of a 0.5-μm-thick Epon-embedded section of the outer portion of the zona fasciculata of the rat adrenal cortex. Three small clusters of medullary chromaffin cells (*mc*) are scattered among adrenocortical cords. × 500.

of rays of medullary tissue and, additionally, described the presence of small clusters of chromaffin cells and single cells in all the three zones of the rat and pig adrenal cortex. At the ultrastructural level, close cellular contacts were observed between cortical and medullary cells, and in some rare instances, chromaffin cells appeared to be releasing their secretory products (probably catecholamines) by exocytosis near adrenocortical cells (Bornstein and Ehrhart-Bornstein, 1992). More recently, these findings were confirmed in the human adrenal glands (Bornstein et al., 1994).

C. Possible Mechanisms Involved in the Paracrine Interactions

The regulatory molecules locally released by medullary chromaffin cells and nerve fibers may affect the function of adrenocortical cells through both direct and indirect mechanisms.

1. Direct mechanism. A regulatory molecule, released inside the cortex, may modulate the secretion and growth of adrenocortical cells by binding to specific receptors located on their plasma membrane and activating intracellular pathways. If such mechanisms are operative, the regulatory molecule is able to affect in vitro steroid secretion of dispersed adrenocortical cells.

2. Indirect mechanisms. a. REGULATION OF ADRENAL BLOOD FLOW. Adrenal blood flow undergoes a very complex regulatory mechanism, involving neural and endocrine components (e.g., both splanchnic-nerve activation and ACTH raise it), so that it does not strictly follow the changes in the systemic blood pressure. The fine regulation of adrenal blood flow possesses an important physiological significance, inasmuch as a strict direct relation exists between the rates of blood flow and steroid-hormone (especially glucocorticoid) release. Accord-

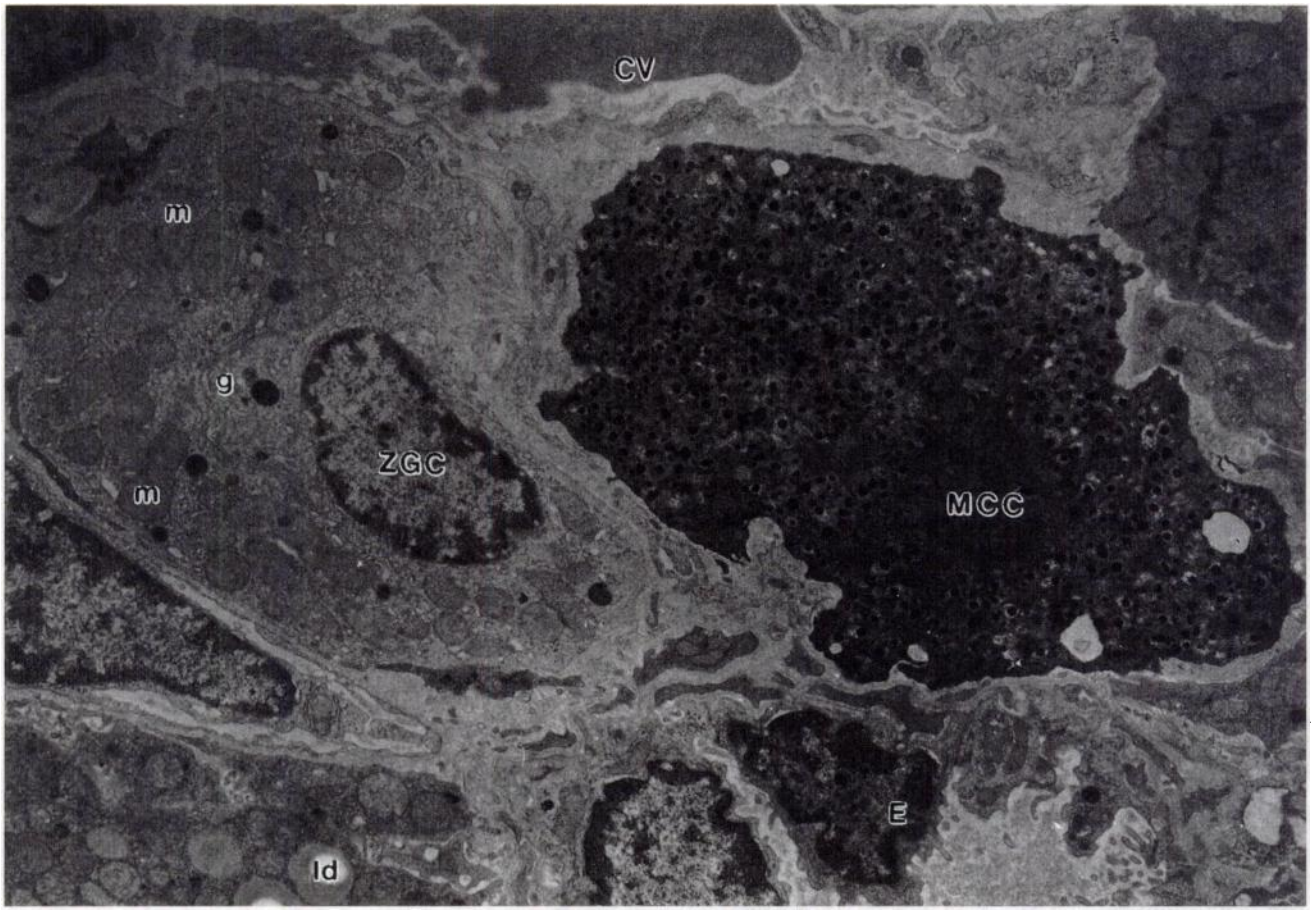

FIG. 2. Electron micrograph showing the close contact between a medullary chromaffin cell (*MCC*) and a zona glomerulosa cell (*ZGC*) in the rat adrenal cortex. *CV*, vessel of the gland capsule; *E*, endothelial cell nucleus; *m*, mitochondria; *g*, Golgi apparatus; *ld*, lipid droplet in an outer zona fasciculata cell. × 11,000.

ing to Vinson and Hinson (1992), the mechanism whereby the increase in the rate of blood (or perfusion medium) flow affects steroid secretion may involve: (*a*) wash-out of secretory products, (*b*) increase in oxygen and substrate supply, (*c*) enhancement in the rate of presentation of agonists and, finally, (*d*) increased release of endothelins by gland endothelium in response to fluid-shear stress. With few exceptions, all the studies investigating such mechanisms were carried out using in situ perfused rat adrenal glands. In this model, the left adrenal is perfused via a cannula introduced into the coeliac artery; perfusion medium reaches an isolated segment of the aorta from which the adrenal arteries arise, then passes through the adrenal gland and into a cannula inserted in the ipsilateral renal vein. It has been demonstrated that the change of the flow rate of the perfusion medium evokes per se a parallel change in the basal rate of glucocorticoid secretion (for review, see Vinson and Hinson, 1992).

b. MODULATION OF INTRA-ADRENAL RAS. There is now a general consensus that an intra-adrenal RAS, mainly located in the capsule-zona glomerulosa, plays an important role in the paracrine control of aldosterone secretion (for review, see Mulrow, 1992). It is not unreasonable to suggest that some intramedullary regulatory peptides, which are able to inhibit kidney renin release, may also control zona glomerulosa function acting on this system.

c. MODULATION OF INTRA-ADRENAL ENDOTHELIN SYSTEM. Evidence is accumulating that endothelins and their receptor subtypes A and B are expressed in the mammalian adrenals and play a role in the stimulation of steroidogenesis, especially in aldosterone secretion (for review, see Nussdorfer et al., 1997). Hence, there is the possibility that some intramedullary regulatory molecules may indirectly affect the function of the cortex by modulating intra-adrenal synthesis and release of endothelins.

d. STIMULATION OF THE RELEASE OF OTHER REGULATORY MOLECULES. A regulatory peptide may act indirectly on adrenocortical cells by eliciting the release by chromaffin cells of other regulatory molecules in a paracrine or autocrine manner, which in turn may control the function of the cortex through one (or more) of the mechanisms described in previous paragraphs. Evidence of such a mechanism is suggested by the observation that the regulatory molecule is able to modulate the secretion and growth of the cortex in adrenal quarters containing

medullary tissue, but not in either dispersed adrenocortical cells or quarters of adrenocortical autotransplants lacking medullary chromaffin cells. A few years ago, Belloni et al. (1990) proposed an experimental model for investigating the role of medullary chromaffin cells in the paracrine regulation of the cortex. Because results obtained with the "adrenal enucleation-regeneration" technique were hampered by frequent remnants of medullary tissue, an autotransplantation procedure was chosen. Rats were bilaterally adrenalectomized, and several fragments of their excised capsular-zona glomerulosa tissue were implanted in the musculus gracilis. After 4-5 months of regeneration, implants gave rise to small nodules of cortical tissue, somewhat arranged in an outer zona glomerulosa-like and an inner zona fasciculata-like layer. These regenerated autotransplants are deprived of chromaffin cells and lack the typical vascular arrangement and the normal nerve-fiber supply of the adrenal glands. Accordingly, they can provide useful information concerning adrenal cortical regulatory mechanisms not involving chromaffin tissue, nervous and vascular influences.

Obviously, the various mechanisms of action described above in the previous paragraphs are not mutually exclusive, so that a regulatory peptide may directly inhibit adrenocortical cells and simultaneously, by stimulating catecholamine release, exert an indirect adrenocortical secretagogue effect. These considerations easily explain why rather different findings can be obtained according to the experimental model used to test the effect of a regulatory molecule on the adrenal cortex: e.g., in vitro versus in vivo, or dispersed adrenocortical cells versus adrenal slices. Moreover, it must be recalled that each circulating molecule (regulatory peptide or true hormone) of extra-adrenal origin, which is able to modulate the activity of adrenal medulla or adrenal blood flow, may indirectly affect the secretion and growth of the adrenal cortex.

D. General Remarks

An intramedullary regulatory molecule reasonably can be assumed to exert a paracrine control of the cortex of physiological or pathophysiological relevance only when it meets the following general criteria:

- The adrenal content of the putative regulatory molecule must be elevated enough to allow it to reach, upon appropriate stimulation of its release, a local concentration not below its minimal effective one in vitro. Mazzocchi et al. (1993d) provided a method to roughly calculate this parameter, based on the evidence that in the fresh adrenal tissue, there is correspondence between weight and volume (specific gravity, 1.039), and that the interstitial space in the mammalian adrenal glands (as calculated by morphometry) is less than 2-3% of the total volume. Thus, if the adrenal content of a regulatory molecule is 100 fmol/g, its 30% release will produce a local concentration of 10^{-9} M. In fact, 30 fmol of the regulatory molecule will be released into about 30 μl of interstitial space (3% of 1 ml, which is the volume of 1 g of fresh adrenal tissue), producing a local concentration of about 1 fmol/μl or 1 nmol/l.
- The administration of a specific antagonist of the putative regulatory molecule, at a dose that is able to produce a plasma concentration near its maximal effective one in vitro, must evoke sizable effects on the adrenal cortex (opposite to those elicited by the regulatory molecule). If this occurs, the endogenous putative regulatory molecule surely plays a physiological role in the functional modulation of adrenal cortex; when the basal blood level of the regulatory molecule is below its minimal effective in vitro concentration, we can reasonably assume that this molecule is locally secreted in the adrenal gland. This contention may be supported by the demonstration that the antagonists of the regulatory molecule affect the function of adrenocortical cells in intact rats, but not in animals bearing adrenocortical autotransplants deprived of medullary chromaffin cells. Obviously, this finding acquires its full relevance only when adrenocortical autotransplants respond to the putative regulatory molecule; unfortunately, this does not always occur, because regenerated adrenocortical cells may not have developed specific receptor for the regulatory molecule, or the regulatory molecule may affect the cortex function via an indirect mechanism requiring the presence of chromaffin cells (see section I).

III. The Involvement of Medullary Monoamines in the Control of the Cortex Function

A. Epinephrine and Norepinephrine

Mammalian adrenocortical cells possess both α and β receptor subtypes for epinephrine and norepinephrine, although a major role appears to be played by β-adrenoceptors.

1. β-Adrenoceptors. Both catecholamines and their β-receptor agonists (e.g., isoprenaline and dobutamine) are able to enhance aldosterone secretion in vitro by zona glomerulosa preparations (dispersed cells, capsular strips or cultured cells), and this effect is blocked by β-adrenoceptor antagonists (e.g., propanolol and L-alprenolol). This was found to occur in humans (Neri et al., 1996), cows (DeLéan et al., 1984b) and rats (Pratt et al., 1985; Horiuchi et al., 1987; Pratt and McAteer, 1989; Vizi et al., 1992; Andreis et al., 1995). Similar results were obtained for zona fasciculata-reticularis preparations and glucocorticoid secretion in calves (Kawamura et al., 1984; Walker et al., 1988; Lightly et al., 1990). Data indicate that β-adrenoceptor activation is coupled with the stimulation of adenylate cyclase (Kawamura et al., 1984). Using cultured pig inner adrenocortical cells,

Ehrhart-Bornstein et al. (1994) showed that epinephrine specifically elicits the release of androstenedione via β-adrenoceptors, a finding suggesting an action of the catecholamine on the zona reticularis cells. It has also been reported that the epinephrine-enhanced cortisol secretion by cultured bovine adrenocortical cells is associated with a marked rise in the messenger ribonucleic acids (mRNAs) of the enzymes catalyzing the conversion of cholesterol to cortisol, including the cholesterol side-chain cleavage cytochrome P450, the 17α-hydroxylase cytochrome P450, the 21-hydroxylase cytochrome P450 and the 11β-hydroxylase cytochrome P450. The maximum rise was observed after 5-6 h exposure to 10^{-5} M epinephrine (Ehrhart-Bornstein et al., 1991b; Güse-Behling et al., 1992). Also, this long-term effect of epinephrine is mediated by cyclic adenosine monophosphate (cAMP) (Güse-Behling et al., 1992).

2. α-Adrenoceptors. This receptor subtype is found in rat adrenals (Wypijewski et al., 1995), and its activation seems to stimulate guanylate cyclase (Jaiswal and Sharma, 1986). The α-agonist guanabenz inhibits agonist-stimulated aldosterone secretion and cAMP production of rat zona glomerulosa cells (Lotshaw et al., 1991a), thereby suggesting that α- and β-adrenoceptors play opposite roles in the regulation of adrenocortical function. The following data, however, appear to conflict with this contention. Mokuda et al. (1992) showed that epinephrine raises cortisol secretion from perfused adrenals of the guinea pig and that the effect is blocked by α-antagonists (e.g., phentolamine), but not by the β-antagonists (e.g., propranol). Interleukins are monokines released by activated macrophages and monocytes during the acute phase of the inflammatory response; interleukins potently stimulate the hypothalamo-pituitary-adrenal (HPA) axis (for review, see Gaillard, 1994). Interleukin-1α enhances corticosterone release by rat adrenal quarters containing medullary tissue, but not by dispersed inner adrenocortical cells (Gwosdow et al., 1992), and its effect is blocked by phentolamine, but not by β-adrenoceptor antagonists (Gwosdow et al., 1992; O'Connell et al., 1994). This strongly suggests that α-adrenoceptor activation stimulates glucocorticoid secretion.

B. Dopamine

Many lines of evidence indicate that the dopaminergic system exerts a maximal tonic inhibitory influence on the zona glomerulosa and aldosterone secretion in many mammalian species, including humans and rats (for review, see Cuche, 1988). This contention stems from the demonstration that the administration of D_2 dopamine antagonists (e.g., metoclopramide) raises plasma aldosterone levels, whereas dopamine agonists (e.g., bromocriptine) are ineffective. In this connection, recall that Rossi et al. (1994) reported that metoclopramide elicits a quantitatively higher aldosterone response in patients with primary aldosteronism than in essential hypertensives. Interestingly, the D_2 agonist dihydroergotoxine decreases plasma aldosterone in the latter patients but causes a paradoxical increase in the former patients, suggesting that these responses may have value for the screening of primary aldosteronism. These findings, however, were obtained in vivo, so that the possibility that dopamine agonists and antagonists act indirectly on the zona glomerulosa by interfering with its complex extra-adrenal multifactorial control cannot be ruled out.

Several lines of evidence strongly support the view that dopamine may be included in the group of medullary paracrine regulators of the cortex function. Dopaminergic fibers (Vizi et al., 1992, 1993) and significant concentrations of dopamine are present in the capsule-zona glomerulosa (Racz et al., 1984a; McCarty et al., 1986; Pratt et al., 1987; Buu and Lussier, 1990). Because the level of circulating dopamine cannot account for these elevated concentrations in the zona glomerulosa (Van Loon and Sole, 1980), adrenal cortex dopamine is likely derived from intrinsic dopaminergic fibers and chromaffin cells.

Abundant dopamine receptors of the D_1 and D_2 subtypes are located on the zona glomerulosa cells of humans (Amenta et al., 1994), cows (Missale et al., 1985; Stern et al., 1986) and rats (Missale et al., 1986; Amenta and Ricci, 1995), but in vitro studies dealing with the effect of their activation gave equivocal results. Dopamine and its agonists did not modify either basal or angiotensin II (ANG-II)-stimulated aldosterone secretion of dispersed zona glomerulosa cells of cows, sheep and rats (McDougall et al., 1981; Zanella and Bravo, 1982; Wilson et al., 1983; DeLéan et al., 1984b; Gallo-Payet et al., 1990; Porter et al., 1992). Conversely, dopamine was reported to inhibit ANG-II-stimulated aldosterone production by dispersed rat and cultured bovine zona glomerulosa cells (Braley et al., 1983; Racz et al., 1984b; Missale et al., 1988), but to increase cAMP and aldosterone synthesis in cultured rat zona glomerulosa cells (Gallo-Payet et al., 1990). These conflicting results may be reconciled in the light of the following observations. Zona glomerulosa cells possess both D_1 and D_2 receptors, and evidence is available that shows that these two receptor subtypes mediate opposite effects. The activation of the D_1 receptor subtype enhances the activity of adenylate cyclase, while the stimulation of the D_2 subtype inhibits adenylate cyclase and phospholipase C activities and reduces Ca^{2+} influx in the rat zona glomerulosa cells (Gallo-Payet et al., 1990, 1991a; Osipenko et al., 1994). Hence, depending on its concentration and on the affinity of each receptor subtype, dopamine may exert opposite effects on the zona glomerulosa cells, because the activation of D_1 may mask that of D_2 receptors and vice versa.

Sound evidence of the involvement of dopamine in the physiological control of zona glomerulosa was provided by Vinson's group by using isolated in situ perfused rat

adrenals. Porter et al. (1992) demonstrated that dopamine infusion (from 10^{-7} to 10^{-4} M) for 10 min results in a transient dose-related decrease in aldosterone secretion (50% of the basal value at 10^{-6} M dopamine). The simultaneous administration of 10^{-6} M haloperidol, a dopamine antagonist, reverses the effect of dopamine, without affecting basal aldosterone release. Electrical field stimulation of the perfused gland lowers aldosterone secretion. Also, this effect is abolished by haloperidol, a fact that strongly suggests that the local release of dopamine may be involved in the inhibitory effect of such experimental procedures.

C. Serotonin

Serotoninergic fibers have been detected in the mouse adrenal cortex (Fernandez-Viveros et al., 1993), and the occurrence of serotonin has been immunocytochemically and biochemically demonstrated in the adrenal medulla of mice (Fernandez-Viveros et al., 1993) and rats (Verhofstad and Jonsson, 1983; Holzwarth et al., 1984; Brownfield et al., 1985; Holzwarth and Brownfield, 1985). In humans, chromaffin cells do not contain serotonin, whose presence in adrenals seems to be restricted to the mast cells (Lefebvre et al., 1992).

Serotonin exerts its physiological effects through four different subtypes of receptors, named 5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4 (for review, see Bradley et al., 1986), and evidence indicates that adrenocortical cells possess 5-HT_2 (Williams et al., 1984; Matsuoka et al., 1985) and 5-HT_4 receptors (Lefebvre et al., 1992, 1993), which are coupled with phospholipase C and adenylate cyclase, respectively (Conn et al., 1986; Dumuis et al., 1988).

Serotonin stimulates basal aldosterone secretion in vitro in zona glomerulosa cells of humans (Lefebvre et al., 1993, 1995) and rats (Matsuoka et al., 1985; Rocco et al., 1992), as well as cortisol production by human inner adrenocortical cells (Racz et al., 1979; Lefebvre et al., 1992). Findings obtained by the use of serotonin-receptor agonists and antagonists suggest that in humans, the secretagogue effects of serotonin are mediated by 5-HT_4 receptors and hence via the activation of adenylate cyclase (Lefebvre et al., 1992, 1993). However, there are indications that serotonin acts on rat zona glomerulosa cells via 5-HT_2 receptors (Matsuoka et al., 1985); accordingly, in this species, serotonin is able to stimulate phospholipase C-mediated cascade (Whitley et al., 1984) and Ca^{2+} influx (Davies et al., 1991). Hinson et al. (1989) showed that serotonin markedly enhances aldosterone and corticosterone release by in situ perfused rat adrenals and raises notably the flow rate of the perfusion medium; therefore, they suggested that the secretagogue effect of serotonin, and especially the glucocorticoid response, may be, at least in part, mediated by an increase in the adrenal blood-flow rate.

The putative role of intra-adrenally produced serotonin in controlling the cortex function seems to be suggested indirectly by the following considerations. The minimal effective concentrations of serotonin able to elicit an in vitro secretory response of adrenocortical cells are in the range of 10^{-8} M, i.e., well above those present in the blood, where this neurotransmitter is rapidly taken up by and stored in platelets (Zinner et al., 1983). Conversely, adrenal serotonin content of about 7-8 nmol/g in the rat (Holzwarth and Brownfield, 1985) may produce local concentrations higher than $10^{-5}/10^{-4}$ M (see Section II D).

D. Summary

Epinephrine and norepinephrine, acting via adenylate cyclase-coupled β-receptors, directly stimulate mineralo- and probably glucocorticoid secretion of adrenocortical cells; they also appear to enhance gene expression of all the enzymes involved in steroid synthesis. The participation of α-receptors in the adrenocortical secretagogue action of the two catecholamines is doubtful. Dopamine, acting via D_1 and D_2 receptors, exerts a direct concentration-dependent biphasic effect on the zona glomerulosa and aldosterone secretion: the activation of D_1 increases the activity of adenylate cyclase, while that of D_2 inhibits it. Under normal circumstances, the inhibitory effect prevails. Serotonin, acting via phospholipase C-coupled 5-HT_2 and adenylate cyclase-coupled 5-HT_4 receptors, directly stimulates aldosterone and glucocorticoid secretion of adrenal cortex; the glucocorticoid secretagogue action seems to be, at least in part, mediated by an increase in the adrenal blood flow.

IV. The Intramedullary Corticotropin-Releasing Hormone-Adrenocorticotropic Hormone System

Adrenal medulla contains sizable amounts of CRH-immunoreactivity (ir) in several mammalian species, including humans (Suda et al., 1984, 1986), cows (Edwards and Jones, 1988; Minamino et al., 1988), dogs (Bruhn et al., 1987a, b) and rats (Hashimoto et al., 1984; Bagdy et al., 1990; Mazzocchi et al., 1993d). The adrenal content of CRH-ir varies from 50 to 300 fmol/g in cows, dogs and rats, and reaches about 3-4 pmol/g in humans. The expression of prepro-CRH gene was demonstrated in human adrenal medulla (Usui et al., 1988). The presence of significant amounts of ACTH-ir has been demonstrated by radioimmunoassay (RIA) in adrenal medulla of humans (Suda et al., 1986), cows (Jones and Edwards, 1990) and rats (Bagdy et al., 1990; Andreis et al., 1992; Mazzocchi et al., 1993d); its content ranges from 50-200 fmol/g in rats to 50 pmol/g in humans. These intra-adrenal contents of CRH and ACTH could produce local concentrations of about 1-3 × 10^{-9} M in rats and about three orders of magnitude higher in humans (see Section II D).

Jones and Edwards (1990) observed that in calves, splanchnic-nerve stimulation elicits adrenal release of both CRH and ACTH in amounts of the same order of magnitude (8 and 12 fmol/min/kg) and suggested the

co-release of the two peptides, because "much higher gearing would be expected if the release of ACTH was a consequence of the release of CRH." However, this does not seem to be the case at least in the rat, because (*a*) CRH is able to evoke a clear-cut release of ACTH-ir by fragments of adrenomedullary tissue (Andreis et al., 1992; Mazzocchi et al., 1993d), and (*b*) medullary chromaffin cells are provided with specific CRH receptors (Dave et al., 1985a; Udelsman et al., 1986; Aguilera et al., 1987). CRH does not stimulate corticosterone secretion by dispersed rat inner adrenocortical cells (Andreis et al., 1991b, 1992; Van Oers et al., 1992), but it does enhance corticosterone output by adrenal slices containing medullary chromaffin cells (Andreis et al., 1991b, 1992; Neri et al., 1991b), and this effect is blocked not only by the specific CRH receptor antagonist α-helical-CRH (α-CRH), but also by the competitive ACTH-receptor antagonist corticotropin-inhibiting peptide (CIP or $ACTH_{7\text{-}38}$). These results were confirmed in vivo by using hypophysectomized rats administered maintenance doses of ACTH (Andreis et al., 1991b, 1992). Interleukin-1β, a cytokine that is well known to stimulate hypothalamo-pituitary CRH-ACTH system (for review, see Gaillard, 1994), does not evoke any secretory response of dispersed rat adrenocortical cells; however, it exerts a marked acute corticosterone secretagogue action on adrenal quarters containing medullary tissue (but not on slices of adrenocortical autotransplants), and this effect is annulled by either α-CRH or CIP (Andreis et al., 1991a). Moreover, α-CRH blocks the interleukin-1β-elicited release of ACTH-ir by rat adrenal medulla fragments (Mazzocchi et al., 1993d).

The view that the intra-adrenal CRH-ACTH system plays a physiological role in the control of adrenal functions is also supported by other findings. The prolonged administration of CRH reduces the adrenocortical atrophy in hypophysectomized rats (Bornstein et al., 1990a). Moreover, the infusion of either α-CRH or CIP to hypophysectomized rats receiving maintenance doses of ACTH causes a marked atrophy of inner adrenocortical cells, as well as a notable reduction of their glucocorticoid secretory capacity; both these effects are reversed by the simultaneous administration of CRH or ACTH (Markowska et al., 1993). These results strongly suggest that when the central branch of the CRH-ACTH system is functionally ineffective, the peripheral intramedullary one plays a role in the maintenance of a normal adrenocortical function and growth.

This last contention is supported by the demonstration that both the adrenal content and the interleukin-1β-stimulated release of CRH-ir and ACTH-ir increase in rats in relation to the number of days elapsed from hypophysectomy. The effect of hypophysectomy requires 48 h to become significant and reaches its maximum after 72 h. ACTH infusion of hypophysectomized animals, at a rate restoring a normal blood level of the hormone, prevents the effect of hypophysectomy on intra-adrenal levels of both CRH-ir and ACTH-ir; similarly, the hypophysectomy-induced rise in intra-adrenal ACTH-ir is abolished by the administration of CRH or dexamethasone (Mazzocchi et al., 1994b). Collectively, these findings suggest that the hypophysectomy-induced lowering of circulating ACTH and the consequent drop in the adrenal secretion of glucocorticoids enhance, by removing a classic negative feedback mechanism, the gene expression of CRH and ACTH in adrenomedullary chromaffin cells.

Before concluding, it must be recalled that some regulatory peptides, contained in adrenal medulla and stimulating glucocorticoid secretion, appear to do this indirectly by activating intramedullary CRH-ACTH system (see Section V).

V. The Medullary Regulatory Peptides Affecting Adrenocortical Cell Function

A. Hypothalamic Peptides

This group of intramedullary peptides includes CRH, arginine-vasopressin (AVP) and oxytocin, which stimulate adrenocortical secretion, and somatotropin release-inhibiting hormone (SRIH) and thyrotropin-releasing hormone (TRH), which inhibit it.

1. Corticotropin-releasing hormone. Scanty data indicate that CRH may specifically affect zona glomerulosa and aldosterone secretion. CRH-positive nerve fibers, originating from adrenomedullary neurons, are present in the rat zona glomerulosa (Rundle et al., 1988). In normal rats infused with ACTH or CRH, a positive linear correlation occurs between the plasma levels of ACTH and corticosterone or aldosterone. Regression curves for corticosterone are similar in both groups of rats, while the regression line for aldosterone is significantly steeper in CRH- than in ACTH-infused rats (Mazzocchi et al., 1989). Neri et al. (1991b) did not observe any effect of CRH on dispersed rat zona glomerulosa cells. Conversely, Hinson and Kapas (1995) showed an aldosterone response that manifests itself only at a CRH concentration of 10^{-7} M, which is well above that which the peptide can attain either in blood or in adrenals (see Section IV). There are also indications that intramedullary CRH enhances rat adrenal glucocorticoid response to ACTH without eliciting any secretory response by dispersed inner adrenocortical cells (Van Oers et al., 1992). CRH has been reported to increase adrenal blood flow in cows (Jones and Edwards, 1992) and the medium flow rate in rat adrenals perfused in situ (Hinson et al., 1994a). Hence, it may be conceived that CRH may indirectly stimulate glucocorticoid release by increasing adrenal blood flow.

2. Arginine-vasopressin. AVP-ir has been detected by RIA in the fresh adrenal medulla of humans (Ang and Jenkins, 1984; Nicholson et al., 1984), cows (Nussey et al., 1987) and rats (Ang and Jenkins, 1984; Nussey et al., 1984); its intra-adrenal concentration ranges from 10

pmol/g in rats to 100 pmol/g in humans. The presence of AVP-ir has been confirmed by immunocytochemistry in adrenal chromaffin cells of humans, cows, rats, hamsters and guinea pigs (Nussey et al., 1984; Ravid et al., 1986; Hawthorn et al., 1987). Recently, it has been demonstrated clearly that human medullary chromaffin cells secrete AVP (Guillon et al., 1995).

a. EFFECTS ON ZONA GLOMERULOSA. There is a general consensus that AVP directly enhances zona glomerulosa cell secretion. Biochemical and autoradiographical studies demonstrated the presence of specific AVP receptors of the V_1 subtype in the zona glomerulosa cells of humans (Guillon et al., 1995), sheep (Lutz et al., 1993) and rats (Balla et al., 1985; Guillon and Gallo-Payet, 1986; Gallo-Payet et al., 1991b). AVP exerts a potent acute aldosterone secretagogue effect on dispersed zona glomerulosa cells (Payet and Lehoux, 1982; Hinson et al., 1987; Quinn et al., 1990; Mazzocchi et al., 1995d), its minimal and maximal effective concentrations being about 10^{-10} M and 10^{-8} M, respectively. Findings are available that demonstrate that AVP shares with ANG-II the mechanism of action, inasmuch as it activates phospholipase C and raises intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (Balla et al., 1985; Gallo-Payet et al., 1986, 1991b; Enyedi et al., 1988; Guillon et al., 1990, 1995; Woodcock et al., 1990). The prolonged administration of AVP enhances zona glomerulosa growth by promoting both hypertrophy and hyperplasia of its parenchymal cells (Payet and Lehoux, 1980; Payet et al., 1984; Mazzocchi et al., 1995e). As expected, all these adrenoglomerulotropic effects of AVP are abolished by the selective V_1 receptor antagonist Des-Gly-[Pha^1, D-Thyr(Et)^2, Lys^6, Arg^8]-AVP, but not by the specific V_2 receptor antagonist [D-$(CH_2)^5$, D-Phe^2, Ile^4, Ala^9-NH_2]-AVP (Guillon et al., 1995; Mazzocchi et al., 1995e). Moreover, it has been shown that the prolonged infusion with the V_1-receptor antagonist strongly depresses the growth and aldosterone secretion of rat zona glomerulosa, a finding suggesting a physiological role of endogenous AVP in the regulation of zona glomerulosa function (Mazzocchi et al., 1993c). Even in the case of extreme stimulation of the pituitary AVP release (e.g., severe hemorrhage or prolonged water restriction), the plasma levels of AVP remain well below its minimal in vitro effective concentration: about 5×10^{-11} M versus 1×10^{-10} M, respectively (for review, see Baylis, 1989). Conversely, after stimulation of its release from medullary chromaffin cells, AVP could conceivably reach intra-adrenal concentrations of about 10^{-7} M in rats and 10^{-6} M in humans (see Section II D). These considerations suggest that the adrenal medulla is the source of endogenous AVP involved in the physiological control of zona glomerulosa function. This contention received support by the experiments of Mazzocchi et al. (1995e). They demonstrated that a V_1 receptor antagonist, when administered alone, although depressing the growth and secretion of rat zona glomerulosa, does not exert any apparent effect on zona glomerulosa-like cells of adrenocortical autotransplants lacking medullary chromaffin cells, which are, however, well responsive to AVP.

b. EFFECTS ON ZONA FASCICULATA-RETICULARIS. **i. Direct mechanism.** The direct effects of AVP on inner adrenocortical cells are very controversial. The bulk of investigations carried out in the rat did not detect any secretagogue action of AVP on dispersed zona fasciculata-reticularis cells (Hinson et al., 1987; Mazzocchi et al., 1995e). However, AVP has been reported to enhance in vitro cortisol production by human and bovine inner adrenocortical cells, acting via V_1 receptors and activating phospholipase C (Bird et al., 1990; Perraudin et al., 1993; Guillon et al., 1995); autoradiography showed the presence of V_1 receptors on human zona fasciculata cells (Guillon et al., 1995). These conflicting findings may be explained by taking into account the fact that human and bovine zona fasciculata cells, in contrast to those of rodents, are sensitive to ANG-II and have an active phosphatidylinositol mechanism of transduction of the secretagogue signals (for review, see Vinson et al., 1992).

ii. Indirect mechanisms. Using in situ perfused intact dog or rat adrenals, Hinson et al. (1987) and Schneider (1988) observed a stimulatory effect of AVP on glucocorticoid secretion. Mazzocchi et al. (1995e) described an AVP-evoked acute net rise in the blood concentration of corticosterone and a small but significant increase in the volume of zona fasciculata and its parenchymal cells after prolonged infusion of AVP to pharmacologically hypophysectomized rats administered maintenance doses of ACTH. In light of these findings, it may be concluded that the acute glucocorticoid secretagogue action and the long-term trophic effect of AVP on the zona fasciculata require the structural integrity of adrenal tissue, at least in those species whose inner adrenocortical cells are not sensitive to ANG-II. The possibility that the mechanism underlying the stimulatory action of AVP on rat inner adrenocortical zones involves activation of the intramedullary CRH-ACTH system is supported by the fact that (*a*) medullary chromaffin cells are provided with AVP receptors of the V_1 subtype (Taylor et al., 1989); (*b*) AVP stimulates glucocorticoid secretion and growth of zona fasciculata cells in normal rats, but not of zona fasciculata-like cells in bilaterally adrenalectomized adrenocortical autotransplant-bearing animals; and (*c*) the in vitro glucocorticoid secretagogue action of 10^{-7} M AVP on adrenal slices, containing medullary tissue, is completely blocked by 10^{-6} M α-CRH or CIP (Mazzocchi et al., 1995e). However, the involvement of endogenous intra-adrenal AVP in the physiological control of inner adrenocortical zones remains very questionable, because the prolonged administration of a V_1-receptor antagonist does not apparently affect zona fasciculata function in rats, as it does in the case of zona glomerulosa (Mazzocchi et al., 1995e).

3. Oxytocin. A low amount of oxytocin-ir has been immunocytochemically demonstrated in human neona-

tal adrenals (Ravid et al., 1986). Conversely, an elevated content of oxytocin-ir has been found by RIA in adult human, cow and rat glands (Ang and Jenkins, 1984; Nicholson et al., 1984; Nussey et al., 1984, 1987); the content varies from 150 pmol/g in humans to 12-17 pmol/g in rats. According to Hawthorn et al. (1987), oxytocin shares the intra-adrenal localization with, but is less abundant than, AVP.

Oxytocin enhances basal and lowers acetylcholine-stimulated secretion of aldosterone of in situ perfused rat adrenals (Hinson et al., 1987; Porter et al., 1988), whereas it has no effect on corticosterone secretion of collagenase-dispersed rat inner adrenocortical cells superfused on a Sephadex bed in an isoprene column (Hinson et al., 1987). However, Warchol et al. (1993) observed a marked stimulatory effect of oxytocin on basal corticosterone release by suspensions of isolated rat zona fasciculata-reticularis cells. Video-imaging analysis revealed the existence of a small population of dispersed cells (about 5%); these cells respond to oxytocin by a two-fold rise in their $[Ca^{2+}]_i$. Single oxytocin-unresponsive cells were also found to move with the stream of the medium toward an oxytocin-responsive one, and, after reaching it, to begin to redistribute intracellular Ca^{2+}, achieving the maximum within 30 sec. Warchol and associates (1993) hypothesized that corticosterone response to oxytocin is cell-contact-dependent and attributable to the spreading of the secretagogue signal from a small population of responsive cells to the large one of insensitive elements. These findings have been recently confirmed. Oxytocin at relatively low concentrations ($10^{-10}/10^{-9}$ M) stimulates, and at high concentrations ($10^{-7}/10^{-6}$ M) does not affect, basal corticosterone output by dispersed rat inner adrenocortical cells; furthermore, the highest concentrations of oxytocin appear to strongly depress maximally ACTH-stimulated corticosterone production (Stachowiak et al., 1995). Given that the level of circulating oxytocin is in the pM range (Nussey et al., 1984) and the release of intramedullary oxytocin may produce in human and rat adrenals concentrations of $10^{-7}/10^{-6}$ M (see Section II D), it seems reasonable to suggest that adrenal medulla is the source of endogenous oxytocin involved in the control of the cortex function.

4. Somatotropin release-inhibiting hormone. SRIH-ir has been detected by RIA in the fresh adrenal medulla of humans (Bucsic et al., 1981), cows (Saito et al., 1984), cats (Corder et al., 1982) and rats (Srikant and Patel, 1985), its concentration being 6-7 μmol/g in humans and 1.6 pmol/g in cats. Saito et al. (1984) reported that the release of SRIH-ir by perfused, isolated bovine adrenals increases, after stimulation by acetylcholine, from 12 to 86 fmol/4 ml/min. The presence of SRIH-ir has been confirmed by immunocytochemistry in human (Osamura et al., 1987), cat (Vincent et al., 1987) and rat (Morel et al., 1990) adrenal medulla. However, in situ hybridization failed to reveal SRIH mRNA in the rat medullary chromaffin cells (Morel et al., 1990), thereby raising the hypothesis that the peptide is not synthesized locally in this species.

a. EFFECTS ON ZONA GLOMERULOSA. **i. Direct mechanism.** Convincing evidence indicates that SRIH exerts a direct inhibitory effect on ANG-II-stimulated aldosterone secretion of zona glomerulosa cells. Zona glomerulosa cells of humans (Epelbaum et al., 1995), cows (Maurer and Reubi, 1986) and rats (Aguilera et al., 1982; Srikant and Patel, 1985; Maurer and Reubi, 1986; Morel et al., 1990) possess specific SRIH receptors; moreover, Kong et al. (1994) showed that rat adrenal gland expresses high levels of SRIH-receptor mRNA. It is probable that SRIH, by binding to its receptors, interferes with the intracellular mechanisms mediating the secretagogue action of ANG-II (Aguilera et al., 1981; Hausdorff et al., 1989), its minimal and maximal effective concentrations being 10^{-10} M and $10^{-8}/10^{-7}$ M, respectively. It was also observed that the prolonged administration of SRIH strongly depresses the growth and steroidogenic capacity of rat zona glomerulosa cells (Mazzocchi et al., 1985) and decreases their basal proliferation rate (Pawlikowski et al., 1990), probably by blocking the basal trophic action of ANG-II. The involvement of endogenous SRIH in the physiological tonic inhibitory control of rat zona glomerulosa has been suggested by the demonstration that the administration of the specific SRIH depletor cysteamine markedly enhances the growth and aldosterone secretory capacity of the zona glomerulosa (Kasprzak et al., 1991). The level of circulating SRIH of various origins (hypothalamic, renal and intestinal) is very low, and well below the in vivo and in vitro minimal effective concentrations of the peptide ($10^{-10}/10^{-9}$ M) (Chiodini et al., 1991); conversely, it may be calculated (see Section II D) that, upon stimulation, SRIH can attain an intra-adrenal concentration of about $10^{-8}/10^{-7}$ M. This suggests that intramedullary SRIH plays a role in the physiological control of the zona glomerulosa function. This contention has been confirmed by Rebuffat et al. (1994a), who used rats bearing adrenocortical autotransplants deprived of chromaffin cells and the specific SRIH-receptor antagonist cyclo-(7-aminoheptanonyl-Phe-D-Trp-Lys-Thr[Bzl]). They observed that SRIH infusion depresses the growth and secretory activity of both adrenal zona glomerulosa cells and zona glomerulosa-like cells of autotransplants, an effect that is abolished by the simultaneous administration of the SRIH antagonist. When infused alone, SRIH antagonist enhances the growth of zona glomerulosa, but does not affect zona glomerulosa-like cells of autotransplants. Because the latter are responsive to SRIH, this finding suggests that medullary chromaffin cells are the local intra-adrenal source of endogenous SRIH involved in the control of zona glomerulosa function.

ii. Indirect mechanisms. SRIH, at a concentration above 10^{-6} M, has been found to inhibit nicotine-induced

epinephrine and norepinephrine release by cultured bovine medullary chromaffin cells (Moeller et al., 1989). SRIH also inhibits kidney renin release (Sieber et al., 1988), so it is conceivable that it may also depress intra-adrenal RAS. It remains to be investigated whether these effects contribute to the inhibitory effect of SRIH on the zona glomerulosa function.

b. EFFECTS ON ZONA FASCICULATA-RETICULARIS. None of the aforementioned investigations has reported either the presence of SRIH receptors on inner adrenocortical cells or an effect of SRIH on glucocorticoid secretion.

5. Thyrotropin-releasing hormone. TRH-ir has been demonstrated by RIA in the rat adrenal medulla (Tal et al., 1984; Mitsuma et al., 1987a, b; Simard et al., 1989; Jaworska-Feil et al., 1994), where its content is about 30 pmol/g. Findings indicate that TRH inhibits glucocorticoid secretion of dispersed rat inner adrenocortical cells; probably, it specifically impairs the late steps of corticosterone synthesis, i.e., 11β-/18-hydroxylation, without affecting the rate-limiting step of this process, i.e., the conversion of cholesterol to pregnenolone (Neri et al., 1993). The physiological relevance of the adrenocortical inhibitory action of TRH remains to be ascertained, although it must be calculated (see Section II D) that the release of intra-adrenal TRH may produce local μM concentrations, which are well above the minimal and maximal effective ones in vitro (10^{-10} M and 10^{-8} M, respectively).

B. Opioid Peptides

Opioid peptides are widely distributed in the central nervous system and are known to modulate the function of the hypothalamo-pituitary axis (Pechnick, 1993). The effects of the three members of opioid peptide family, i.e., enkephalins, endorphins and dynorphins, on the secretory activity of the adrenal cortex are rather controversial, and this might depend on the fact that opioids may act via both direct and indirect mechanisms.

1. Enkephalins. Enkephalinergic fibers are present in the rat zona glomerulosa (Holzwarth et al., 1987), and an impressive mass of data indicates that medullary chromaffin cells synthesize, store and release enkephalins. The presence of enkephalins and their related peptides met-enkephalin and leu-enkephalin in the adrenal medulla has been demonstrated by RIA in several mammalian species, including humans (Evans et al., 1985a; Parmer and O'Connor, 1988), monkeys (Evans et al., 1985a), cows (Kilpatrick et al., 1980; Evans et al., 1985a; Fischer-Colbrie et al., 1986; Hook and Liston, 1987; Bastiaensen et al., 1988), sheep (Boarder and McArdle, 1985; Evans et al., 1985a; Coulter et al., 1989), dogs (Hexum et al., 1980a, b; Evans et al., 1985a; Damase-Michel et al., 1993, 1994), cats (Corder et al., 1982; Vindrola et al., 1988), rabbits (Evans et al., 1985a), guinea pigs (Evans et al., 1985b) and rats (Dewald and Lewis, 1983; Evans et al., 1985a; Jackson et al., 1985; Bhargava et al., 1988). The adrenal content of met-enkephalin and leu-enkephalin varies according to the species and the method of assay. It is very elevated in humans and cows (from 110 to 220 pmol/g) and low in rats (10-20 pmol/g). Thus, enkephalins, upon stimulation of their intra-adrenal release, may attain local concentrations ranging from $10^{-6}/10^{-5}$ M in humans to about 10^{-7} M in rats (see Section II D). Enkephalin-ir has also been detected in cultures of bovine medullary chromaffin cells (Livett et al., 1981; Adams and Boarder, 1987; Cherdchu and Hexum, 1991). Immunocytochemistry confirmed the presence of enkephalin-ir in medullary chromaffin cells of humans (Linnoila et al., 1980; Hervonen et al., 1989), dogs (Kobayashi et al., 1983), cats (Kobayashi et al., 1983; Pelto-Huikko et al., 1987), rabbits (Wikström et al., 1996) and rats (Kobayashi et al., 1983; Pelto-Huikko et al., 1985a). The intra-adrenal synthesis of enkephalins was demonstrated by the detection of their specific mRNAs in the medullary chromaffin cells of cows (Mar et al., 1992; Suh et al., 1992, 1993; Bacher et al., 1994), rabbits (Martinez et al., 1991) and rats (Kilpatrick et al., 1984; Zhu et al., 1992; Brimijoin et al., 1995).

a. EFFECTS ON ZONA GLOMERULOSA. **i. Direct mechanism.** Racz et al. (1980) did not find any effect of enkephalins on basal aldosterone secretion of dispersed bovine zona glomerulosa cells, whereas Bevilacqua et al. (1982) and Bruzzone and Marusic (1988) reported a net stimulatory action. Racz et al. (1980) described a strong inhibitory effect of met-enkephalin on rat zona glomerulosa cells (minimal and maximal effective concentrations, 10^{-10} M and 10^{-5} M), whereas Guaza and Borell (1984) did not observe any apparent effect; conversely, Frisina et al. (1985) showed that met-enkephalin (from 10^{-8} to 10^{-6} M) increases both basal and agonist (ACTH or ANG-II)-stimulated aldosterone secretion, and that this effect is blocked by the enkephalin receptor antagonist naloxone. These last findings were recently confirmed by Kapas et al. (1995), who also demonstrated that both met-enkephalin and leu-enkephalin act via μ opioid receptors. Furthermore, Hinson and Kapas (1995) showed that previous sodium depletion enhances aldosterone response of dispersed rat zona glomerulosa cells to D-Ala^2-Met-enkephalin (DALA), a long-acting analogue of met-enkephalin. This effect was prevented by naloxone, but not by saralasin, indicating that opioid receptors, but not ANG-II receptors, mediate the sodium restriction-enhanced aldosterone secretagogue action of enkephalins. In vivo studies confirmed the aldosterone secretagogue effect of enkephalins in the rat. Robba et al. (1986a) reported a clear-cut acute aldosterone-stimulating action of DALA in hypophysectomized ACTH-replaced rats; an analogous effect of enkephalins was observed by Hinson et al. (1994a) in the rat adrenal perfused in situ, met-enkephalin being about three times more potent than leu-enkephalin.

ii. Indirect mechanisms. Jarry et al. (1989) showed that enkephalins inhibit catecholamine release by rat

chromaffin cells. Hence, the possibility remains to be investigated that this effect may mask, at least in part, the in vivo aldosterone secretagogue action of enkephalins in this species.

b. EFFECTS ON ZONA FASCICULATA-RETICULARIS. **i. Direct mechanism.** Evidence indicates that enkephalins (at concentrations greater than 10^{-8} M) inhibit ACTH-enhanced glucocorticoid secretion of cultured human inner adrenocortical cells (Lamberts et al., 1983) and dispersed rat zona fasciculata-reticularis cells (Racz et al., 1980; Guaza and Borrell, 1984), apparently without affecting the basal release rate. In contrast, Kapas et al. (1995) provided findings that $10^{-6}/10^{-5}$ M met-enkephalin or DALA (but not leu-enkephalin) raise basal corticosterone production by collagenase-dispersed rat zona fasciculata cells. The prolonged (5-7 day) administration of DALA was found to elicit in chemically hypophysectomized rats administered maintenance doses of ACTH a net hypertrophy of zona fasciculata cells, coupled with a parallel increase in their basal and maximally agonist-stimulated corticosterone secretory capacity (Andreis et al., 1988).

ii. Indirect mechanisms. Robba et al. (1986b) did not observe significant changes in plasma corticosterone levels of hypophysectomized ACTH-treated rats after the acute administration of DALA. In contrast, Hinson et al. (1994b) reported an increased release of corticosterone by in situ perfused rat adrenals after both met-enkephalin and leu-enkephalin administration, an effect that could be (at least partly) attributable to the enkephalin-induced increase in the flow rate of the perfusion medium.

2. Endorphins. The pro-opiomelanocortin (POMC)-derived endorphins, especially β-endorphin-ir, have been demonstrated by RIA in the adrenal medulla of humans (Boarder and McArdle, 1985; Evans et al., 1985a) and rats (Bhargava et al., 1988; Bagdy et al., 1990), with concentrations ranging from 150 pmol/g in humans to 15 fmol/g in rats. Immunocytochemistry confirmed the presence of β-endorphin-ir in the adrenal chromaffin cells of rats and mice (Arefolov et al., 1986). β-Endorphin mRNA has been detected in the human adrenal medulla (De Bold et al., 1988).

Specific β-endorphin receptors have been found in both dispersed (Dave et al., 1985b) and cultured rat adrenocortical cells (Gelfand et al., 1995). According to Dave et al. (1985b), β-endorphin receptors are coupled with adenylate cyclase. However, the results of investigations dealing with the effect of endorphins on the in vitro functioning of adrenocortical cells are apparently very conflicting. No effect on adrenocortical secretion (either basal or agonist-stimulated) was reported in guinea pigs (Matsuoka et al., 1981; Pham-Huu-Trung et al., 1982; O'Connell et al., 1993) and rats (Goverde et al., 1988; Hung and LeMaire, 1988). A stimulatory action of β-endorphin on glucocorticoid, but not aldosterone, production has been described in humans (Eggens et al., 1987), guinea pigs (Bruni et al., 1985) and rats (Shanker and Sharma, 1979; Guaza et al., 1986; Kapas et al., 1995). In contrast, Szalay and Stark (1981) observed a β-endorphin-induced inhibition of corticosterone (but not aldosterone) secretion of dispersed rat adrenocortical cells. Further studies provided a clue to reconcile the above reviewed contrasting findings, because they showed that the effects of β-endorphin are tightly linked to its concentration (Szalay, 1990, 1993). From 10^{-11} to 10^{-5} M, β-endorphin decreases basal corticosterone output by dispersed rat zona fasciculata cells, but not aldosterone secretion of zona glomerulosa cells; at a higher concentration (10^{-4} M), both corticosterone and aldosterone yields are raised. β-Endorphin from 10^{-9} to 10^{-7} M inhibits the ACTH-stimulated production of both corticosterone and aldosterone. To explain her findings, Szalay (1993) advanced the hypothesis that β-endorphin can bind to both opioid and ACTH receptors located on adrenocortical cells. Both receptors are coupled to adenylate cyclase, ACTH receptors positively and opioid receptors negatively, and a "cross talk" exists between the two receptor systems. Hence, the net effect of β-endorphin on steroidogenesis depends on the proportion of its binding to opioid or ACTH receptors. It is likely that, given the relative affinity for the two receptors, at low concentrations of β-endorphin, the binding to opiate receptors prevails, with ensuing inhibition of adenylate cyclase, which reduces both basal and ACTH-stimulated steroid secretion. At higher concentrations, the aspecific binding of β-endorphin to ACTH receptors may activate steroidogenesis. In conclusion, it appears that β-endorphin plays a role in the control of adrenal steroid secretion, especially directed on the zona fasciculata-reticularis and glucocorticoid secretion. Under physiological conditions, the action of β-endorphin is mainly inhibitory, because it may be calculated (see Section II D) that the intra-adrenal content of this peptide can produce local concentrations not higher than 10^{-6} M in humans and 10^{-9} M in rats.

3. Dynorphins. Dynorphin-ir has been found by RIA in the adrenal medulla of rats (Akil et al., 1984; Vincent et al., 1984; Bhargava et al., 1988) and guinea pigs (Evans et al., 1985a, b), where its content (of about 110-130 pmol/g) could give rise to local concentrations greater than 10^{-6} M (see Section II D). Watson et al. (1981) detected dynorphin-ir in rat adrenomedullary chromaffin cells by immunocytochemistry. Day et al. (1991) showed the expression of the pro-dynorphin gene in the rat adrenal cortex by in situ hybridization. Unfortunately, light micrographs do not allow us to ascertain whether pro-dynorphin mRNA is contained in adrenocortical cells or in chromaffin cells scattered in the cortex.

The presence of specific receptors for dynorphin has been reported in both the cortex and medulla of rat adrenals (Quirion et al., 1983). However, in vitro studies using dispersed rat adrenocortical cells gave equivocal

results. Guaza et al. (1986) found that dynorphin$_{1-17}$ (from 10^{-8} to 10^{-6} M) does not affect basal corticosterone secretion, although it increases inner-cell responsiveness to submaximal effective concentrations (10^{-12}/10^{-11} M) of ACTH. By contrast, Neri et al. (1990a) showed that dynorphin concentration-dependently lowers basal aldosterone and corticosterone productions by dispersed outer and inner adrenocortical cells, respectively, its minimal effective concentration being 10^{-9} M; the maximal effective concentration of dynorphin (10^{-6} M) depresses both basal and ACTH-stimulated post-progesterone steps of steroid synthesis, specifically affecting the conversion of progesterone to 11-deoxycorticosterone (21-hydroxylation). Mazzocchi et al. (1990) observed that dynorphin infusion for 3 h significantly decreases blood levels of aldosterone and corticosterone in rats and markedly restrains their ACTH- or ANG-II-evoked rises.

C. Neuromedins

Neuromedins are regulatory peptides, originally isolated from spinal cord, that stimulate the contractility of smooth-muscle cells. They are widely distributed in the central nervous system and HPA axis, and many of them have been found to variously affect adrenocortical secretion by acting on the central branch of the CRH-ACTH system (for review, see Malendowicz and Markowska, 1994). Mammalian neuromedins are distinguished in four groups: kassinin-like tachykinins, bombesin-like neuromedins, neurotensin-like neuromedins and neuromedins U.

1. Kassinin-like tachykinins. Mammalian tachykinins include neuromedin K (neurokinin B), neuromedin L (neurokinin A), substance P, structurally related to neuromedin L and encoded by the same gene, and neuropeptide K, which is the N-terminally extended form of neuromedin L (for review, see Maggio, 1988). Substance P-positive nerve fibers are present in the adrenal cortex of rabbits, guinea pigs and rats (Kuramoto et al., 1985; Holzwarth et al., 1987; Heym et al., 1995; Wikström et al., 1996). RIA showed that tachykinin-ir is contained in the human, cow, cat, rabbit, guinea pig and rat adrenal medulla (Saria et al., 1980; Bucsics et al., 1981; Vaupel et al., 1988; Basile et al., 1992; Fischer-Colbrie et al., 1992; Cheung et al., 1993; Hinson and Kapas, 1996). The adrenal content of substance P is higher than that of other tachykinins, ranging from 8 to 12 pmol/g in humans to 4 to 5 pmol/g in rats. Substance P-ir (Kuramoto et al., 1985) and neuromedin L-ir (Wang et al., 1994) have been immunocytochemically evidenced in medullary chromaffin cells of rats and pigs, respectively.

Tachykinins act via three subtypes of receptors, named NK_1, NK_2 and NK_3, which show the highest affinity for substance P, neuromedin L and neuromedin K, respectively. Their activation induces transient stimulation of phospholipase C and intracellular Ca^{2+} mobilization, increased prostaglandin E_2 formation, and stimulation of adenylate cyclase (for review, see Maggio, 1988).

a. EFFECTS ON ZONA GLOMERULOSA. **i. Direct mechanism.** Neri et al. (1990b) and Mazzocchi et al. (1995b) reported that substance P depresses aldosterone production of dispersed rat zona glomerulosa cells by specifically inhibiting the late steps of its synthesis (i.e., the conversion of corticosterone to aldosterone). However, this inhibitory effect was observed only at μM peptide concentrations, a finding casting doubts on its physiological relevance, inasmuch as it may be calculated (see Section II D) that intra-adrenal release of substance P can produce a local concentration not higher than 10^{-7} M.

ii. Indirect mechanisms. Convincing evidence is available that substance P is involved in the physiological regulation of zona glomerulosa growth and secretion, probably acting through indirect mechanisms. Substance P evokes a marked rise in the plasma level of aldosterone in rats, whose HPA axis and RAS had been interrupted to exclude an indirect action of the peptide via pituitary ACTH and ANG-II (Nussdorfer et al., 1988). Moreover, substance P induces a moderate rise in aldosterone release by in situ perfused rat adrenals (Hinson et al., 1994a). Substance P, at nM concentrations, enhances basal aldosterone output by adrenal quarters including medullary tissue (Mazzocchi et al., 1995b). In addition to acutely enhance aldosterone secretion, substance P also stimulates the growth and steroidogenic capacity of rat zona glomerulosa (Nussdorfer et al., 1988). Mazzocchi et al. (1995a) confirmed the physiological relevance of this long-term effect of substance P. A 7-day intraperitoneal infusion with the specific substance P antagonist [D-Pro^4, D-Trp^{7,9}]-SP$_{4-11}$ significantly lowers plasma aldosterone concentration, causes atrophy of zona glomerulosa and its parenchymal cells, and markedly decreases either basal or maximally agonist-stimulated aldosterone secretion of dispersed zona glomerulosa cells. The simultaneous infusion with substance P abolished all these effects. Under normal circumstances, substance P mainly acts as a neurotransmitter, and its plasma levels are too low to account for a systemic action (Maggio, 1988). Hence, adrenal medulla is conceivably the source of endogenous substance P exerting adrenoglomerulotrophic effect. The contention that medullary chromaffin cells are involved in the mediation of aldosterone secretagogue effect of substance P in rats is supported by the demonstration that this peptide does not affect steroid release by quarters of regenerated adrenocortical autotransplants deprived of chromaffin cells (Mazzocchi et al., 1995b). Medullary chromaffin cells have NK_1 receptors (Maggio, 1988; Geraghty et al., 1990), and substance P (like other tachykinins) is involved in the modulation of catecholamine release by cow and rat adrenal chromaffin cells, probably by activating phosphatidylinositol phosphate metabolism and increasing $[Ca^{2+}]_i$ (Minenko and Oehme,

1987; Khalil et al., 1988; Zhou et al., 1991). The β-adrenoceptor antagonist L-alprenolol (10^{-7} M) was found to block not only isoprenaline (10^{-8} M)-induced aldosterone release by rat adrenal quarters, but also the aldosterone secretagogue effect of 10^{-8} M substance P (Mazzocchi et al., 1995b).

b. EFFECTS ON ZONA FASCICULATA-RETICULARIS. **i. Direct mechanism.** Yoshida et al. (1992) reported that tachykinins concentration-dependently raise cortisol output by cultured bovine zona fasciculata-reticularis cells, the potency order being as follows: substance P > neuromedin L > neuromedin K. Minimal and maximal effective concentrations of substance P were 10^{-10} M and 10^{-8} M, respectively, and the secretagogue effect of substance P was prevented by a calmodulin inhibitor, thereby suggesting that it is dependent upon intracellular Ca^{2+} redistribution. Conversely, other investigations showed that none of the tachykinins, including neuropeptide K, is able to alter basal corticosterone secretion of dispersed rat inner adrenocortical cells (Mazzocchi et al., 1994a; Malendowicz et al., 1995a, b). Malendowicz et al. (1996b) recently reported that both substance P and its antagonist spantide (from 10^{-8} to 10^{-6} M) inhibit maximally ACTH-stimulated corticosterone yield by dispersed rat zona fasciculata-reticularis cells, an observation strongly suggesting a receptor-independent interference of the tachykinin molecule with the intracellular mechanisms transducing the ACTH secretagogue signal. In conclusion, the above findings cast doubts on the physiological relevance of the direct effects of tachykinins on inner adrenocortical cells and suggest that, probably, they must be considered pharmacological in nature.

ii. Indirect mechanisms. Tachykinins affect the secretion and growth of inner adrenocortical cells, acting through indirect mechanisms. Neuropeptide K evokes a significant increase in the plasma corticosterone concentration of hypophysectomized rats administered maintenance dose of ACTH (Mazzocchi et al., 1994a). Substance P also evokes a sizable rise in corticosterone release by in situ perfused rat adrenals (Hinson et al., 1994b), which is coupled with only a minor effect on the perfusion-medium flow rate (Hinson et al., 1994c). Neuromedin K, neuromedin L and neuropeptide K, at nM concentrations, enhance basal corticosterone secretion by adrenal slices including medullary tissue (Mazzocchi et al., 1994a; Malendowicz et al., 1995a, b). Mammalian medullary chromaffin cells are provided with NK_1 receptors (Maggio, 1988; Geraghty et al., 1990), and there is evidence that neuropeptide K stimulates HPA axis (Kalra and Kalra, 1993). Mazzocchi et al. (1994a) demonstrated that neuropeptide K elicits corticosterone secretion by rat adrenal slices (threshold concentration 10^{-9} M), and 10^{-8} M neuropeptide K potentiates corticosterone response to 10^{-12} M ACTH, but not that to higher concentrations of the hormone. The corticosterone secretagogue effect of 10^{-8} M neuropeptide K is abolished by both 10^{-6} M α-CRH and CIP, thereby suggesting that the glucocorticoid secretagogue effect of this peptide is indirectly mediated by the activation of the intramedullary CRH-ACTH system. In this connection, it must be recalled that substance P, when infused at high doses (20 pmol/kg/min), inhibits acetylcholine-induced release of CRH by adrenal glands of conscious calves with pharmacologically interrupted HPA axis (Edwards and Jones, 1994b).

2. Other neuromedins. Bombesin is a peptide originally isolated from the skin of the frog *Bombina bombina*, whose mammalian counterpart is the gastrin-releasing peptide. The two bombesin-like neuromedins are neuromedin B and neuromedin C, the latter of which is the C-terminal decapeptide of gastrin-releasing peptide. Both neuromedins share structural homologies and exert similar biological effects, including regulation of the release of gastrointestinal hormones, smooth-muscle contraction and modulation of neuronal activity (for review, see Malendowicz and Markowska, 1994). Neuromedin N is a hexapeptide, whose C-terminal tetrapeptide sequence is identical to that of neurotensin. It is widely distributed and evokes neurotensin-like effects, such as analgesia, hypothermia and neuronal activation (for review, see Malendowicz and Markowska, 1994). Two forms of neuromedin U, containing 25- and 8-amino acid residues, have been isolated from mammalian tissues. The C-terminus of neuromedin U-25 contains the entire sequence of neuromedin U-8. Neuromedin U-ir has been found in the central nervous system, including the hypothalamo-pituitary axis, but its physiological role is not yet entirely known, although its stimulatory effect on the smooth-muscle cells and pituitary ACTH release is well established.

Neuromedin B-ir and neuromedin C-ir are present in the cow (Lemaire et al., 1989) and rat adrenal medulla (Domin et al., 1989). Elevated concentrations of neuromedin N-ir (about 13 pmol/g) are present in the cat adrenal medulla (Carraway and Mitra, 1987). No reports are available on the presence of neuromedin U-ir in the adrenal glands (for review, see Domin et al., 1989; Malendowicz and Markowska, 1994).

Despite the many papers dealing with the effects of these three groups of neuromedins on the HPA axis in vivo and in vitro (for review, see Malendowicz and Markowska, 1994), studies on their direct effect on adrenocortical cells are very scarce to the best of my knowledge. Only neuromedin U-8 was tested and found to affect, within the concentration range from 10^{-8} to 10^{-6} M, neither steroid secretion nor intracellular Ca^{2+} redistribution of dispersed rat adrenocortical cells (Malendowicz et al., 1994a, b). However, evidence is available that this peptide may affect adrenal cortex through indirect mechanisms. In fact, neuromedin U-8 (from 10^{-8} to 10^{-6} M) was found to stimulate steroid secretion by rat adrenal slices, but not by quarters of regenerated adrenocortical autotransplants (Malendowicz et al.,

1994a). This strongly suggests the involvement of medullary chromaffin cells in the mediation of the secretagogue action of this peptide. An accurate high performance liquid chromatography analysis of steroid hormones secreted showed that the effects vary according to the concentration of neuromedin U-8. At all concentrations tested, the peptide raises the production of both pregnenolone and total post-pregnenolone steroids. The increase in total post-pregnenolone steroid output induced by a concentration of 10^{-8} M is attributable to similar rises in the yields of non-18-hydroxylated steroids (progesterone, 11-deoxycorticosterone and corticosterone) and 18-hydroxylated hormones (18-hydroxycorticosterone and aldosterone); conversely, the increase elicited by a concentration of $10^{-7}/10^{-6}$ M is almost exclusively attributable to the rise in the yield of 18-hydroxylated steroids. The stimulating effect of neuromedin U-8 on pregnenolone output, i.e., the early rate-limiting step of steroid synthesis, is blocked by both 10^{-6} M α-CRH and CIP. In light of these findings, Malendowicz et al. (1994a) drew the following conclusions: (a) at all concentrations tested, neuromedin U-8 stimulates intramedullary CRH-ACTH system, thereby increasing the activity of the early step of steroidogenesis and consequently the production of the entire spectrum of post-pregnenolone steroids; and (b) at concentrations greater than 10^{-8} M, neuromedin U-8 also elicits the release from chromaffin cells of a factor capable to specifically enhance the late steps of aldosterone synthesis. The possibility that catecholamines are such a factor does not seem to be convincing, because they are known to enhance all the steps of aldosterone synthesis (see Section III A).

D. Pancreatic Polypeptide Family

This family of 36-amino acid peptides includes pancreatic polypeptide, peptide YY and neuropeptide Y. Pancreatic polypeptide and peptide YY are present in the pancreas and gastrointestinal mucosa, where they exert important endocrine and paracrine actions involved in the control of digestive functions (for review, see Taylor, 1989). Neuropeptide Y is ubiquitously distributed in the neurons of the whole body and possesses multiple and important physiological functions, including the regulation of brain-endocrine axes (for review, see McDonald and Koening, 1993).

1. Pancreatic polypeptide. Pancreatic polypeptide-ir has been recently immunocytochemically detected in the medulla (but not in the cortex) of the rat adrenals (Malendowicz et al., 1996a), and specific receptors for this peptide have been demonstrated in the rat zona fasciculata-reticularis and adrenal medulla (Whitcomb et al., 1992). Andreis et al. (1993) reported that pancreatic polypeptide raises both basal and submaximally ACTH-stimulated corticosterone production of dispersed rat inner adrenocortical cells (minimal effective concentration being in the nM range), without affecting aldosterone secretion of dispersed zona glomerulosa cells. In a subsequent in vivo study, Mazzocchi et al. (1995c) demonstrated that pancreatic polypeptide may play an important role as modulator of rat adrenocortical responses to insulin-induced hypoglycemic stress.

2. Peptide YY. Peptide YY-ir is contained in the adrenal gland of rats and guinea pigs (for review, see Taylor, 1989), and receptors for this peptide are present in the rat zona fasciculata-reticularis cells (Whitcomb et al., 1992). Peptide YY (10^{-6} M) was reported to depress both basal and ACTH-stimulated output of 18-hydroxylated steroids by dispersed rat zona glomerulosa cells (Neri et al., 1991a). However, Bernet et al. (1994b) did not find any effect of this peptide (from 10^{-10} to 10^{-6} M) on aldosterone and corticosterone yields by rat capsule-zona glomerulosa preparations.

3. Neuropeptide Y. Neuropeptide Y-positive nerve fibers are present in the adrenal cortex of cows and rats (Varndell et al., 1984; Majane et al., 1985; Kuramoto et al., 1986; Maubert et al., 1990; Oomori et al., 1994). The fibers ending in the rat adrenal capsule and zona glomerulosa arise, at least in part, from adrenal medulla, inasmuch as neuropeptide Y-ir content in the zona glomerulosa markedly lowers after demedullation (Maubert et al., 1993). Large amounts of neuropeptide Y-ir have been shown by RIA in the fresh adrenal medulla of humans (Lundberg et al., 1985, 1986c), cows (Allen et al., 1984; Majane et al., 1985; Fischer-Colbrie et al., 1986; Bastiaensen et al., 1988), dogs (Briand et al., 1990a, b; Damase-Michel et al., 1993, 1994), cats (Lundberg et al., 1986b) and rats (De Quidt and Emson, 1986; Higuchi et al., 1988; Hinson and Kapas, 1996). Neuropeptide Y-ir adrenal content displays a great variability according to the species: it is very high in mice and cats (250-500 pmol/g), moderate in cows and guinea pigs (60-100 pmol/g) and low in humans and rats (5-20 pmol/g). These intra-adrenal contents could produce local concentrations of neuropeptide Y ranging from 6×10^{-8} to 6×10^{-6} M (see Section II D). Immunocytochemistry confirmed the presence of neuropeptide Y-ir in the adrenal medulla of rabbits, rats and mice (Majane et al., 1985; Schalling et al., 1988; Pelto-Huikko, 1989; Steiner et al., 1989; Fernandez-Vivero et al., 1993; Wolfensberger et al., 1995; Malendowicz et al., 1996a; Wikström et al., 1996). Neuropeptide Y mRNA has been detected in the rat adrenal medulla (Schalling et al., 1988, 1991; Higuchi et al., 1990, 1991a). Of interest, in the rat, medullary neuropeptide Y protein and mRNA display a marked age-dependent increase that is coupled with only a minor rise in the plasma level of this peptide (Higuchi et al., 1988, 1991b).

a. EFFECTS ON ZONA GLOMERULOSA. **i. Direct mechanism.** Binding sites for neuropeptide Y have been autoradiographically demonstrated in the bovine zona glomerulosa (Torda et al., 1988); this finding, coupled with the presence of abundant neuropeptide Y-positive nerve fibers, strongly suggests that the specific anatomical

and physiological target of this peptide is zona glomerulosa and mineralocorticoid secretion, respectively. Mazzocchi et al. (1996a) showed that neuropeptide Y dose-dependently raised aldosterone, but not corticosterone, plasma concentration in rats with pharmacologically interrupted HPA axis and RAS, an effect reversed by the neuropeptide Y-receptor antagonists PYX-1 and PYX-2. Furthermore, Hinson et al. (1994a) observed that neuropeptide Y evokes a moderate increase in aldosterone release by in situ perfused rat adrenals. However, a receptor-mediated direct stimulatory action of this peptide on the zona glomerulosa cells is controversial. Neri et al. (1990b) demonstrated that 10^{-6} M neuropeptide Y strongly depresses basal 18-hydroxylated steroid output by dispersed rat zona glomerulosa cells. By contrast, Hinson and Kapas (1995) did not find any effect over a concentration range from 10^{-11} to 10^{-6} M, nor a moderate stimulatory action at higher concentrations; however, 10^{-8} M neuropeptide Y appears to stimulate aldosterone secretion by dispersed zona glomerulosa cells from sodium-depleted rats. Interestingly, Hinson et al. (1995) showed that neuropeptide Y biphasically modulates aldosterone response to ACTH of rat zona glomerulosa cells: 10^{-6} M neuropeptide Y magnifies the response to submaximal concentrations of ACTH (less than 10^{-10} M) and reduces those to maximally effective concentrations of the hormone (greater than 10^{-10} M). It also moderately enhances the response to supramaximal concentrations of ANG-II (10^{-7}/10^{-6} M), but not to K^+. Thus, they hypothesized that neuropeptide Y regulates the sensitivity of rat zona glomerulosa cells to their main peptide agonists. The physiological relevance of these findings is doubtful, because it is difficult to conceive that in the rat adrenals, neuropeptide Y may reach a concentration higher than 10^{-8} M (see above). The prolonged (7-day) intraperitoneal infusion of neuropeptide Y was found to induce a marked stimulation of the growth and secretory capacity of zona glomerulosa in rats with pharmacologically interrupted HPA axis and RAS (Mazzocchi et al., 1996a), but not in intact animals (Lesniewska et al., 1990). Because this peptide inhibits renin release, both in vivo and in vitro (see next paragraph), this discrepancy may be ascribed to the neuropeptide Y-induced chronic suppression of ANG-II production that could have masked its adrenoglomerulotropic action. Mazzocchi et al. (1996a) showed that the simultaneous infusion of PYX-2 completely abolishes the stimulatory effect of neuropeptide Y on the growth of rat zona glomerulosa. However, when administered alone, PYX-2 did not evoke any apparent effect, a finding that causes doubts about the involvement of endogenous neuropeptide Y in the physiological maintenance of the zona glomerulosa growth in rats.

ii. Indirect mechanisms. Neuropeptide Y and its Y_1 and Y_2 receptor agonists (from 10^{-8} to 10^{-6} M) were found to raise aldosterone secretion by rat capsule-zona glomerulosa preparations (Bernet et al., 1994b). This is keeping with the findings of Mazzocchi et al. (1996a) in rat adrenal slices including medullary tissue, where aldosterone secretagogue action of neuropeptide Y is blocked by its receptor antagonists. The mechanism underlying the adrenoglomerulotropic action of neuropeptide Y may involve the stimulation of catecholamine release by adrenal medulla, at least in the rat. In fact, neuropeptide Y elicits catecholamine release by chromaffin cells and adrenergic fibers (Bernet et al., 1994a; Di Maggio et al., 1994) and enhances catecholamine synthesis in the rat (Hong et al., 1995). In addition, Bernet et al. (1994a) showed that 10^{-7} M atenolol, a β_1-adrenoceptor antagonist, but not the β_2-antagonist ICI-18,551 hydrochloride, hampers 10^{-7} M neuropeptide Y-induced aldosterone release by rat capsule-zona glomerulosa preparations. Finally, it is worth recalling that neuropeptide Y strongly depresses renin release by kidney juxtaglomerular cells (Hackenthal et al., 1987; Corder et al., 1989; Aubert et al., 1992). Hence, the possibility that the inhibition of intra-adrenal RAS may counteract the adrenoglomerulotropic action of this peptide awaits exploration.

b. EFFECTS ON ZONA FASCICULATA-RETICULARIS. Scarce and rather conflicting data are available on the direct effect of neuropeptide Y on inner adrenocortical cells. Malendowicz et al. (1990) described an inhibitory effect of neuropeptide Y (3×10^{-7} M) on basal and ACTH-stimulated corticosterone secretion of dispersed rat zona fasciculata-reticularis cells, but neither Neri et al. (1990b) nor Hinson et al. (1995) observed any significant effect. Moreover, no change in corticosterone output by adrenal slices were observed after exposure to neuropeptide Y (Mazzocchi et al., 1996a). In vivo studies also failed to detect any acute or chronic action of neuropeptide Y on the zona fasciculata and glucocorticoid secretion in rats (Mazzocchi et al., 1996a). At variance with these results, Hinson et al. (1994b, c) reported a small increase in corticosterone release by in situ perfused rat adrenal only after the administration of nanomolar doses of neuropeptide Y, coupled with a sizable rise in the flow rate of the perfusion medium.

E. Vasoactive Intestinal Peptide and Pituitary Adenylate Cyclase-Activating Polypeptide

VIP and pituitary adenylate cyclase-activating polypeptide (PACAP) are 28- and 38-amino acid-residue peptides, originally isolated from the pig intestine and sheep hypothalamus, respectively, then found in many other tissues. Because it has been demonstrated that the N-terminal 28-amino acid sequence of PACAP has a 68% identity with VIP, PACAP and VIP are now considered to belong to a family of structurally related regulatory peptides that also includes secretin, glucagon, gastric inhibitory peptide and somatotropin-releasing hormone. VIP and PACAP share several physiological functions, including neurotransmission and stimulation of pituitary ACTH release, regulation of vascular tone and

modulation of the activity of the gastrointestinal tract (for review, see Fahrenkrug, 1989; Arimura and Shioda, 1995). The structural homology of VIP and PACAP easily explains why several subtypes of receptors can interact with both peptides (Harmar and Lutz, 1994).

1. Vasoactive intestinal peptide. VIP-ergic fibers are present in the zona glomerulosa of pigs (Kong et al., 1989) and rat adrenals (Hökfelt et al., 1981; Holzwarth, 1984; Maubert et al., 1990; Oomori et al., 1994). The presence of VIP-ir has been detected by RIA in the pig (Ehrhart-Bornstein et al., 1991a) and rat adrenal medulla (Hinson and Kapas, 1996), where its content of about 4-9 pmol/g could give rise to local concentrations of $10^{-8}/10^{-7}$ M (see Section II D). The presence of VIP-ir has been also demonstrated by RIA in cultures of bovine adrenomedullary cells (Pruss et al., 1985), and by immunocytochemistry in the adrenal medulla of humans (Linnoila et al., 1980), sheep (Cheung and Holzwarth, 1986) and rats (Holzwarth, 1984; Kondo et al., 1986; Oomori et al., 1994).

a. EFFECTS ON ZONA GLOMERULOSA. **i. Direct mechanism.** Specific binding of VIP has been autoradiographically demonstrated in the rat adrenal capsule and zona glomerulosa (Cunningham and Holzwarth, 1989). The studies dealing with VIP effects on dispersed zona glomerulosa cells gave controversial results. Enyedi et al. (1983) and Hinson et al. (1992) reported no significant aldosterone response. However, a moderate effect of 10^{-6} M VIP on basal aldosterone secretion of dispersed rat zona glomerulosa cells was observed by Hinson and Kapas (1995). They also reported a more potent effect on cells obtained from sodium-deprived animals (threshold concentration 10^{-9} M). Because saralasin did not affect the response to VIP, ANG-II receptors, which are upregulated by sodium restriction, are not involved. It was therefore hypothesized that sodium depletion may upregulate VIP receptors in the rat zona glomerulosa cells. A general consensus exists on the marked aldosterone response to VIP of rat capsule-zona glomerulosa preparations, minimal effective concentrations ranging from 10^{-8} to 10^{-7} M (Cunningham and Holzwarth, 1988; Hinson et al., 1992; Bernet et al., 1994a). Mazzocchi et al. (1993a) showed that VIP enhances basal aldosterone secretion of rat adrenal slices, its minimal and maximal effective concentrations being 10^{-10} M and 10^{-8} M, respectively. Compelling evidence also indicates that VIP exerts an acute and chronic stimulatory action on zona glomerulosa secretion both in vivo in rats (Nussdorfer and Mazzocchi, 1987; Rebuffat et al., 1994b) and in pig (Ehrhart-Bornstein et al., 1991a) and rat adrenals perfused in situ (Hinson et al., 1992, 1994a). Rebuffat et al. (1994b) showed that the prolonged administration of VIP specifically enhances the growth and aldosterone secretory capacity of zona glomerulosa in rats with pharmacologically interrupted HPA axis and RAS. They also demonstrated that VIP stimulates the growth and secretion of zona glomerulosa-like cells of rat adrenocortical autotransplants, and that the effect of this peptide on both adrenals and autotransplants are annulled by the simultaneous administration of the VIP-receptor antagonist [4-Cl-D-Phe^6, Leu^{17}]-VIP. Moreover, the administration of the VIP antagonist alone for 7 days was found to depress the growth and secretory capacity of the zona glomerulosa, but not of zona glomerulosa-like cells of autotransplants. By taking into account that intramedullary release may allow VIP to achieve local concentrations higher than its minimal effective ones in vitro, Rebuffat and associates (1994b) suggested that the source of endogenous VIP involved in the direct regulation of the zona glomerulosa function in rats is most likely adrenal medulla.

ii. Indirect mechanisms. Compelling evidence indicates that an indirect mechanism, involving the release of catecholamines, plays an important role in the stimulatory action of VIP on the rat zona glomerulosa. VIP appears to act as a neurotransmitter in rat adrenal medulla, being a potent activator of adenylate cyclase and catecholamine release (Cheung and Holzwarth, 1986; Malhotra and Wakade, 1987; Wakade et al., 1991; Bernet et al., 1994a). The specific β-adrenoceptor antagonists L-alprenolol and atenolol (10^{-7} M) block VIP ($10^{-7}/10^{-5}$ M)-induced release of aldosterone by rat capsule-zona glomerulosa preparations (Hinson et al., 1992; Bernet et al., 1994a). Moreover, Mazzocchi et al. (1993a) reported that 10^{-7} M L-alprenolol lowers by 70% 10^{-8} M VIP-stimulated aldosterone output by rat adrenal slices, without affecting corticosterone release. They also observed that the ACTH-receptor antagonist CIP (10^{-6} M) abolishes VIP-induced corticosterone secretion, but only decreases (by about 50%) aldosterone production; when added together, CIP and L-alprenolol abrogate both aldosterone and corticosterone responses to VIP. In light of these findings, Mazzocchi and associates concluded that a two-fold mechanism underlies the aldosterone secretagogue action of VIP in the rat: (*a*) direct aspecific and weak stimulation of zona glomerulosa cells via ACTH receptors, and (*b*) specific and potent stimulation through the enhancement of catecholamine release by chromaffin cells and adrenergic fibers contained in zona glomerulosa.

b. EFFECTS ON ZONA FASCICULATA-RETICULARIS. Li et al. (1990) described a weak, but significant, stimulatory effect of VIP on the bovine inner adrenocortical cells (minimal and maximal effective concentrations, 10^{-10} M and 10^{-8} M, respectively). A glucocorticoid response to $10^{-10}/10^{-8}$ M VIP by rat adrenal-slice preparations was also found (Mazzocchi et al., 1993a). VIP raises cortisol and androstenedione and, respectively, corticosterone release by in situ perfused pig (Ehrhart-Bornstein et al., 1991a; Bornstein et al., 1993) and rat adrenal glands (Hinson et al., 1992, 1994b). In vivo studies did not reveal any acute or chronic effect of VIP on corticosterone secretion and zona fasciculata growth in rats (Rebuffat et al., 1994b). However, according to Bloom et al.

(1987a), VIP administration increases the blood level of cortisol in hypophysectomized calves, but not in intact animals. These discrepancies were reconciled by Mazzocchi et al. (1994c), who showed that (*a*) VIP concentration-dependently increases basal, but not submaximally ACTH-stimulated, corticosterone yield by dispersed rat inner adrenocortical cells, and (*b*) both a VIP-receptor antagonist and CIP completely block the secretagogue effect of VIP. Because the VIP-receptor antagonist partially reduces also the ACTH-enhanced corticosterone production, these authors hypothesized that the weak glucocorticoid secretagogue action of VIP may be mediated by the ACTH receptors present in zona fasciculata-reticularis cells. Of interest, Li et al. (1990) reported that VIP competes only with a subtype of ACTH receptors, i.e., those recognizing the $ACTH_{11-24}$ sequence. This could explain why the VIP-receptor antagonist does not abolish the secretagogue effect of the entire ACTH molecule and also the findings of Bloom et al. (1987a). In fact, the tonic activation of ACTH receptors by circulating ACTH may mask in vivo their VIP-induced aspecific activation, which can manifest itself only when hypophyseal ACTH release is suppressed by hypophysectomy. Finally, it must be recalled that VIP was found to increase the medium flow rate of in situ perfused rat adrenals (Hinson et al., 1994c), thereby suggesting that the rise in adrenal blood flow rate may contribute to the glucocorticoid secretagogue action of VIP.

2. Pituitary adenylate cyclase-activating polypeptide. Two PACAP amidated forms exist, with 38- and 27-amino acid residues (PACAP-38 and PACAP-27), the latter deriving from the N-terminal 27 of the former. Both are equipotent in stimulating pituitary adenylate cyclase (Arimura and Shioda, 1995). Occasional PACAP-positive nerve fibers have been observed in the rat adrenal medulla (Frodin et al., 1995). PACAP-ir was detected in rat (Arimura et al., 1991; Ghatei et al., 1993; Tabarin et al., 1994; Frodin et al., 1995), cow, pig, hamster and mouse adrenal medulla (Tabarin et al., 1994). The highest adrenal content of PACAP was observed in the mouse and hamster (9-12 pmol/g) and the lowest in the cow and pig (0.7-0.8 pmol/g). In the rat, PACAP adrenal content is about 2-4 pmol/g and could produce a local concentration of about $2\text{-}3 \times 10^{-8}$ M. Immunocytochemistry confirmed the presence of PACAP-ir in the medullary chromaffin cells (Tabarin et al., 1994; Shiotani et al., 1995). The demonstration of PACAP mRNA in the rat adrenal medulla (Ghatei et al., 1993) is consistent with a local synthesis of both peptides.

a. EFFECTS ON ZONA GLOMERULOSA. PACAP-38 does not affect the secretory activity of dispersed human (Neri et al., 1996) and rat zona glomerulosa cells (Andreis et al., 1995). However, this peptide stimulates aldosterone secretion of human and rat adrenal slices, containing chromaffin cells, the minimal effective concentration of PACAP-38 being 10^{-12} M in rats and 10^{-8} M in humans (Andreis et al., 1995; Neri et al., 1996). In the rat, aldosterone response is abolished by 10^{-6} M $PACAP_{6-38}$, an antagonist of PACAP-38 (Andreis et al., 1995). PACAP enhances the secretion of catecholamines from human (Neri et al., 1996) and rat adrenal medulla (Chowdhury et al., 1994; Guo and Wakade, 1994), as well as from cultured cow (Houchi et al., 1994; Perrin et al., 1995), pig (Isobe et al., 1993) and rat medullary chromaffin cells (Watanabe et al., 1992). The effect of PACAP is equipotent on epinephrine and norepinephrine release and is not blocked by cholinergic antagonists, thereby suggesting that the action of this peptide is direct and not mediated by the release of acetylcholine (Watanabe et al., 1995). More recently, Isobe et al. (1996) demonstrated that PACAP, at nM concentrations, coordinately up-regulates gene expression of the catecholamine-synthesizing enzymes in cultured porcine adrenomedullary cells, by activating both cAMP and protein kinase C signalling pathways. L-Alprenolol (10^{-6} M) reverses, partially in the rat and completely in humans, aldosterone response of adrenal slices to 10^{-8} M PACAP-38 (Andreis et al., 1995; Neri et al., 1996). Quarters of regenerated rat adrenocortical autotransplants, deprived of chromaffin cells, although displaying an aldosterone response to isoprenaline (10^{-7} M), are insensitive to PACAP-38 (Andreis et al., 1995). Taken together, these results indicate that the aldosterone secretagogue action of PACAP is almost exclusively mediated by indirect mechanisms involving medullary catecholamine release.

b. EFFECTS ON ZONA FASCICULATA-RETICULARIS. PACAP-38 does not affect glucocorticoid secretion of dispersed human and rat inner adrenocortical cells, but it enhances corticosterone production when rat adrenal slices, containing medullary chromaffin cells, are used (Andreis et al., 1995; Neri et al., 1996). PACAP was found to increase cortisol secretion in functionally hypophysectomized calves, an effect that may be due, at least in part, to an enhancement in blood-flow rate (Edwards and Jones, 1994a), and/or an aspecific activation of ACTH receptors, attributable to the strict homology of PACAP and VIP.

Evidence is available that shows that PACAP-38 elicits glucocorticoid secretion in rats by stimulating intramedullary CRH-ACTH system. However, whereas catecholamine-mediated aldosterone response of adrenal slices to PACAP already occurs at pM peptide concentrations, corticosterone response requires μM concentrations. Andreis et al. (1995) showed that CIP (10^{-6} M) completely prevents corticosterone secretion elicited by 10^{-6} M PACAP-38. It remains to be settled whether this mechanism may also account for the glucocorticoid response to PACAP of bovine adrenals, but it must be recalled that this peptide has been found to evoke a sizable release of CRH by calf adrenal glands (Edwards and Jones, 1994a). In human adrenal slices, Neri et al. (1996) did not observe any cortisol response to PACAP-

38, but only a relatively low concentration (10^{-8} M) of the peptide has been tested. The requirement of μM concentrations of PACAP to evoke a sizable glucocorticoid response renders the physiological relevance of these findings very doubtful.

F. Galanin

Galanin is a 29-amino-acid peptide originally isolated from pig intestine, that is widely distributed in both central and peripheral nervous systems. Galanin variously affects smooth-muscle cells, depending on its localization in the different organs, acting as a local neuromodulator of the pancreatic function (for review, see Bedecs et al., 1995). The colocalization of galanin with CRH in paraventricular-nucleus neurons and with ACTH in pituitary corticotropes (for review, see Merchenthaler et al., 1993), as well as its ability to increase ACTH and corticosterone plasma levels (Malendowicz et al., 1994c), suggest its involvement in the regulation of the central branch of the HPA axis.

Galanin-ir was detected by RIA in the fresh adrenal medulla of humans (Bauer et al., 1986b), pigs and cats (Bauer et al., 1986a; Holst et al., 1991), rabbits (Wikström et al., 1993) and rats (Zentel et al., 1990; Fischer-Colbrie et al., 1992; Mazzocchi et al., 1995c). The adrenal content of galanin, which varies from 3 pmol/g in humans to 90-115 pmol/g in pigs and rats, could give rise to local concentrations ranging from 10^{-8} to 10^{-6} M (see Section II D). Immunocytochemistry confirmed the presence of galanin-ir in the medullary chromaffin cells (Pelto-Huikko, 1989; Zentel et al., 1990; Elfvin et al., 1994). Galanin mRNA has also been demonstrated in the adrenal medulla of cows (Rökaeus and Carlquist, 1988; Rökaeus et al., 1990; Anouar et al., 1994) and rats (Anouar and Eiden, 1995).

Evidence of the presence of specific galanin receptors in the adrenal cortex is not yet available. However, galanin raises both basal and submaximally ACTH-stimulated corticosterone secretion by dispersed rat inner adrenocortical cells, threshold concentration being 10^{-8} M (Mazzocchi et al., 1992b). This effect is suppressed by the specific galanin antagonist galantide, but not by CIP, thereby ruling out the possibility that galanin interferes with ACTH receptors. Aldosterone secretion of dispersed rat zona glomerulosa cells is not affected by galanin, that, however, is able to enhance both aldosterone and corticosterone secretion by rat adrenal slices containing chromaffin cells (Mazzocchi et al., 1992b). Galanin increases the release of both mineralo- and glucocorticoids by in situ perfused pig adrenals (Holst et al., 1991), as well as their plasma concentrations in pharmacologically hypophysectomized ACTH-replaced rats, without inducing changes in natremia, kalaemia and plasma renin activity (Mazzocchi et al., 1992b).

Mazzocchi et al. (1995d) reported that, in pharmacologically hypophysectomized rats, a 7-day infusion of galanin causes a marked increase in corticosterone plasma level and an evident hypertrophy of the zona fasciculata and its cells; dispersed zona fasciculata cells from galanin-infused animals also show a significant increase in both basal and maximally ACTH-stimulated corticosterone secretion. The simultaneous infusion of galantide completely reverses all the effects of galanin. The prolonged administration of galantide alone causes a clear-cut lowering in the level of circulating corticosterone, along with atrophy of zona fasciculata cells and reduction in their steroidogenic capacity. Taken together, these findings indicate that endogenous galanin plays a physiological role in the regulation of the secretion and growth of inner adrenocortical zones in the rat.

G. Neurotensin

Neurotensin is a 13-amino-acid peptide, which exerts various central and peripheral effects, including hypotension, hypothermia, analgesia and reduced locomotor activity (for review, see Ferris, 1989). Neurotensin has been localized in the CRH-secreting paraventricular-nucleus neurons and pituitary corticotropes (Miyoshi et al., 1985; Merchenthaler and Lennard, 1991), and there is evidence that this neuropeptide is able to stimulate the hypothalamo-pituitary CRH-ACTH system (Nussdorfer et al., 1992; Rowe et al., 1995).

Neurotensin-ir has been detected by RIA in the adrenal medulla of cows, cats, rabbits, guinea pigs and rats (Corder et al., 1982; Lundberg et al., 1982; Rökaeus et al., 1982; Goedert et al., 1983; Terenghi et al., 1983; Ferris et al., 1986; Fischer-Colbrie et al., 1992), its concentration being very high in cats (more than 60 pmol/g) and low in rats and guinea pigs (1.5 pmol/g). Cytochemistry evidenced neurotensin-ir in the medullary chromaffin cells of cats (Pelto-Huikko et al., 1987) and hamsters (Pelto-Huikko et al., 1985b).

Neurotensin-specific binding sites have been demonstrated in the rat adrenals, especially at the cortico-medullary junction (Goedert et al., 1984). Mazzocchi et al. (1991) provided evidence that neurotensin concentration-dependently inhibits aldosterone response of dispersed rat zona glomerulosa cells to both ANG-II and K^+ (threshold concentration, 10^{-7} M), but not basal or ACTH-stimulated aldosterone secretion. This finding may suggest the interference of neurotensin with the transduction mechanisms of agonist-evoked secretagogue signals involving the rise in the $[Ca^{2+}]_i$. Neurotensin (6×10^{-8} M) was also reported to lower basal, but not ACTH-stimulated, corticosterone production by dispersed rat zona fasciculata-reticularis cells (Malendowicz et al., 1991). The physiological relevance of the direct inhibitory effect of neurotensin on rat adrenocortical cells remains to be addressed, inasmuch as it may be calculated (see Section II D) that the release of intramedullary stored neurotensin can give rise to local concentrations of the peptide near its minimal effective ones in vitro.

Neurotensin, despite its direct (probably pharmacological) inhibitory effect on adrenocortical cells, was found to cause a moderate increase in both aldosterone and corticosterone release by in situ perfused rat adrenals (Hinson et al., 1994a, b). This was associated with a sizable rise in the flow rate of the perfusion medium (Hinson et al., 1994c), which may, at least in part, account for the increase in corticosterone, but not aldosterone, release. It seems conceivable that in vivo, when the architecture of the adrenal gland is preserved, some indirect mechanisms are operative that overcome the direct inhibitory action of neurotensin on adrenocortical cells.

H. Calcitonin Gene-Related Peptide and Adrenomedullin

Calcitonin gene-related peptide (CGRP) is a 37-amino-acid peptide widely distributed in the central and peripheral nervous systems. It has been also identified in nerve fibers to various peripheral organs, especially of the gastrointestinal tract (for review, see Owyang and Louie, 1989). Adrenomedullin is a recently discovered 52-amino-acid peptide, originally isolated from human pheochromocytomas (Kitamura et al., 1993a, b); rat adrenomedullin has 50-amino-acid residues, with a 2-amino-acid deletion and 6 substitutions compared with human peptide (Sakata et al., 1993). The amino acid sequences of CGRP and adrenomedullin display a slight homology (Kitamura et al., 1993a, b), and the competition of human adrenomedullin and CGRP for the same receptor site has been recently demonstrated in the rat heart and lungs (Owji et al., 1995). CGRP is a potent vasodilator involved in cardiovascular regulation (Brain et al., 1985). Adrenomedullin also exerts a strong and long-lasting hypotensive effect in rats (Ishiyama et al., 1993; Gardiner et al., 1995) and predominantly increases blood flow rate in organs, where its gene is highly expressed (He et al., 1995). There is evidence that the hypotensive effects of adrenomedullin are, at least in part, mediated by $CGRP_1$ receptors (Nuki et al., 1993; Eguchi et al., 1994; Hall et al., 1995).

1. Calcitonin gene-related peptide. CGRP-positive nerve fibers have been found in the adrenal cortex of pigs (Kong et al., 1989), rabbits (Wikström et al., 1996), guinea pigs (Heym et al., 1995) and rats (Kuramoto et al., 1987). CGRP-ir has been detected by immunocytochemistry in medullary chromaffin cells of the rat (Kuramoto et al., 1987).

Findings indicate that adrenocortical cells possess CGRP receptors (Goltzman and Mitchell, 1985). In vitro studies have shown that CGRP (from 10^{-9} to 10^{-7} M) specifically inhibits basal and ANG-II-stimulated aldosterone secretion of dispersed dog zona glomerulosa cells (Murakami et al., 1989). In the rat, CGRP does not affect basal aldosterone secretion by dispersed zona glomerulosa cells, nor does it change corticosterone production (either basal or agonist-stimulated) by isolated zona fasciculata-reticularis cells (Hinson and Vinson, 1990; Mazzocchi et al., 1996c). However, Mazzocchi et al. (1996c) demonstrated that 10^{-7} M CGRP inhibits ANG-II-stimulated aldosterone secretion by rat zona glomerulosa cells and that this effect is blocked by $CGRP_{8-37}$, an antagonist of $CGRP_1$ receptors. In vivo studies gave controversial results that cannot be accounted for exclusively by interspecific differences. CGRP was found to raise cortisol secretion in hypophysectomized calves (Bloom et al., 1989), but to lower aldosterone plasma concentration in dogs (Murakami et al., 1989, 1991) and rats (Mazzocchi et al., 1992a).

CGRP enhances both aldosterone and corticosterone release by the isolated perfused rat adrenal gland in situ, as well as the perfusion-medium flow rate (Hinson and Vinson, 1990; Mazzocchi et al., 1996b). This last effect may explain the glucocorticoid response, but not the aldosterone stimulatory effect that overcomes the in vitro inhibitory action of CGRP. Mazzocchi et al. (1996b) showed that the CGRP-evoked rise in aldosterone, but not glucocorticoid, release by in situ perfused rat adrenals is blocked by the simultaneous administration of L-alprenolol, thereby suggesting that it is mediated by locally secreted catecholamines.

2. Adrenomedullin. Adrenomedullin-ir has been demonstrated by RIA in the adrenal medulla of humans (Ichiki et al., 1994; Satoh et al., 1995) and rats (Sakata et al., 1993, 1994), where its content, ranging from 12 to 50 pmol/g, could produce local concentrations around 10^{-7} M (see Section II D). Adrenomedullin-ir has been also shown in cultures of bovine chromaffin cells (Katoh et al., 1994). Washimine et al. (1995) and Satoh et al. (1996) provided the immunocytochemical demonstration of the presence of adrenomedullin-ir in medullary chromaffin cells of humans, pigs and rats. Specific adrenomedullin mRNA has been detected in rat medullary chromaffin cells (Sakata et al., 1993).

The investigations dealing with the presence of adrenomedullin receptors in adrenals and adrenomedullin effects on adrenocortical cells are still scarce. Iwasaki et al. (1996) showed a relative abundance of specific binding sites for proadrenomedullin N-terminal 20 peptide in the rat adrenals. Yamaguchi et al. (1995) reported that 10^{-10} M adrenomedullin inhibits ANG-II- and K^+-stimulated aldosterone secretion of dispersed rat zona glomerulosa cells, without apparently affecting either basal or ACTH-stimulated one; they also showed that the inhibitory effect of adrenomedullin on zona glomerulosa-cell response to ANG-II is blocked by the Ca^{2+} ionophore A23187, a finding indicating that adrenomedullin interferes with the agonist-elicited elevation of $[Ca^{2+}]_i$. Mazzocchi et al. (1996c) confirmed those findings, but observed that the threshold concentration of adrenomedullin is rather high (10^{-7} M); they also demonstrated that 10^{-6} M $CGRP_{8-37}$ blocks the adrenomedullin effect, which suggests that this peptide acts via the $CGRP_1$ receptor subtype. No effect of ad-

renomedullin was found on basal or ACTH-stimulated corticosterone secretion of dispersed rat inner adrenocortical cells.

Mazzocchi et al. (1996b) showed that adrenomedullin dose-dependently induces a rise in aldosterone and corticosterone release by the in situ perfused rat adrenal gland, along with a net increase in the flow rate of the perfusion medium. The minimal effective dose of adrenomedullin that evokes a significant aldosterone response was three orders of magnitude less than those able to cause the corticosterone response and two orders of magnitude less than those able to cause the medium flow rate response. As in the case of CGRP, the adrenomedullin-evoked rise in aldosterone release by in situ perfused rat adrenals is counteracted by the simultaneous administration of L-alprenolol, a finding strongly suggesting that adrenomedullin may indirectly stimulate rat zona glomerulosa cells by eliciting catecholamine release. However, it must be noted that there are indications that proadrenomedullin N-terminal 20 peptide inhibits catecholamine secretion from cultures of bovine adrenomedullary cells (Katoh et al., 1995; Niina et al., 1995).

I. Natriuretic Peptide Family

The first member of natriuretic peptide family was originally discovered in the late 1970s in the secretory granules of atrial myocytes and was named atriopeptine or atrial natriuretic factor. Since then, an impressive mass of investigations demonstrated that atriopeptine, in addition to its action on the kidney, also possesses vasodilatatory effects and a potent inhibitory action on the renin-angiotensin-aldosterone system (for review, see Cantin and Genest, 1985). Subsequently, atriopeptine-like peptides were also found in extra-atrial tissues, including heart ventricles, brain, pulmonary veins, lung and endocrine glands. More recently, other two members of natriuretic peptide family were isolated from porcine brain. Thus, this family of peptides consists of at least three members: atrial natriuretic peptide (ANP), brain or B-type natriuretic peptide (BNP) and C-type natriuretic peptide (CNP), with CNP being far less potent than the other two. Specific receptors for natriuretic peptide have been isolated and characterized: A subtype (binding potency, ANP = BNP > CNP) and B subtype (specific for CNP) are coupled to cGMP production, whereas C subtype (binding potency, ANP = BNP = CNP) is not coupled to guanylate cyclase, probably inhibits adenylate cyclase and is considered a clearance receptor (for review, see Rosenzweig and Seidman, 1991; Levin, 1993).

ANP-ir has been detected by RIA in the adrenal medulla of humans (Lee et al., 1993), cows (Ong et al., 1987; Mukoyama et al., 1988; Okazaki et al., 1989) and pigs (Duntas et al., 1993), where its concentration is 1-3 pmol/g. ANP-ir has also been demonstrated by immunocytochemistry in the medullary chromaffin cells of rats (McKenzie et al., 1985; Inagaki et al., 1986; Wolfensberger et al., 1995) and guinea pigs (Wolfensberger et al., 1995). ANP mRNA has been detected in the adrenal medulla of humans (Lee et al., 1993), cows (Gardner et al., 1986; Nunez et al., 1990) and rats (Morel et al., 1988), as well as in cultures of bovine adrenomedullary cells (Pruss and Zamir, 1987). In situ hybridization studies revealed that, at least in the rat, ANP is preferentially expressed in norepinephrine-containing cells (Morel et al., 1988). BNP-ir has been shown by RIA in the adrenal medulla of humans (Lee et al., 1994) and cows (De Léan et al., 1985), where its concentrations average 0.2 pmol/g and 0.8 pmol/g, respectively. BNP-ir was also detected in cultures of bovine adrenomedullary cells, where it was identified with the so-called aldosterone secretion inhibitory factor (Nguyen et al., 1989). BNP mRNA has been found by in situ hybridization and Northern blot analysis in human adrenal medulla (Lee et al., 1994). CNP-ir has been demonstrated by RIA in the adrenal medulla of humans (Totsune et al., 1994), where its concentration is about 0.5 pmol/g. It is also synthesized and secreted by bovine chromaffin cells cultured in vitro (Babinski et al., 1992). CNP protein and mRNA have been evidenced by immunocytochemistry and reverse transcription polymerase-chain reaction (RT-PCR) in the zona glomerulosa of cow (Kawai et al., 1996) and rat adrenals (Minamino et al., 1993). These techniques, however, did not allow us to ascertain whether CNP is contained in the zona glomerulosa cells or in the medullary chromaffin cells scattered in the zona glomerulosa.

1. Effects on zona glomerulosa. Mammalian zona glomerulosa cells possess specific receptors for the three members of the natriuretic peptide family, and most evidence indicates that the activation of the A and B receptor subtypes inhibits both basal and agonist (especially ANG-II)-stimulated aldosterone secretion with the threshold concentration being in the nM range (for review, see Hawata et al., 1991; Ganguly, 1992). Natriuretic peptides seem to impair Ca^{2+} influx into the zona glomerulosa cells through a selective action on the voltage-dependent Ca^{2+} channels (Barret et al., 1991; Lotshaw et al., 1991b; and for review, see Ganguly 1992). The inhibitory action of natriuretic peptides affects the early steps of the steroidogenic pathway, the transport of cholesterol to the mitochondria and/or the intramitochondrial conversion of cholesterol to pregnenolone. According to Matsuoka et al. (1987), the mechanism of transduction of the inhibitory signal of ANP does not involve the activation of the guanylate cyclase. However, Kawai et al. (1996) reported that CNP concentration-dependently raises basal cGMP release by cultured bovine zona glomerulosa cells and inhibits ACTH-induced increase in cAMP and aldosterone release, a finding indicating that this peptide acts by both stimulating guanylate-cyclase and suppressing adenylate-cyclase activity. The prolonged ANP administration has been also

shown to specifically inhibit the growth and steroidogenic capacity of adrenal zona glomerulosa in RAS-suppressed rats (Rebuffat et al., 1988). All the aforementioned findings are compatible with the view that natriuretic peptides act as true hormones, reaching their target cells via the systemic circulation. However, the involvement of intramedullary natriuretic peptides in the paracrine control of the cortex function seems to be very probable, based on the following considerations. The minimal concentrations of natriuretic peptides that are able to affect in vitro zona glomerulosa cells are in the nM range, whereas their plasma levels are about one or two orders of magnitude less (for review, see Ganguly, 1992). It may be calculated (see Section II D) that the intra-adrenal release of the three natriuretic peptides may assure local concentrations of $10^{-7}/10^{-6}$ M, which are well above their minimal effective ones in vitro.

Before concluding, it must be recalled that natriuretic peptide receptors of the A subtype are present in bovine medullary chromaffin cells (Heisler and Morrier 1988; Niina et al., 1996) and that natriuretic peptides inhibit medullary catecholamine release in cows (Fernandez et al., 1992; Babinski et al., 1995), dogs (Holtz et al., 1987) and rats (Vatta et al., 1994). Evidence is also available showing that natriuretic peptides inhibit kidney renin release (Obana et al., 1985; Tagaki et al., 1988; Ishimitsu et al., 1992) and endothelin secretion of cultured endothelial (Hu et al., 1992), mesangial (Kohno et al., 1993) and smooth-muscle cells (Bokemeyer et al., 1994). Hence, the possibility that the inhibition of both intra-adrenal RAS and endothelin release may concur to the antiadrenoglomerulotropic action of natriuretic peptides has yet to be explored.

2. Effects on zona fasciculata-reticularis. The possible effects of natriuretic peptides on the zona fasciculata and glucocorticoid secretion are more controversial. Although most studies did not report any effect (for review, see Cantin and Genest, 1985; Ganguly, 1992), specific ANP receptors have been demonstrated in the zona fasciculata cells of cows (Higuchi et al., 1986a) and rats (Mulay et al., 1995), and evidence is available that ANP and BNP depress basal and especially ACTH-stimulated glucocorticoid secretion of in vitro cultured inner adrenocortical cells of humans (Higuchi et al., 1986b; Naruse et al., 1987; Carr and Mason, 1988) and cows (DeLéan et al., 1984a; Hashiguchi et al., 1988; Hawata et al., 1991). The threshold concentration, ranging from 10^{-9} to 10^{-7} M, is well below that which natriuretic peptide can locally attain upon stimulation of their release from chromaffin cells.

J. Summary

The main action of intramedullary CRH appears to be the paracrine/autocrine stimulation of ACTH release by chromaffin cells, the intramedullary CRH-ACTH system being mainly (if not exclusively) involved in the maintenance and stimulation of the secretion and growth of inner adrenocortical zones. The direct steroidogenic effect of CRH is doubtful and, in any case, mainly related to its effect on adrenal blood flow. AVP, acting via phospholipase C-coupled receptors, plays an important and direct role in the maintenance and stimulation of the zona glomerulosa growth and mineralocorticoid secretion. AVP also stimulates glucocorticoid secretion and the growth of zona fasciculata, probably by activating intramedullary CRH-ACTH system; however, the physiological relevance of this last action of AVP is questionable. Oxytocin directly stimulates basal steroid secretion of adrenocortical cells, but it inhibits ACTH-induced glucocorticoid secretion in rats. SRIH, acting via specific receptors that interfere with the intracellular mechanisms transducing ANG-II signals, exerts a direct and specific inhibitory action on the zona glomerulosa that seems to have a notable physiological relevance, inasmuch as the prolonged administration of SRIH antagonists enhances zona glomerulosa growth and secretory capacity. TRH specifically and directly inhibits the late steps of glucocorticoid synthesis in the rat.

Enkephalins exert a direct stimulatory action on the secretion and growth of the adrenal cortex that seems to be mediated by μ opioid receptors. Whether this effect of enkephalins is modulated by their inhibitory action on catecholamine release by chromaffin cells and to which extent their in vivo glucocorticoid secretagogue effect may be related to the increase in adrenal blood flow remain to be explored. β-endorphin seems to directly inhibit glucocorticoid secretion of inner adrenocortical zones, at least under physiological conditions. Dynorphin directly inhibits steroidogenesis of rat adrenocortical cells by specifically impairing 21-hydroxylase activity.

The direct effect of tachykinins on adrenocortical cells is probably pharmacological in nature. Substance P is likely to play a physiological role in the maintenance and stimulation of the secretion and growth of zona glomerulosa by enhancing catecholamine release from medullary chromaffin cells. Neuropeptide K stimulates glucocorticoid secretion of inner adrenocortical zones by activating the intramedullary CRH-ACTH system. Neuromedin U-8 seems to indirectly enhance the secretion of the cortex both by activating the intramedullary CRH-ACTH system and by eliciting the release by chromaffin cells of a hypothetical factor specifically stimulating the late steps of aldosterone synthesis.

Pancreatic polypeptide, at nM concentrations, directly enhances glucocorticoid secretion in rats and probably plays a major role in the adrenal response to hypoglycemic stress. Peptide YY, at μM concentrations, directly depresses aldosterone secretion, but this effect appears to be pharmacological in nature. Intramedullary neuropeptide Y is involved in the fine-tuning of zona glomerulosa secretion under physiological or pathophysiological conditions requiring a rather elevated release of aldosterone. The mechanisms are both direct and indi-

rect, the latter involving the stimulation of catecholamine release by medullary chromaffin cells or adrenergic fibers. Endogenous neuropeptide Y does not play a prominent role in the physiological maintenance of the growth and secretory capacity of the zona glomerulosa, at least in the rat. The effects of neuropeptide Y on the inner adrenocortical zones are doubtful and pharmacological in nature, and the in vivo glucocorticoid response to this peptide appears to be related to its vasodilatory effect.

Intramedullary VIP is involved in the physiological regulation of zona glomerulosa functions. The acute aldosterone secretagogue effect is either direct or indirect and mediated by the stimulation of medullary catecholamine release, whereas the long-term trophic effect is only direct via specific receptors on zona glomerulosa cells. VIP may also aspecifically activate ACTH receptors located on both outer and inner adrenocortical cells; however, under normal conditions, this action is masked by circulating ACTH, which tonically activates such receptors. The glucocorticoid response to VIP, observed in rat adrenals perfused in situ, is indirect and must probably be ascribed to its vasodilatory action. PACAP stimulates both mineralocorticoid and glucocorticoid secretion indirectly, by eliciting catecholamine release by medullary chromaffin cells and by activating the intramedullary CRH-ACTH system, respectively; the physiological relevance of this last effect of PACAP is very doubtful.

Intramedullary galanin seems to be directly involved in the maintenance and stimulation of the secretion and growth of inner adrenocortical zones. It also exerts an indirect aldosterone secretagogue effect, the mechanism and physiological relevance of which remain to be ascertained. Neurotensin directly inhibits aldosterone secretion of zona glomerulosa cells through its receptor-mediated interference with the transduction mechanisms of agonists raising $[Ca^{2+}]_i$, but this effect is probably pharmacological in nature. When the integrity of the adrenal structure is preserved, neurotensin increases both mineralo- and glucocorticoid secretions, an effect that may be at least partly related to the increase in the gland blood flow.

CGRP and adrenomedullin exert a direct inhibitory effect on the ANG-II-stimulated aldosterone secretion of zona glomerulosa cells that is mediated by a common receptor of the $CGRP_1$ subtype; the activation of this receptor interferes with the agonist-stimulated redistribution of intracellular Ca^{2+}. The physiological relevance of these effects of CGRP and adrenomedullin is questionable, because these effects can be observed only at very high concentrations of the peptides. CGRP and adrenomedullin enhance both mineralo- and glucocorticoid release from in situ perfused rat adrenals, via mechanisms overcoming their direct inhibitory effects, probably involving a release of catecholamine by medullary chromaffin cells and an increase in adrenal blood flow, respectively.

Intramedullary natriuretic peptides exert a physiologically relevant direct receptor-mediated inhibition of the secretion and growth of zona glomerulosa that appears to involve a selective impairment of voltage-dependent Ca^{2+} channels, as well as the activation of guanylate cyclase and the inhibition of adenylate cyclase. Their effect on glucocorticoid secretion is rather controversial. Probably, natriuretic peptides may also indirectly affect the function of the cortex by inhibiting catecholamine release by medullary chromaffin cells and perhaps by depressing intra-adrenal RAS and endothelin secretion.

VI. Control of the Secretion of Intramedullary Regulatory Molecules

Splanchnic nerves are certainly the main regulators of the function of adrenal medulla. Their basal or stress-induced activity has been found to modulate not only catecholamine release, but also the synthesis and secretion of many intramedullary regulatory peptides. However, although far less investigated, other regulatory mechanisms appear to be involved.

A. Splanchnic Nerve Activation

Splanchnic-nerve activation elicits the release of catecholamines by medullary chromaffin cells and raises the rate of corticosteroid secretion in various species, including calves, sheep, pigs and dogs. Conversely, splanchnotomy depresses the secretory response of adrenal cortex to its main agonist ACTH (Edwards et al., 1986; Edwards and Jones 1987a, b; Engeland and Gann 1989; Bornstein et al., 1990b). Charlton (1995) recently proposed that noradrenergic innervation and epinephrine and norepinephrine are responsible for the control of basal steroid secretion of the adrenal cortex; however, this hypothesis appears to conflict with most evidence showing that no relevant alterations in the basal levels of circulating corticosteroid hormones occur in splanchnotomized animals. Splanchnic-nerve activation has been reported to stimulate the release and/or co-release with catecholamines of many other intramedullary regulatory molecules, that in turn may directly or indirectly stimulate or inhibit steroid secretion.

1. Dopamine and serotonin. Indirect evidence suggests that splanchnic-nerve fibers stimulate dopamine release by chromaffin cells. Electrical field stimulation of the in situ perfused rat adrenal gland lowers aldosterone secretion (see Section III B), and this effect is blocked by the dopamine-receptor antagonist haloperidol (Porter et al., 1992). Findings are also available indicating that serotonin is co-released with catecholamines upon splanchnic-nerve activation in rats (Brownfield et al., 1985).

2. Regulatory peptides. Splanchnic-nerve stimulation elicits the adrenal release of (*a*) CRH in cows (Edwards and Jones, 1988) and dogs (Bruhn et al., 1987b); (*b*)

enkephalins in cows (Bloom et al., 1987b) and dogs (Hexum et al., 1980a; Damase-Michel et al., 1993); (*c*) substance P in rats (Fischer-Colbrie et al., 1992); (*d*) neuropeptide Y in cows (Allen et al., 1984), dogs (Briand et al., 1990a; Damase-Michel et al., 1993) and cats (Lundberg et al., 1986b); (*e*) VIP in cows (Bloom et al., 1987b); (*f*) galanin in pigs (Holst et al., 1991); (*g*) neurotensin in cats (Rökaeus et al., 1982; Ferris et al., 1986; Gaumann et al., 1989b) and rats (Fischer-Colbrie et al., 1992); and (*h*) ANP in pigs (Duntas et al., 1993). The innervation may also modulate gene expression of some intramedullary regulatory peptides. Splanchnotomy was found to increase adrenal content of enkephalin-ir, substance P-ir and neuropeptide Y-ir in cows and rats (Lewis et al., 1981; Fleminger et al., 1984; Hinson and Kapas, 1996), as well as enkephalin and neuropeptide Y mRNAs in rats (Kilpatrick et al., 1984; Schalling et al., 1991; Henion and Landis, 1992; De Cristofaro et al., 1993). Insulin-induced reflex splanchnic-nerve stimulation evokes a rapid (4 h) and long-lasting (6 days) increase in galanin mRNA in rat adrenal medulla (Anouar and Eiden, 1995). Nicotinic activation elicits the co-release of catecholamines and adrenomedullin (Katoh et al., 1994) or neuropeptide Y in rats (Shimoda et al., 1993); moreover, it stimulates the release of ANP (Mukoyama et al., 1988; Nguyen et al., 1988, 1990) and BNP by cultured bovine medullary cells, BNP response being significantly higher than that of ANP (Nguyen et al., 1990). Reserpine enhances the release by medullary chromaffin cells of neuropeptide Y in cats (Lundberg et al., 1986a) and guinea pigs (Nagata et al., 1987), as well as gene expression of this peptide in rat adrenal medulla (Higuchi et al., 1990; Schalling et al., 1991). γ-Aminobutyric acid (GABA) and conceivably GABA-ergic fibers raise the release of enkephalins by dog adrenal medulla (Fujimoto et al., 1987), and GABA antagonists inhibit thyrotropin-releasing hormone (TRH) secretion of rat chromaffin cells (Mitsuma et al., 1987b).

B. Stressful Conditions

Various types of stresses elicit enkephalin release by adrenals in dogs (Damase-Michel et al., 1994) and rats (Jarry et al., 1985). Hemorrhage raises adrenomedullary secretion of CRH in dogs (Bruhn et al., 1987a), of enkephalins in dogs (Engeland et al., 1986) and cats (Gaumann et al., 1987, 1989a), and of neuropeptide Y in dogs (Briand et al., 1990b). Exercise causes the cosecretion of catecholamines and neuropeptide Y in humans (Lundberg et al., 1985; Taylor, 1989), and lipopolysaccharide-evoked inflammatory responses enhance pro-enkephalin gene expression in the rat adrenals (Behar et al., 1994).

C. Other Possible Mechanisms

Dopamine appears to inhibit TRH release by rat adrenal glands (Mitsuma et al., 1987a). ANG-II and potassium raise enkephalin content in the adrenal medulla of cows (Siegel et al., 1985; Suh et al., 1992), and, in this species, potassium stimulates adrenal release of both ANP and BNP (Nguyen et al., 1990). Substance P and VIP increase adrenal medulla content of enkephalins in sheep (Wilson, 1987), and substance P infusion inhibits acetylcholine-induced enkephalin release by the adrenal glands of pharmacologically hypophysectomized calves administered maintenance doses of ACTH (Edwards and Jones, 1994b). Finally, glucocorticoids raise the medullary content of enkephalin in rats (Inturrisi et al., 1988; Aloyz et al., 1992; Henion and Landis, 1992), but lower the medullary neuropeptide Y mRNA and protein content in this species (Laborie et al., 1995). However, hypophysectomy does not alter neuropeptide Y mRNA and protein content in the rat adrenal medulla (Fischer-Colbrie et al., 1988). Although endothelins are commonly found to stimulate the secretion of natriuretic peptides (for review, see Rubanyi and Polokoff, 1994), low doses of endothelin-1 inhibit ANP secretion in the rat (Shirakami et al., 1993). Hence, it is possible that blockade of the synthesis and release of intramedullary natriuretic peptides may take a part in the adrenoglomerulotropic action of endothelins.

D. Summary

The main modulators of the release of intramedullary regulatory molecules are splanchnic nerves: this has been demonstrated not only for catecholamines, but also for serotonin, CRH, enkephalins, neuropeptide Y, VIP, galanin, neurotensin, natriuretic peptides, and perhaps adrenomedullin and TRH. Several stressful conditions also raise the release by medullary chromaffin cells of CRH, enkephalins and neuropeptide Y.

VII. The Possible Involvement of the Cortico-Medullary Paracrine Interactions in the Pathophysiology of the Adrenal Gland

Some data suggest that the cortico-medullary paracrine interactions can play a role in the pathogenesis of some diseases.

Neuropeptide Y potentiates the vasoconstriction induced by several agonists, including catecholamines, and is thought to be involved in the development of some forms of human hypertension, where there is not a tight direct correlation between intramedullary content and plasma concentration of neuropeptide Y-ir (Michel and Rascher, 1995). This peptide is co-released with norepinephrine by human pheochromocytomas upon ANG-II exposure (Grouzmann et al., 1994). Because pheochromocytoma patients frequently display high levels of circulating neuropeptide Y-ir (Tabarin et al., 1992), Grouzmann et al. (1994) hypothesized that RAS (also the intra-adrenal one?) elicits neuropeptide Y secretion by tumors possessing ANG-II receptors, thereby contributing to trigger hypertensive crisis.

Human pheochromocytomas synthesize and secrete, in addition to catecholamines, several regulatory peptides, which are able to affect the secretion and growth of

the adrenal cortex. RIA and immunocytochemical studies demonstrated the presence in chromaffin cells of pheochromocytomas of POMC-derived peptides (Oishi et al., 1992), SRIH-ir (Osamura et al., 1987; Oishi et al., 1992), enkephalin-ir (Osamura et al., 1987; Parmer and O'Connor, 1988; Oishi et al., 1992), tachykinin-ir (Bucsics et al., 1981), neuropeptide Y-ir (Lundberg et al., 1986c; Osamura et al., 1987; Grouzmann et al., 1994), VIP-ir (Oishi et al., 1992), PACAP-ir (Takahashi et al., 1993), galanin-ir (Bauer et al., 1986b), CGRP-ir (Conlon et al., 1989), adrenomedullin-ir (Kitamura et al., 1993a, b; Kuwasako et al., 1995; Satoh et al., 1995) and natriuretic peptide-ir (Chien et al., 1990; Totsune et al., 1994). The presence in pheochromocytomas of the specific mRNAs of prepro-CRH (Usui et al., 1988; Liu et al., 1994), POMC (Liu et al., 1994), enkephalins (Konoshita et al., 1994), β-endorphin (De Bold et al., 1988), neuropeptide Y (Higuchi et al., 1994; Senanayake et al., 1995), CGRP (Conlon et al., 1989) and adrenomedullin (Kitamura et al., 1993b) has also been detected by both biochemical and in situ hybridization techniques. Most of the regulatory molecules expressed by human pheochromocytomas are able to stimulate the secretion and growth of the cortex and especially of the zona glomerulosa (see Section V). Hence, these findings may explain the pathophysiological basis of some cases of Conn's adenomas or idiopathic primary aldosteronism associated with secreting pheochromocytomas (Wajiki et al., 1985; Inoue et al., 1986; Gordon et al., 1994; Tan et al., 1996).

VIII. Conclusions and Perspectives

Since the discovery in the early 1980s that adrenal medulla contains and releases, in addition to catecholamines, many other regulatory molecules, a huge mass of data has been accumulated indicating that medullary chromaffin cells exert a paracrine control on the function and growth of the adrenal cortex. These regulatory molecules may exert stimulatory or inhibitory effects, act directly or indirectly on the cortex, and specifically affect the function of the zona glomerulosa or zona fasciculata-reticularis. Stimulatory molecules include epinephrine and norepinephrine, serotonin, CRH-ACTH, AVP, oxytocin, enkephalins, some tachykinins, neuropeptide Y, pancreatic polypeptide, VIP, PACAP and galanin. Inhibitory molecules include dopamine, SRIH, TRH, β-endorphin, dynorphin, peptide YY and natriuretic peptides. Neurotensin, CGRP and adrenomedullin appear to exert direct inibitory and indirect stimulatory effects.

Despite these extensive experimental investigations, the physiological and pathophysiological relevance of the paracrine control of the cortex by adrenal medulla largely remains to be established. Available findings cast doubts about the possibility that medullary chromaffin cells play a major role in the fine-tuning of the cortex function under basal conditions, at least in the rat. Animals bearing adrenocortical autotransplants, deprived of chromaffin cells, respond normally to ACTH and ANG-II and exhibit only moderately reduced basal levels of circulating mineralo- and glucocorticoids; these levels may be accounted for, at least in part, by the reduced, moderately low adrenocortical tissue mass. These findings are consistent with those obtained in the rat (Okamoto et al., 1992), in patients bilaterally adrenalectomized for Cushing's disease (Demeter et al., 1990) and with Addison's disease transplanted with embryonic adrenal tissue (Patñio and Fenn, 1993). It is conceivable that, under basal conditions, the stimulatory and inhibitory paracrine effects exerted by adrenal medulla on the cortex are in balance and annul each other. This contention entails that each experimental procedure altering such a balance should be able to evoke sizable effect on the cortex function. In fact, the physiological involvement of regulatory peptides in the maintenance of the cortex function appears to be suggested by experiments demonstrating that the prolonged administration of specific antagonists of such peptides induces marked alteration in the secretion and growth of the cortex. At present, this has been shown for the intramedullary CRH-ACTH system and galanin, as far as zona fasciculata-reticularis is concerned, and for AVP, SRIH, substance P and VIP, as to the zona glomerulosa.

The paracrine control of the cortex by adrenal medulla may become fully evident under various paraphysiological or pathological conditions, such as the following:

- Severe impairment of normal extra-adrenal mechanisms controlling the cortex function. A good example of this possibility stems from the experiments showing that in hypophysectomized rats, the intramedullary CRH-ACTH system undergoes a potent activation (see Section IV).
- Increased need of steroid production (e.g., during inflammatory, physical or emotional stresses). Evidence indicates that the adrenal gland synthesizes and releases interleukins (Murakami et al., 1993; Gadient et al., 1995; Gonzales-Hernandez et al., 1994, 1995; Judd and Ritchie, 1995), as well as expresses interleukin receptors and their endogenous antagonists (Gadient et al., 1995; Schultzberg et al., 1995). Hence, it is very tempting to hypothesize that interleukins, locally produced during inflammatory or immune responses, may directly stimulate glucocorticoid secretion by activating catecholamine release (see Section III A) and/or the intramedullary CRH-ACTH system (see Section IV). This contention received support by a recent study of You-Ten et al. (1995), who showed a several-fold increase in POMC mRNA by RT-PCR and detected high mRNA levels for murine prohormone convertase 1 (the enzyme that cleaves POMC into ACTH) in the adrenal gland of mice undergoing

graft-versus-host disease; accordingly, plasma corticosterone concentration remains elevated in diseased mice compared with syngenic controls, despite the decreased level of circulating ACTH. Intramedullary galanin appears to play a crucial role in mediating glucocorticoid response to the insulin-induced hypoglycemic stress (Fischer-Colbrie et al., 1992; Anouar and Eiden, 1995), and the same seems to be true for pancreatic polypeptide (Mazzocchi et al., 1995c), substance P and neurotensin (Fischer-Colbrie et al., 1992). There is indirect evidence that intramedullary neuropeptide Y may be somehow involved in the maintenance of blood pressure, not only by acting on systemic blood vessels, but also by stimulating mineralocorticoid secretion. Conversely, Higuchi et al. (1993) demonstrated a marked decrease in neuropeptide Y mRNA in spontaneously hypertensive rats during the early stage of development of the hypertension, as compared with age-matched Wistar-Kyoto controls. They hypothesized the existence of a compensatory mechanism, aimed at reducing the stimulatory action of neuropeptide Y on the zona glomerulosa. Glucocorticoids decrease intramedullary neuropeptide Y mRNA, whereas metyrapone (an inhibitor of 11β-hydroxylase) increases it (Laborie et al., 1995), a finding suggesting the existence of an intra-adrenal feedback mechanism regulating neuropeptide Y synthesis and release.

- When an excess of steroid production has to be counteracted. A good demonstration of this possibility stems from the investigations of Lee et al. (1993, 1994) showing that in the adrenal medulla of human patients with primary aldosteronism, ANP and BNP protein and mRNA content is higher than in the medulla of glands obtained from kidney donors.

In light of these considerations, it is my belief that one of the most exciting challenges for the investigators of the physiology of the adrenal gland will be the full understanding of the mechanisms whereby the synthesis and release of the intramedullary regulatory molecules are differentially regulated to face the various physiological and physiopathological conditions of increased or decreased demand of steroid hormones. Many formidable problems must be addressed in the next years, among which I wish to mention the following:

- Are there intraglandular negative feedback mechanisms allowing, under normal conditions, the maintenance of the balance between the cortical effects of intramedullary stimulatory and inhibitory molecules?
- Can adrenal medulla selectively tune the release of the different cortical hormones (e.g., is it able to enhance mineralocorticoid secretion and simultaneously depress glucocorticoid release)?
- Are splanchnic-nerve fibers specifically sorted to stimulate or inhibit discrete pools of medullary chromaffin cells, each of which secretes one or more regulatory molecules?
- How does the central nervous system selectively control splanchnic-nerve fiber outflow to evoke the release by chromaffin cells of specific regulatory molecules?

The answer to these and many other basic questions, along with the continuous development of new potent and selective agonists and antagonists of intramedullary regulatory molecules, will not only open new frontiers in our knowledge of adrenal cytophysiology, but, more importantly, will shed light on new perspectives in the therapy of adrenal diseases.

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