Role of 5-HT in the regulation of the brain-pituitary-adrenal axis: effects of 5-HT on adrenocortical cells[1]

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Abstract: Serotonin (5-HT) plays a pivotal role in the regulation of the brain-pituitary-adrenal axis. In particular, 5-HT has been shown to control the activity of hypothalamic CRF neurons and pituitary corticotrope cells through activation of 5-HT_{1A} and (or) 5-$HT_{2A/2C}$ receptor subtypes. 5-HT, acting through 5-HT_2 receptors, can also trigger the renin-angiotensin system by stimulating renin secretion and consequently can enhance aldosterone production. At the adrenal level, 5-HT produced locally stimulates the secretory activity of adrenocortical cells through a paracrine mode of communication. The presence of 5-HT in the adrenal gland has been demonstrated immunohistochemically and biochemically in various species. In the frog, rat, and pig adrenal gland, 5-HT is synthesized by chromaffin cells, while in the mouse adrenal cortex, 5-HT is contained in nerve fibers. In man, 5-HT is present in perivascular mast cells. In vivo and in vitro studies have shown that 5-HT stimulates corticosteroid secretion in various species (including human). The type of receptor involved in the mechanism of action of 5-HT differs between the various species. In frogs and humans, the stimulatory effect of 5-HT on adrenocortical cells is mediated through a 5-HT_4 receptor subtype positively coupled to adenylyl cyclase and calcium influx. In the rat, the effect of 5-HT on aldosterone secretion is mediated via activation of 5-HT_7 receptors. Clinical studies indicate that 5-HT_4 receptor agonists stimulate aldosterone secretion in healthy volunteers and in patients with corticotropic insufficiency and primary hyperaldosteronism. Local serotonergic control of corticosteroid production may be involved in the physiological control of the activity of the adrenal cortex as well as in the pathophysiology of cortisol and aldosterone disorders.

Key words: HPA axis, renin-angiotensin system, adrenal gland, corticosteroid secretion, serotonergic receptors.

Résumé : La sérotonine (5-HT) joue un rôle important dans le contrôle de l'axe hypothalamo-hypophyso-surrénalien. Il a été montré que la 5-HT pouvait moduler l'activité des neurones à CRF ainsi que les cellules corticotropes de l'hypophyse en activant des récepteurs sérotoninergiqes de type 5-HT_{1A} et (ou) 5-$HT_{2A/2C}$. La 5-HT active également le système rénine-angiotensine, et peut de ce fait stimuler la production d'aldostérone, via l'activation de récepteurs sérotoninergiques de type 2. Au niveau surrénalien, la 5-HT produite localement stimule l'activité sécrétrice des cellules corticosurrénaliennes par un mécanisme de type paracrine. La présence de 5-HT a été montrée dans la glande surrénale de nombreuses espèces de vertébrés par des approches immunohistochimique et biochimique. Chez la grenouille, le rat et le porc, la 5-HT est localisée dans les cellules chromaffines, alors que chez la souris, la 5-HT est présente dans des fibres nerveuses qui innervent toute la glande. Chez l'homme, la 5-HT est contenue dans des mastocytes disseminés dans le cortex surrénalien. Des études in vivo et in vitro ont montré que la 5-HT stimule la sécrétion des corticostéroïdes chez de nombreuses espèces, y compris chez l'homme. Le type de récepteur sérotoninergique présent au niveau surrénalien diffère selon l'espèce. Chez la grenouille et chez l'homme, les effets corticotropes de la 5-HT font intervenir un récepteur 5-HT_4 positivement couplé à l'activité adénylyl cyclasique et à un influx de calcium, alors que chez le rat, la 5-HT stimule la production d'aldostérone par l'intermédiaire de récepteurs 5-HT_7. Des études in vivo révèlent que les agonistes des récepteurs 5-HT_4 stimulent la production d'aldostérone chez des volontaires sains, ainsi que chez des patients atteints d'insuffisance corticotrope ou d'hyperaldostéronisme primaire. Le contrôle sérotoninergique local de la sécrétion des corticostéroïdes pourrait être impliqué dans la physiologie et la physiopathologie du cortex surrénalien.

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Mots clés : l'axe HPS, système rénine-angiotensine, glande surrénale, sécrétion des corticostéroïdes, récepteurs sérotoninergique.

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Introduction

The activity of adrenocortical cells is primarily regulated by circulating peptide hormones, i.e., adrenocorticotropin (ACTH) and angiotensin II (AII), and by variations of kalemia. However, there is now evidence that corticosteroid secretion can also be modulated by intra-adrenal regulatory systems. In particular, various neurotransmitters and neuropeptides, released by nerve fibers ending in the adrenal cortex or released by neighbouring chromaffin cells, may control corticosteroid production through neuroendocrine or paracrine interactions. Indirect mechanisms, including regulation of adrenal blood flow and modulation of intra-adrenal renin-angiotensin or endothelin systems, also contribute to the local control of steroid secretion (Gallo-Payet 1993; Lesouhaitier et al. 1995; Mulrow and Franco-Saenz 1996; Nussdorfer 1996; Bornstein and Vaudry 1998; Ehrhart-Bornstein et al. 1998).

The neurotransmitter serotonin (5-HT) is produced in various endocrine glands including the pituitary (Montage and Calas 1988), thyroid (Tamir et al. 1989), pancreas (Teff and Young 1988), testis (Aguilar et al. 1995; Tinajero et al. 1993), and ovary (Amenta et al. 1992). In several of these organs, 5-HT has been shown to modulate hormone secretion by several types of endocrine cells such as gonadotrophs in the anterior pituitary (Hery et al. 1978), melanotrophs in the pars intermedia (Randle et al. 1983; Lamacz et al. 1989), follicular cells in the thyroid (Tamir et al. 1992), Leydig cell in the testis (Csaba et al. 1998), and granulosa cells in the ovary (Tanaka et al. 1993). These observations indicate that, besides its neurotransmitter role in the central nervous system, 5-HT may act as a paracrine factor locally regulating the secretory activity of a variety of endocrine cells.

The aim of the present review is to update current knowledge on the role of 5-HT in the regulation of adrenal steroid secretion. First, we will describe the roles of 5-HT at the different levels of the hypothalamo-pituitary adrenal (HPA) axis as well as on the renin-angiotensin system (RAS). We will then focus on the local production, effects, and mechanism of action of 5-HT within the adrenal gland. Finally, we will discuss in some detail the physiological and pathophysiological relevance of the intra-adrenal regulatory functions of 5-HT.

5-HT receptors classification synopsis

The multiple physiological effects of 5-HT are mediated by specific interactions of the amine with a wide variety of receptors. Historically, the operational characteristics of 5-HT receptors were considered sufficient for their classification. The scheme proposed by Bradley and co-workers (1986), which defined the 5-$HT_{1\text{-}like}$, 5-HT_2, and 5-HT_3 receptors, was based on the need to reconcile data from functional pharmacological studies with those from radioligand binding studies; this provided a useful and common system of nomenclature for the 5-HT receptors. However, a new source of complexity has been generated by molecular cloning of multiple 5-HT receptor isoforms for which selective ligands are not always available (Boess and Martin 1994).

The current classification and naming of 5-HT receptors have been described elsewhere in detail (Zifa and Fillion 1992; Humphrey et al. 1993; Hoyer et al. 1994; Hoyer and Martin 1997) and here we will summarize the operational, structural, and transductional criteria used to characterize the receptors. (*i*) Initially, receptors were characterized primarily on the basis of operational data, and binding studies with a suitable radiolabeled ligand allowed the determination of the relative affinity of the binding sites for antagonists and for agonists. (*ii*) The cloning and sequencing of the cDNA encoding a receptor provides the definitive identification of its unique protein structure. Sequence homologies between receptor proteins can be used to define receptor families and subfamilies (Table 1). It should be noted, however, that characterization of a receptor cDNA does not necessarily demonstrate the existence of a functional gene product. Therefore, functional and biochemical characterization of the native receptor is required to demonstrate that the mRNA of a given receptor is actually translated into a receptor protein. In term of nomenclature, lower case letters will be used to designate recombinant receptors (Hoyer and Martin 1997). (*iii*) Characterization of the transduction system provides important information for classification into the receptor superfamilies (i.e., ligand-gated ion channel or G protein-linked). This may be useful data to indicate the preferred transduction system for native receptor in its natural tissue versus transfected recombinant receptors in cell lines.

All 5-HT receptors belong to the superfamily of 7-transmembrane domain G protein-coupled receptors (GPRs) except for 5-HT_3 receptors, ligand-gated ion channel receptors (Table 1) that induce neuronal depolarization upon activation (Zifa and Fillion 1992). Among the GPR superfamily, 3 subfamilies are positively coupled to adenylyl cyclase: the 5-HT_4 receptor, which has been cloned in several species (Hegde and Eglen 1996) and two other receptors that were isolated by screening of cDNA libraries with probes derived from sequences of other GPRs, named 5-HT_6 (Ruat et al. 1993*a*; Kohen et al. 1996) and 5-HT_7 receptors (Ruat et al. 1993*b*; Heidmann et al. 1997). Although the 5-ht_6 and 5-HT_7 receptors are coupled to the same transduction system, these two receptors exhibit little sequence homology (Boess and Martin 1994). Several splice variants have been characterized in the case of 5-HT_4 (Claeysen et al. 1999) and 5-HT_7 receptors (Heidmann et al. 1997). All the subtypes of the 5-HT_1 receptor family and the 5-ht_{5A} receptor are negatively coupled to adenylyl cyclase (Hoyer and Martin 1997). The members of the 5-HT_2 receptor family are all

coupled to phospholipase C (PLC) (Baxter et al. 1995), and exhibit strong sequence homology to each other (Table 1).

Involvement of 5-HT in the control of neuroendocrine systems regulating corticosteroid secretion

There is now strong evidence that 5-HT is involved in the modulation of all neuroendocrine and endocrine components of the HPA axis including corticotropin-releasing factor (CRF)-producing neurons of the paraventricular nucleus (PVN) of the hypothalamus and corticotrope cells of the anterior pituitary (Fig. 1), as well as in the regulation of the activity of juxtaglomerular cells of the kidney (Fig. 2).

Serotonergic control of the HPA axis

Immunocytochemical studies have shown that 5-HT-containing nerve terminals form axo-dendritic and axo-somatic synapses with CRF-containing cell bodies in the rat PVN (Liposits et al. 1987). Remarkably, the density of 5-HT fibres and terminals is higher in the parvocellular aspect of the PVN than in the magnocellular part (Montage and Calas 1988). The exact origin of these serotonergic terminals has not yet been determined but they likely originate from three distinct cell groups of the raphe nuclei, namely the B7, B8, and B9 nuclei (Sawchenko et al. 1983).

The occurrence of 5-HT has also been demonstrated in the pituitary gland. In the distal lobe, 5-HT-like immunoreactivity is located in at least two types of endocrine cells, the corticotrophs (Pearse and McGregor 1964) and the gonadotrophs (Payette et al. 1986). In the intermediate lobe, 5-HT-containing fibers innervate melanotrope cells (Friedman et al. 1983; Leranth et al. 1983; Saland et al. 1986; Ubink et al. 1999). In addition, the intermediate lobe also contains 5-HT-immunoreactive mast-like cells (Palkovits et al. 1986). Finally, 5-HT-positive fibres have been identified in the neural lobe by immunocytochemistry and autoradiographic labeling (Payette et al. 1985; Ubink et al. 1999).

Early studies have demonstrated that the secretion of CRF from isolated rat hypothalami is stimulated by 5-HT (Calogero et al. 1989). There is also strong evidence that in vivo administration of 5-HT precursors (5-HTP), releasers (*p*-chloroamphetamine, fenfluramine) and uptake inhibitors (fluoxetine) activates the HPA axis in the rat (Fuller and Snoddy 1990; Fuller 1992, 1996). In human, it has been reported that oral administration of 5-HTP or fenfluramine produce a significant increase in plasma cortisol concentrations (Lewis and Sherman 1984; Shenker et al. 1985*a*, *b*). It has also been found that the effect of fenfluramine on ACTH and cortisol levels are attenuated by the non-selective 5-HT receptor antagonist cyproheptadine, suggesting the involvement of a serotonergic control of corticotrope and (or) adrenocortical cells (Lewis and Sherman 1984).

Pharmacological studies with 5-HT have shown that at least two different receptor subtypes can mediate the stimulatory effect of 5-HT on pituitary-adrenocortical secretions (Fig. 1). It has been initially reported that the 5-HT_1 receptor agonist 8-OH-DPAT increases serum ACTH and corticosterone levels in the rat (Koenig et al. 1987) and it has been subsequently found that this effect is mimicked by ipsapirone, buspirone, and gepirone (Koenig et al. 1988). The secretory responses induced by these 5-HT_1 receptor agonists can be blocked by several 5-HT_{1A} receptor antagonists, including pindolol and penbutolol (Fuller and Snoddy 1990; Lesch et al. 1990). Additional observations indicate that 5-HT_{1A} receptors are likely located post-synaptically on target neurons rather than presynaptically as autoreceptors on 5-HT neurons. For instance, the effect of 8-OH-DPAT on serum corticosterone is not affected by pretreatment with neurotoxic doses of *p*-chloroamphetamine (Fuller 1996), suggesting that the 5-HT_{1A} receptor agonist does not act on 5-HT neurons. Concurrently, it has been shown that local application of 8-OH-DPAT into the PVN increases plasma corticosterone levels (Haleem et al. 1989). It has also been found that 8-OH-DPAT stimulates CRF release from the isolated rat hypothalamus in vitro (Calogero et al. 1989). Collectively, these data indicate that the effects of 8-OH-DPAT are mediated through activation of postsynaptic 5-HT_{1A} receptors.

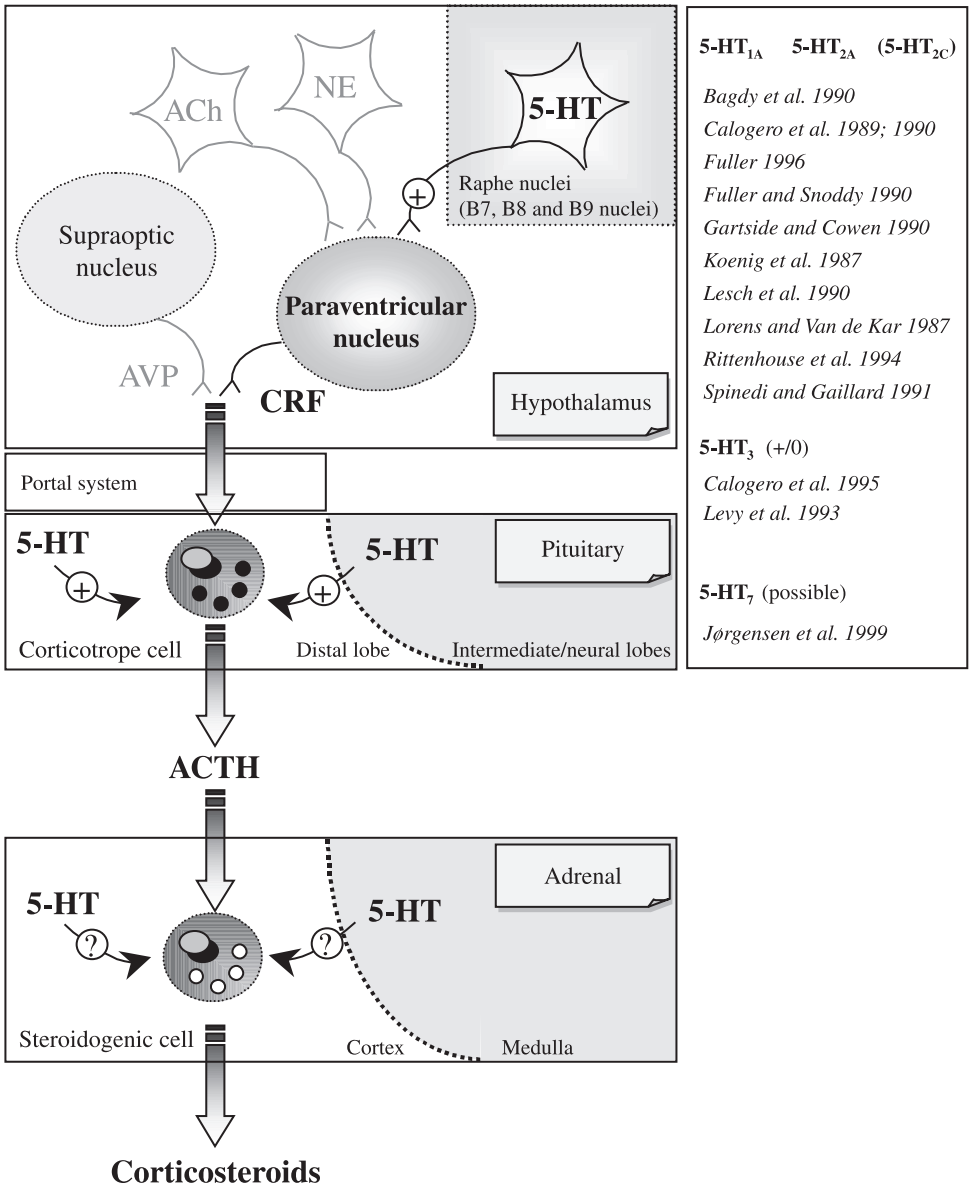
Pharmacological discrimination between 5-HT_{2A} and 5-HT_{2C} receptors is difficult because of the lack of selective ligands that could differentially modulate these two receptors. It has been shown that MK-212 and quipazine, two 5-HT_{2A}/5-HT_{2C} receptor agonists, provoke an increase in plasma corticosterone level (Lorens and Van de Kar 1987; Fuller and Snoddy 1990), but the type of receptor involved remains questionable. A good correlation ($r = 0.69$) has been observed between the potencies of 11 receptor antagonists in blocking quipazine-induced elevation of serum corticosterone in rat and their affinities for the 5-HT_{2A} receptor, whereas a weaker correlation ($r = 0.50$) was observed with their affinities for the 5-HT_{2C} receptor (Fuller 1996), suggesting that the stimulatory effect of quipazine is likely mediated through 5-HT_{2A} receptors. Nevertheless, the possibility remains that 5-HT_{2C} receptors are involved in the serotonergic control of pituitary-adrenocortical functions since *m*-chloro- and *m*-trifluoromethylphenylpiperazine, two 5-HT_{2C} receptor agonists that have little or no agonistic activity at 5-HT_{2A} receptors, stimulate corticosterone secretion in the rat (Fuller 1996).

At the pituitary level, the presence of 5-HT_2 receptors has been demonstrated by quantitative autoradiography, suggesting that 5-HT plays a physiological role in the control of adenohypophysial hormone secretion (De Souza 1986). In support of this hypothesis, it has been shown that 5-HT triggers ACTH release from rat anterior pituitary cells in primary culture (Spinedi and Negro-Vilar 1983). The fact that both 8-OH-DPAT and DOI can both mimic this effect indicates that the 5-HT-induced stimulation of corticotrope cells is mediated through 5-HT_1 and 5-HT_2 receptors (Bagdy et al. 1990). The possible involvement of 5-HT_3 receptors in the control of ACTH secretion is currently a matter of debate. In vivo studies have shown that MDL 72222 does not impair the effect of *p*-chloroamphetamine, 5-HT, or 2-Me-5-HT on plasma ACTH concentration in freely moving rat (Levy et al. 1993), suggesting that 5-HT_3 receptors do not contribute to the stimulatory action of 5-HT on ACTH secretion. Recent in vivo data have confirmed that 5-HT_3 receptors are not implicated in the serotonergic activation of the HPA axis

Table 1. Classification and nomenclature for 5-HT receptors.

Nomenclature (previous name)	Locus in human	Structure	Effector/transduction systems	Radioligands	Selective compounds	
					Agonists	Antagonists
5-HT$_1$ Family						[Methiothepin, non selective]
5-HT$_{1A}$	5q11-q13	h 421aa 7TM		[^{3}H]-WAY 100635 ^{3}H]-8-OH-DPAT	5-CT, 8-OH-DPAT, 5-MeO-DMT >metergoline	WAY 100135, WAY 100635 SDZ 21625
5-HT$_{1B}$ (5-HT$_{1D\beta}$)	6q13	h 390 aa 7TM		[^{125}I]-GTI	5-CT, metergoline, CP 93129 >>8-OH-DPAT	SDZ 21009, GR 127935
5-HT$_{1D}$ (5-HT$_{1D\alpha}$)	1p34-p36	h 377 aa 7TM	$G_{i/o}$ ⊖ adenylyl cyclase	[^{3}H]-sumatriptan	5-CT>metergoline>sumatriptan	
5-ht$_{1E}$	6q14-q15	h365 aa 7TM	⊕ K^+ conductance	[^{3}H]-5-HT (in cloned cells)		[non selective compounds]
5-ht$_{1F}$	3p12	h 366 aa 7TM		[^{3}H]-sumatriptan [^{125}I]-LSD (with mesulergine and 5-CT)	Sumatriptan	[non selective compounds]
5-HT$_2$ Family						
5-HT$_{2A}$ (D/5-HT$_2$)	13q14-q21	h 471 aa 7TM		[^{3}H]-ketanserin	DOI, α-Me-5-HT, quipazine >>8-OH-DPAT	Ketanserin, methiothepin, mesulergine>ritanserin
5-HT$_{2B}$ (5-HT$_{2F}$)	2q36-q37	h 479 aa 7TM	$G_{\alpha/11}$ ⊕ phospholipase C	[^{3}H]-5-HT (in cloned cells)	αMe-5-HT	SB 200646, SB 204741
5-HT$_{2C}$ (5-HT$_{1C}$)	Xq24	h 485 aa 7TM		[^{3}H]-mesulergine	αMe-5-HT, DOI, RU 24969	Metergoline, SB 200646, mesulergine>methiothepin> ketanserin
5-HT$_3$ (M)	11q23	m 487 aa 4TM	Ligand-gated Cation channel	[^{3}H]-zacopride [^{125}I]-zacopride	2-Me-5-HT, m-chlorophenylbiguanide	ICS 205930, zacopride, MDL 722222
5-HT$_4$	5q31-q33	7TM *several isoforms*	G_s ⊕ adenylyl cyclase ⊖ K^+ conductance ⊕ Ca^{2+} influx	[^{3}H]-GR 113808 [^{125}I]-SB 207710	Cisapride, zacopride, BIMU 8>>5-CT	GR 113808, DAU 6285, SB 204070, RS 23597-190 >> ICS 205930 (methiothepin inactive)
5-ht$_5$ Family						
5-ht$_{5A}$	7q34-q36	h 357 aa 7TM	⊖ adenylyl cyclase	[^{3}H]-5-HT (in cloned cells)		
5-ht$_{5B}$	2q11-q13	r 371 aa 7TM	n.d.			
5-ht$_6$	1p35-p36	h 440 aa 7TM	G_s ⊕ adenylyl cyclase	[^{3}H]-5-HT (in cloned cells)	5-MeOT>5-HT>tryptamine> 2-Me-tryptamine>5-CT	SB 271046, Ro 04-6790 Methiothepin>lisuride>clozapine> mianserin>metergoline
5-HT$_7$	10q21-q24	7TM *several isoforms*	G_s ⊕ adenylyl cyclase	[^{3}H]-5-HT (in the presence of pindolol) [^{3}H]-5-CT	5-CT>5-HT>5-MeOT>8-OH-DPAT	LY 215840 Methiothepin>lisuride>metergoline> clozapine>mesulergine>ketanserin

Fig. 1. Schematic representation summarizing the role of serotonin (5-HT) in the regulation of the hypothalamo-pituitary-adrenal (HPA) axis. The subtypes of 5-HT receptors possibly involved in the regulation of the HPA axis at the hypothalamic and pituitary levels are indicated. NE, noradrenaline; ACh, acetylcholine; ACTH, adrenocorticotropin; AVP, vasopressin; CRF, corticotropin-releasing factor.



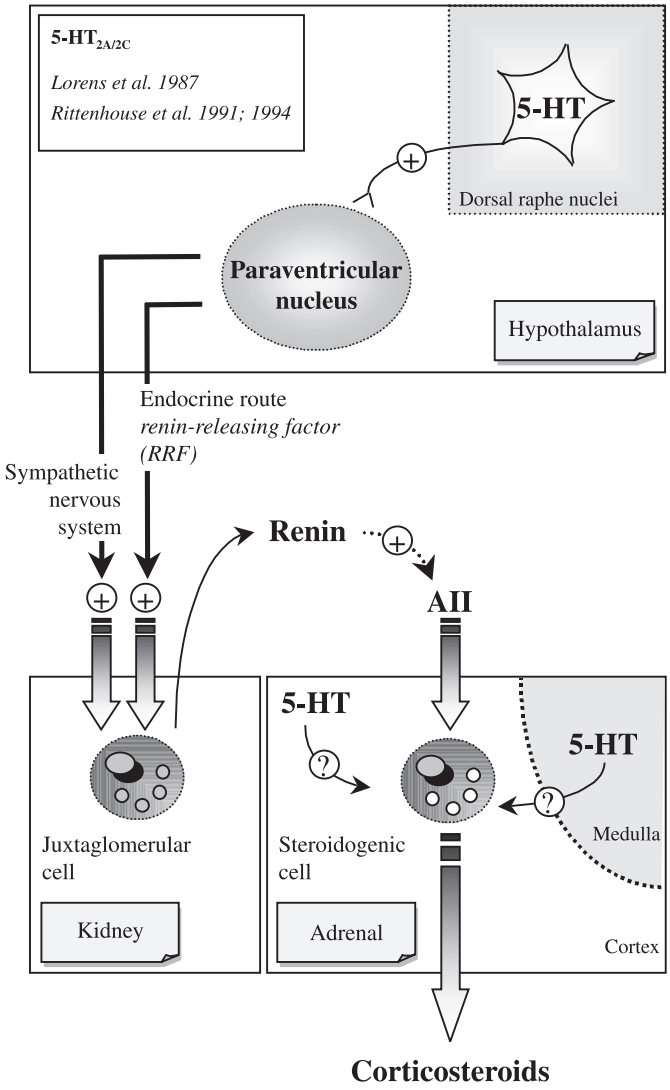
(Jorgensen et al. 1999). In contrast, in vitro studies have shown that 5-HT and the 5-HT_3 receptor agonist *m*-chlorophenylbiguanide stimulate ACTH release in a concentration-dependent manner, and that these effects are antagonized by ICS 205930 and MDL 72222 (Calogero et al. 1995). Further studies are clearly required to solve the discrepency between in vivo and in vitro data and to determine whether 5-HT_3 receptors are implicated in the control of the HPA axis. The involvement of 5-HT_4 receptors in the action of 5-HT on ACTH secretion has been suggested in the rat (Jorgensen et al. 1999). In contrast, administration of the selective 5-HT_4 receptor agonist zacopride does not affect plasma ACTH level in humans (Lefebvre et al. 1996*a*). The possible involvement of 5-HT_7 receptors in the effects of 5-HT receptor agonists on ACTH secretion has been proposed in rat (Jorgensen et al. 1999) but pharmacological studies with more selective agonists and antagonists are needed in order to assess the potential implication of 5-HT_7 receptors in the stimulatory effect of 5-HT on corticotrope cell activity.

Serotonergic control of renin secretion

Renin secretion from the kidney is regulated by different homeostatic mechanisms including alterations in renal perfusion pressure and sodium transport in the distal convoluted tubules, as well as by activation of β-adrenoreceptors (Skott and Jensen 1993). Beside these mechanisms, serotonergic neurons have also been shown to contribute to the regulation of renin secretion (Fig. 2).

Activation of 5-HT receptors in the PVN stimulates renin secretion from the kidney. Destruction of serotonergic neurons in the dorsal raphe nucleus using the neurotoxin 5,7-dihydroxytryptamine prevents the increase in renin release

Fig. 2. Schematic representation summarizing the role of serotonin (5-HT) in the regulation of renin secretion. The subtypes of 5-HT receptors possibly involved in the regulation of renin secretion are indicated.



induced by *p*-chloroamphetamine (Van de Kar et al. 1982). Cell selective lesion with ibotenic acid in the PVN inhibits the stimulatory effect of the 5-HT_{2C} receptor agonist RU 24969 on renin secretion (Rittenhouse et al. 1992). Conversely, direct injection of RU 24969 into the PVN of conscious rat increases plasma renin concentration (Rittenhouse et al. 1994). In healthy volunteers, administration of the 5-HT precursor tryptophan induces a substantial increase in plasma renin activity (Modlinger et al. 1979). The transmission of the neuronal message from the hypothalamus to the kidney can be accounted for by two distinct mechanisms. The involvement of the sympathetic pathway is supported by the observation that β-adrenoreceptor antagonists, such as sotalol and atenolol, inhibit the effects of *p*-chloroamphetamine or fenfluramine on renin release (Van de Kar et al. 1985; Van de Kar and Richardson-Morton 1986). However, it has been found that adrenal medullectomy combined with chemical sympathectomy (using 6-hydroxytryptamine) or spinal transection proximal to the exit of the adrenal and renal nerves (Van de Kar et al. 1985; Van de Kar and Richardson-Morton 1986) fails to inhibit the effect of *p*-chloroamphetamine on renin secretion. Alternatively, it has been proposed that a hormone-like substance called renin-releasing factor (RRF) may be involved in the stimulatory effect of serotonergic neurons on renin release (Urban et al. 1985). In particular, it has been found that *p*-chloroamphetamine or MK-212 provoke a concomitant increase in the plasma concentrations of RRF and renin (Van de Kar et al. 1996). Studies on the distribution of RRF in the brain indicate that the hypothalamus and the cerebellum contain the highest concentrations of the peptide (Urban et al. 1992). Incubation of rat kidney slices with hypothalamic extracts produced a dose-dependent increase in renin release. Furthermore, depolarization of hypothalamic explants with high concentrations of potassium increases the release of RRF in the medium, suggesting that the peptide is located in neurons and could be released into the circulation (Urban et al. 1992). It is conceivable that 5-HT could stimulate the secretion of renin through activation of RRF-producing neurons.

The type of serotonergic receptors involved in the effect of 5-HT on renin secretion has also been investigated (Fig. 2). Intracerebroventricular injection of low doses of the 5-HT_2 receptor agonists DOI or RU 24969 causes an increase in plasma renin (Rittenhouse et al. 1991, 1994). The effect of DOI and RU 24969 on renin release is inhibited by ritanserin, suggesting that the effects of both agonists are mediated through activation of central 5-HT_{2A} and (or) 5-HT_{2C} receptors (Rittenhouse et al. 1991). It is of note that, when injected systemically, DOI and quipazine can act also at the periphery to increase arterial blood pressure and to decrease renal blood flow, thereby activating the renal baroreceptor reflex yielding to an increase in renin release (Alper and Snider 1987). Concurrently, it has been clearly demonstrated that neither 5-HT_{1A} receptors (Lorens and Van de Kar 1987) nor 5-HT_3 receptors (Levy et al. 1993) are involved in the serotonergic regulation of renin secretion.

Involvement of intra-adrenal 5-HT in the regulation of corticosteroid secretion

Occurrence of 5-HT in the adrenal gland

The presence of 5-HT-like immunoreactivity has been visualized in the cytoplasm of adrenaline-producing cells in the frog interrenal gland (Delarue et al. 1988*a*) and in the rat medulla (Verhofstad and Jonsson 1983; Holzwarth and Brownfield 1985). In contrast, in the pig adrenal gland, most 5-HT is localized in noradrenaline-containing cells (Kong et al. 1989). At the ultrastuctural level, 5-HT-immunoreactivity appears to be sequestered in secretory vesicles within rat (Brownfield et al. 1985; Holzwarth and Brownfield 1985) and frog (Delarue et al. 1988*a*) chromaffin cells, suggesting that 5-HT might be released together with catecholamines under splanchnic nerve stimulation. In the mouse, uptake of 5-HT has been demonstrated by autoradiography in chromaffin cells (Kent and Coupland 1984) as well as in fibers innervating the whole gland (Fernandez-Vivero et al. 1993). In the rat adrenal gland, it oposed that mast cells scarnal 5-HT (Hinson et al. 1989). In human, 5-HT is not produced in chromaffin cells but is instead restricted to perivascular mast cells (Lefebvre et al. 1992).

Table 2. Serotonin content as determined by HPLC in adrenal glands of various species.

Species		5-HT content in whole adrenal, μg/g wet tissue (nmol/g wet tissue)	5-HT content in chromaffin tissue, μg/g wet tissue	Local concentration estimation with 30 % release	5-HT/adrenaline, %	References
Frog *Rana ridibunda*		0.194 ± 0.012 (1.11 ± 0.01)	0.58	~ 10 μM	0.02	Delarue et al. 1988*a*, 1992; Leboulenger et al. 1993
Rat	Wistar	0.57 ± 0.03 (3.16 ± 0.16)		~ 30 μM		Verhofstad and Jonsson 1983
	Sprague-Dawley	0.45 ± 0.04 (1.4 ± 0.11[a])			0.4	Verhofstad and Jonsson 1983
	Sprague-Dawley	1.4 ± 0.2 [a] (7.7 ± 0.1)	14	~ 100 μM	2.55	Holzwarth and Brownfield 1985
Human		0.124 ± 0.034 (0.68 ± 0.18)		1–10 μM		Lefebvre et al. 1992

[a]Decapsulated glands.

Biochemical identification of 5-HT has been performed in frog (Delarue et al. 1988*a*), rat (Verhofstad and Jonsson 1983; Holzwarth and Brownfield 1985), and human (Lefebvre et al. 1992) adrenal gland extracts by combination of high performance liquid chromatography (HPLC) analysis and electrochemical detection (Table 2). In the frog adrenal gland, the concentration of 5-HT (0.2 μg/g wet tissue) represents about 0.02% of the concentration of adrenaline, whereas, in rat, the concentration of 5-HT (0.5 μg/g wet tissue) reaches 0.4-2.5% of the level of adrenaline. The human adrenal gland also contains substantial amounts of 5-HT (Table 2). The occurrence of 5-hydroxyindolacetic acid (5-HIAA) in frog (Delarue et al. 1988*a*), rat (Verhofstad and Jonsson 1983), and human (Lefebvre et al. 1992) adrenal tissue indicates that adrenal cells are also capable of metabolizing 5-HT. In agreement with this hypothesis, monoamine oxydase activity has been demonstrated within the human adrenal gland (Lefebvre et al. 1996*b*).

Origin of 5-HT in the adrenal gland

Studies have been conducted to determine whether 5-HT is synthesized locally or taken up from the blood. In the frog adrenal gland, it has been demonstrated that 5-HT can be synthesized from [^{3}H]tryptophan, indicating that frog chromaffin cells exhibit trypophan hydroxylase activity (Delarue et al. 1992). In contrast, in the rat adrenal gland, it has been reported that 5-HT can be formed only by decarboxylation of 5-hydroxytryptophan (Verhofstad and Jonsson 1983). Accordingly, the enzyme L-aromatic amino acid decarboxylase (L-AAAD) has been localized by immunocytochemistry in the adrenal medulla as well as in the zona glomerulosa and zona fasciculata (Burns et al. 1996), suggesting that 5-HT can be synthesized within the rat adrenal cortex by circulating 5-HTP. Nevertheless, active uptake of exogenous 5-HT by chromaffin cells has been documented in the frog (Delarue et al. 1992) and the rat (Verhofstad and Jonsson 1983). In humans, phaeochromocytoma cells also possess a 5-HT uptake mechanism (Yoffe and Borchardt 1982), but the presence of 5-HT has never been reported in normal adrenochromaffin cells. These observations indicate that 5-HT present in the adrenal gland can be both newly synthesized within the tissue and uptaken from the circulation.

Effect of 5-HT on steroid secretion

The stimulatory effect of 5-HT on the adrenal cortex has been first reported by Rozenkrantz (1959). Since then, a number of studies performed in various species have shown that 5-HT is a potent stimulator of corticosteroid secretion (Muller and Ziegler 1968; Haning et al. 1970; Al-Dujaili et al. 1982; Lefebvre et al. 1998). In vitro experiments in the rat have shown that 5-HT$_5$ stimulates both corticosterone and aldosterone secretion (Fig. 3A) from freshly prepared capsular cells, i.e., glomerulosa cells (Haning et al. 1970). In contrast, 5-HT is devoid of effect on aldosterone secretion from cultured rat capsular cells (Gallo-Payet et al. 1993; V. Contesse, S. Lenglet, and H. Vaudry 1998, unpublished observations), indicating that after several days in culture the responsiveness of rat glomerulosa cells to 5-HT disappears. It has been demonstrated

Fig. 3. Comparison of the effects of serotonin (5-HT) and serotonergic agonists on aldosterone secretion in different species. (A) Effect of graded concentrations of 5-HT on aldosterone secretion by cultured human adrenocortical cells (●), perifused rat adrenocortical slices (□), perifused frog adrenocortical slices (△) and cultured bovine adrenocortical cells (◇). (B) Effect of graded concentrations of two 5-HT receptor agonists, zacopride (▽;▼) and 8-OH-DPAT (○;●) on aldosterone secretion by perifused frog adrenocortical slices (open symbols) and perifused rat adrenocortical slices (black symbols). Results are expressed as a percentage of the maximum response induced by 5-HT in each model. Standard error of the mean have been omitted for more clarity. (Adapted from Contesse et al. 1994 with permission from Eur. J. Pharmacol., Elsevier Science Publishers; Contesse et al. 1999 with permission from Mol. Pharmacol., The American Society for Pharmacology and Experimental Therapeutics; Delarue et al. 1988*b* with permission from J. Steroid Biochem., Pergamon Press; Idres et al. 1991 with permission from Mol. Brain Res., Elsevier Science Publishers.)

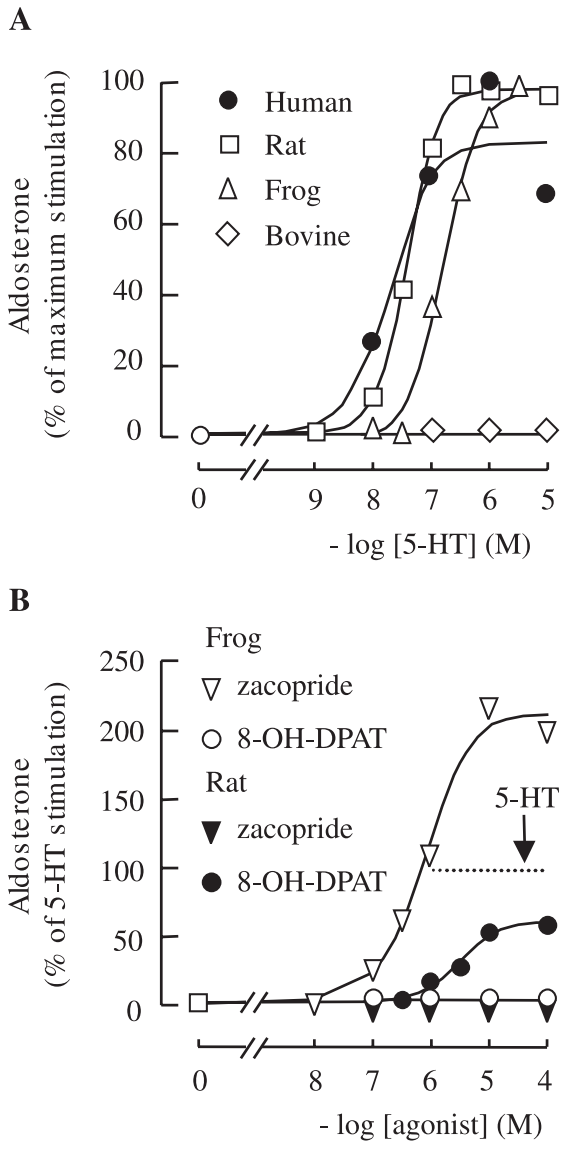


Table 3. Effect of serotonergic receptor antagonists on 5-HT-induced steroid secretion from perifused adrenal cortex.

Ligands	Frog (pK_i)	Rat (pK_B)	Human
Methiothepin	inactive	10.13	
Lisuride		9.83	
Pimozide		8.75	
Metergoline	inactive	8.57	
Clozapine		8.33	
Mesulergine	inactive	8.18	
Methysergide	inactive		inactive
Mianserin		7.93	
Ketanserin	inactive	7.37	inactive
Cyproheptadine		7.18	
1NP		6.92	
MDL 72222	inactive		
GR 113808	10.34[a]	inactive	active[b]
DAU 6285	7.84[a]	inactive	
ICS 205930	6.20[a]		active[c]

Note: from Contesse et al. 1994, 1999; Idres et al. 1989; Lefebvre et al. 1992, 1996*b*.

[a] against zacopride.

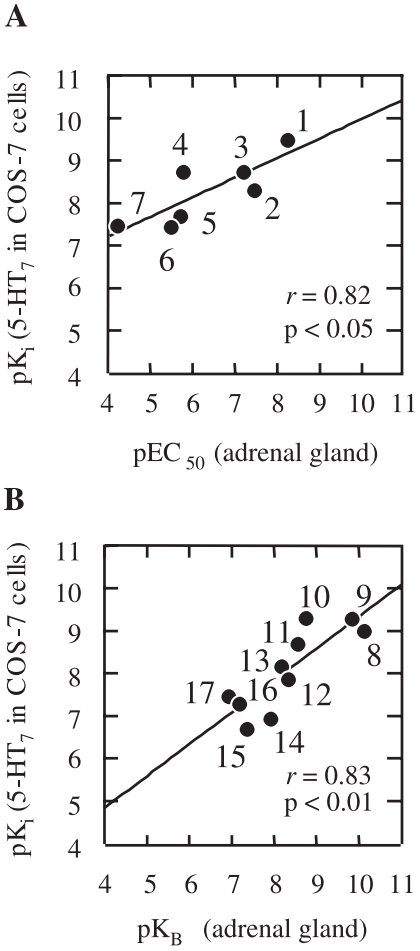
[b] 1 μM GR 113808 abolished the effect of compound 48/80 on aldosterone secretion.

[c] 1 μM ICS 205930 abolished the effect of 5-HT on cortisol secretion.

that 5-HT markedly enhances steroid release by in situ perfused rat (Hinson et al. 1989) and mouse (Yang et al. 1995) adrenal glands. The observation that 5-HT raises the flow rate of the perfusion medium in rat (Hinson et al. 1989) suggests that 5-HT is capable of indirectly stimulating steroid release by increasing the adrenal blood-flow. In amphibians, 5-HT directly stimulates corticosterone and aldosterone secretion (Fig. 3A) in a dose-dependent manner (Delarue et al. 1988*b*). Some studies have also been conducted in humans to investigate the effect of 5-HT on corticosteroid secretion. In vitro experiments have shown that 5-HT stimulates corticosterone production by adrenal cells derived from patients with Cushing's disease (Racz et al. 1979) and aldosterone secretion by adrenal cells from aldosterone-producing adenomas (Shenker et al. 1985*a*). It has been subsequently demonstrated that 5-HT triggers aldosterone (Fig. 3A) and cortisol secretion from normal human adrenocortical cells (Lefebvre et al. 1992). Surprisingly, 5-HT is totally devoid of effect on aldosterone (Fig. 3A) or cortisol secretion from bovine cultured adrenocortical cells (V. Contesse, V. Carpentier-Turquir, and H. Vaudry 1999, unpublished observations).

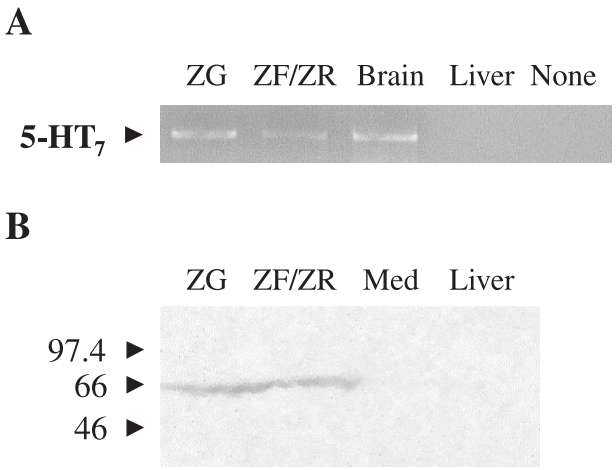
In the blood, a large proportion of 5-HT is sequestered in platelets so that the concentration of free 5-HT (0.4 to 2.5 ng/mL, i.e., about 5 nM) is lower than the minimal effective concentration required to stimulate corticosteroid secretion. Thus, it can be assumed that only 5-HT locally secreted in the adrenal gland, i.e., from chromaffin and (or) mast cells, can exert a paracrine control of steroid secretion. The concentration of 5-HT in the pericellular fluid of frog, rat, and human adrenal glands, evaluated according to the method proposed by Mazzocchi and co-workers (1993) to determine the local concentrations of regulatory molecules,

Fig. 4. Correlations between the affinities of various serotonergic ligands for the recombinant rat 5-HT_7 receptor and the pEC_{50} or pK_B values on aldosterone secretion by perifused rat adrenocortical slices. (A) Agonists: 1, 5-carboxamidotryptamine; 2, pergolide; 3, 5-HT; 4, 5-methoxytryptamine; 5, *N*,*N*-dimethyl-5-methoxytryptamine; 6, 8-OH-DPAT; 7, tryptamine. (B) Antagonists: 8, methiothepin; 9, lisuride; 10, pimozide; 11, metergoline; 12, clozapine; 13, mesulergine; 14, mianserin; 15, ketanserin; 16, cyproheptadine; 17, 1-(1-naphtyl)piperazine. (Adapted from Contesse et al. 1999 with permission from Mol. Pharmacol., The American Society for Pharmacology and Experimental Therapeutics.)



would range from 1 to 100 μM (Table 2), suggesting that 5-HT may play a physiological role within the adrenal gland. In support of this hypothesis, it has been found that the mast cell-stimulating compound 48/80 provokes the release of 5-HT and a subsequent increase in aldosterone secretion from perifused human adrenocortical slices (Lefebvre et al. 1996*b*) and from the perfused rat adrenal gland (Hinson et al. 1989). In contrast, no effect of compound 48/80 on adrenal secretion was detected in the perfused mouse adrenal gland model (Yang et al. 1995). These findings suggest that 5-HT released within the adrenal gland stimulates the secretory activity of adrenocortical cells through a paracrine mode of communication.

Fig. 5. Expression of 5-HT_7 receptors in rat tissues. (A) RT-PCR analysis of 5-HT_7 receptor mRNA. Primers specific for the rat 5-HT_7 receptor were used to amplify DNA fragments of 963 bps. (B) Western blot analysis of rat tissues. Proteins from tissue samples were analyzed by SDS-PAGE followed by immunoblotting with specific antibodies raised against the rat 5-HT_7 receptor (Diasorin, Stillwater, Minn.). Molecular mass markers (in kDa) are indicated on the left. ZG, zona glomerulosa; ZF/ZR, zona fasciculata/reticularis; Med, adrenal medulla. None, no DNA was added to the amplification mixture. (Adapted from Contesse et al. 1999 with permission from Mol. Pharmacol., The American Society for Pharmacology and Experimental Therapeutics.)

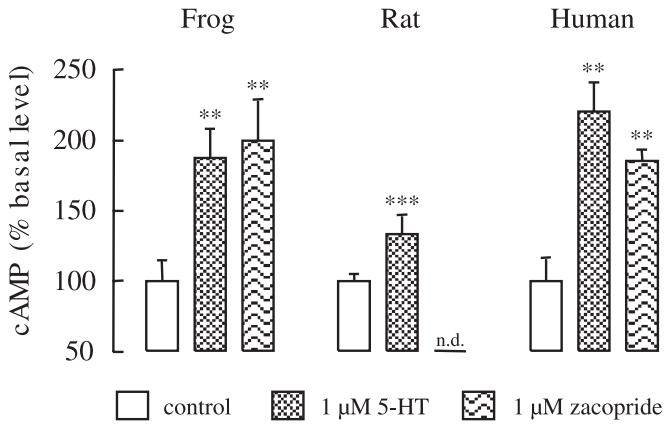


Characterization of adrenal serotonergic receptor

In the frog adrenal gland, the stimulatory effect of 5-HT on steroid secretion is not affected by various serotonergic receptor antagonists such as methiothepin, methysergide, ketanserin, or MDL 72222 (Table 3) (Idres et al. 1989). Furthermore, 8-OH-DPAT (Fig. 3B) or 2-Me-5-HT cannot mimic the action of 5-HT on corticosteroid production (Idres et al. 1991). In contrast, a series of substituted benzamide derivatives (cisapride, BRL 24924, zacopride) and benzimidazolone derivatives (BIMU 8, BIMU 1) induce a dose-dependent stimulation of corticosteroid secretion from perifused frog adrenal slices (Contesse et al. 1994). In this model, it was found that zacopride exhibits a higher efficacy than 5-HT (Fig. 3B). The 5-HT_3/5-HT_4 receptor antagonist ICS 205930, and the selective 5-HT_4 receptor antagonists GR 113808 and DAU 6285 induce a dose-dependent inhibition of the zacopride-evoked stimulation of steroid secretion (Contesse et al. 1994). The pK_i values (Table 3) of these different compounds are in good agreement with those reported in various models used to characterize 5-HT_4 receptors (Hegde and Eglen 1996). Altogether, these data strongly suggest that a 5-HT_4-like receptor is involved in the mechanism of action of 5-HT on frog adrenocortical cells.

In humans, the pharmacological profile of the receptor involved in the corticotropic action of 5-HT is very similar to that characterized in amphibians. In particular, neither methysergide nor ketanserin can affect the response to 5-HT, while ICS 205930 inhibits 5-HT-induced cortisol production (Lefebvre et al. 1992). In humans, as in frogs, zacopride

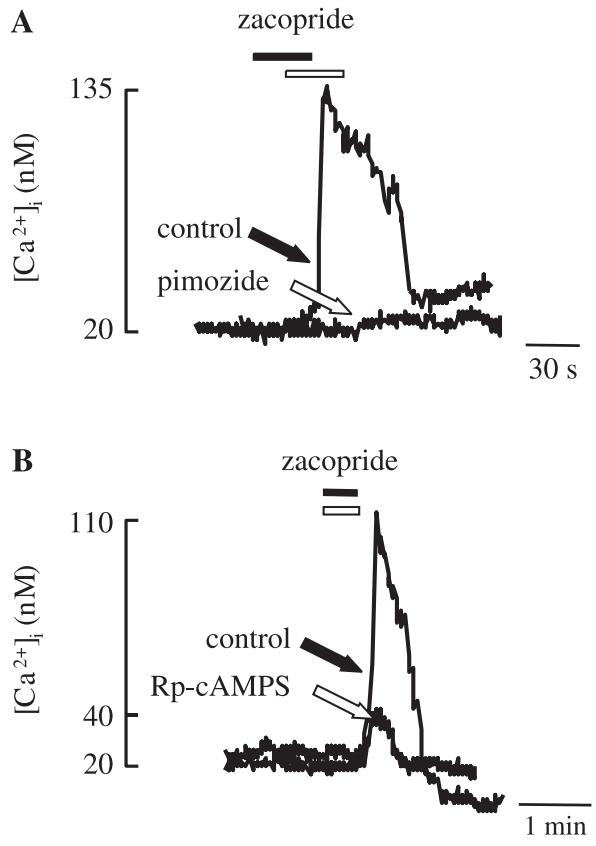
Fig. 6. Effect of 5-HT (1 μM) and zacopride (1 μM) on cAMP production by frog, rat, and human adrenocortical fragments. $^{**}P < 0.01$; $^{***}P < 0.001$; nd, not determined. (Adapted from Idres et al. 1991 with permission from Mol. Brain Res., Elsevier Science Publishers, Lefebvre et al. 1992 with permission from Neuroscience, Pergamon Press, Matsuoka et al. 1985 with permission from Am. J. Physiol., The American Physiological Society.)



causes in vitro a dose-dependent increase in both cortisol (Lefebvre et al. 1992) and aldosterone secretion (Lefebvre et al. 1993). Moreover, the stimulatory effect of compound 48/80 on aldosterone production from perifused human adrenal slices (see above) is abolished by concomitant administration of GR 113808 (Lefebvre et al. 1996*b*), supporting the view that 5-HT released within the adrenal gland stimulates steroid secretion through activation of a 5-HT_4 receptor subtype.

The type of receptor mediating the corticotropic effect of 5-HT on rat glomerulosa cells has long been puzzling. Early studies had shown that 5-HT stimulates aldosterone secretion and that this effect was associated with activation of adenylyl cyclase (Fujita et al. 1979; Williams et al. 1984; Matsuoka et al. 1985). Based on the single observation that ketanserin inhibits the effects of 5-HT on both aldosterone production and cAMP formation (Williams et al. 1984; Matsuoka et al. 1985; Rocco et al. 1986), it has been proposed that the action of 5-HT is mediated by a 5-HT_2 receptor positively coupled to adenylyl cyclase. However, it is now firmly established that stimulation of 5-HT_2 receptors causes activation of PLC and does not affect cAMP formation (Table 1). The fact that 5-HT has no effect on phospholipid hydrolysis in rat adrenocortical cells (Rocco et al. 1990) strongly suggests that the action of 5-HT on aldosterone secretion cannot be mediated through activation of 5-HT_2 receptors. To elucidate this paradox, a series of 22 ligands has been recently used to thoroughly characterize the receptor (Contesse et al. 1999). This study revealed that the potent 5-HT_2 receptor agonist DOI is totally devoid of effect on aldosterone secretion. In contrast to what has been observed in the frog (Idres et al. 1991), 5-HT receptor agonists such as 8-OH-DPAT displayed agonistic activity (Contesse et al. 1999) whereas zacopride had no effect on aldosterone secretion in the rat (Fig. 3B). Studies with 5-HT receptor antagonists clearly demonstrated that the pharmacological profile of the rat adrenal serotonergic receptor is very different

Fig. 7. Effect of the T-type calcium channel blocker pimozide (10 μM) (A) and the PKA inhibitor Rp-cAMPS (30 μM) (B) on zacopride-induced $[Ca^{2+}]_i$ rise in cultured frog adrenocortical cells. (Adapted from Contesse et al. 1996 with permission from Mol. Pharmacol., The American Society for Pharmacology and Experimental Therapeutics.)

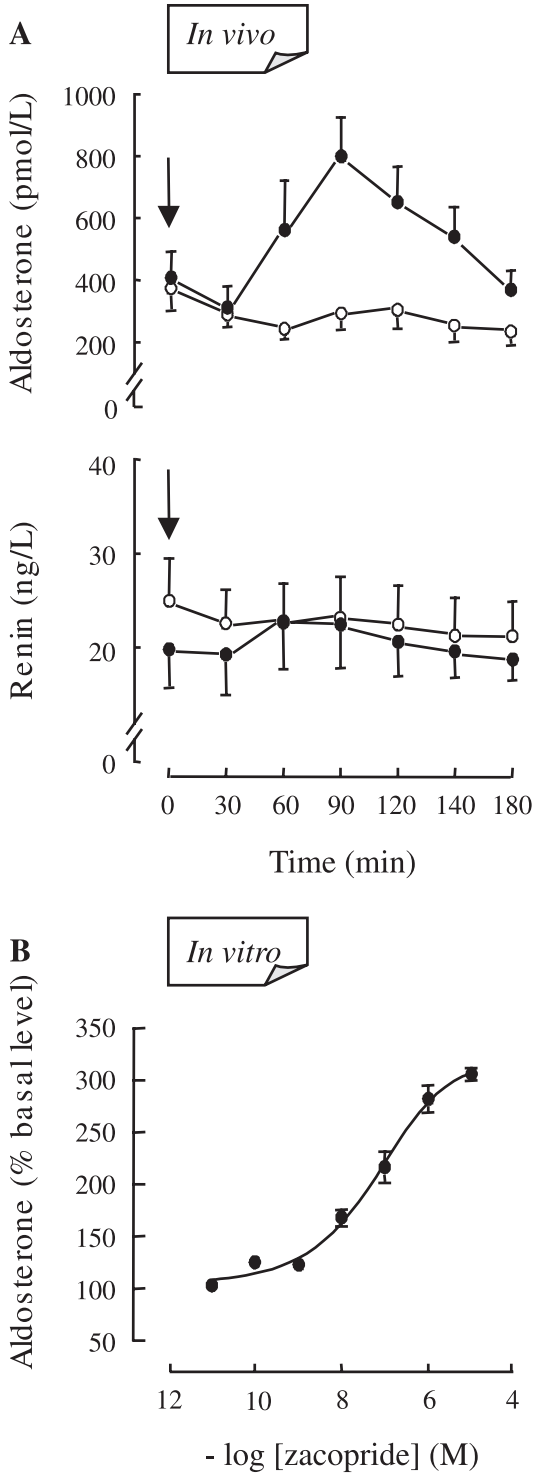


from those of frog and human adrenal receptors (Table 3). No significant correlation ($0.01 < r < 0.52$) was found between the relative affinities of these agonists and antagonists for the 5-HT_1 to 5-ht_6 receptors and their effects on rat glomerulosa cells. In contrast, there was a significant correlation between the affinity of the compounds for 5-HT_7 receptors and their agonistic (Fig. 4A) and antagonistic activity (Fig. 4B) on aldosterone secretion by the rat adrenal gland (Contesse et al. 1999). To confirm the involvement of a 5-HT_7 receptor subtype, molecular characterization of the serotonergic receptor was performed using polymerase chain reaction (RT-PCR) and Western blot analysis. It was found that 5-HT_7 receptor mRNA (Fig. 5A) and receptor protein (Fig. 5B) are present in the zona glomerulosa of the adrenal gland. Altogether, these data demonstrate that in the rat the stimulatory effect of 5-HT on aldosterone secretion can be accounted for by activation of a 5-HT_7 receptor subtype (Contesse et al. 1999).

Transduction mechanisms associated with adrenal serotonergic receptors

In frog (Idres et al. 1991), rat (Fujita et al. 1979; Williams et al. 1984; Matsuoka et al. 1985), and human (Lefebvre et al. 1992) adrenocortical cells, 5-HT stimulates adenylyl cyclase activity (Fig. 6). The stimulatory effect of 5-HT on

Fig. 8. Effect of the 5-HT_4 receptor agonist zacopride on aldosterone secretion in humans. (A) In vivo effect of zacopride on plasma aldosterone and renin levels in dexamethasone-suppressed subjects. A single dose of placebo (○) or 0.4 mg zacopride (●) was administered orally (arrows). (B) In vitro effect of zacopride on aldosterone production by perifused human adrenocortical slices. (Adapted from Lefebvre et al. 1993 with permission from J. Clin. Endocrinol. Metab., The Endocrine Society.)



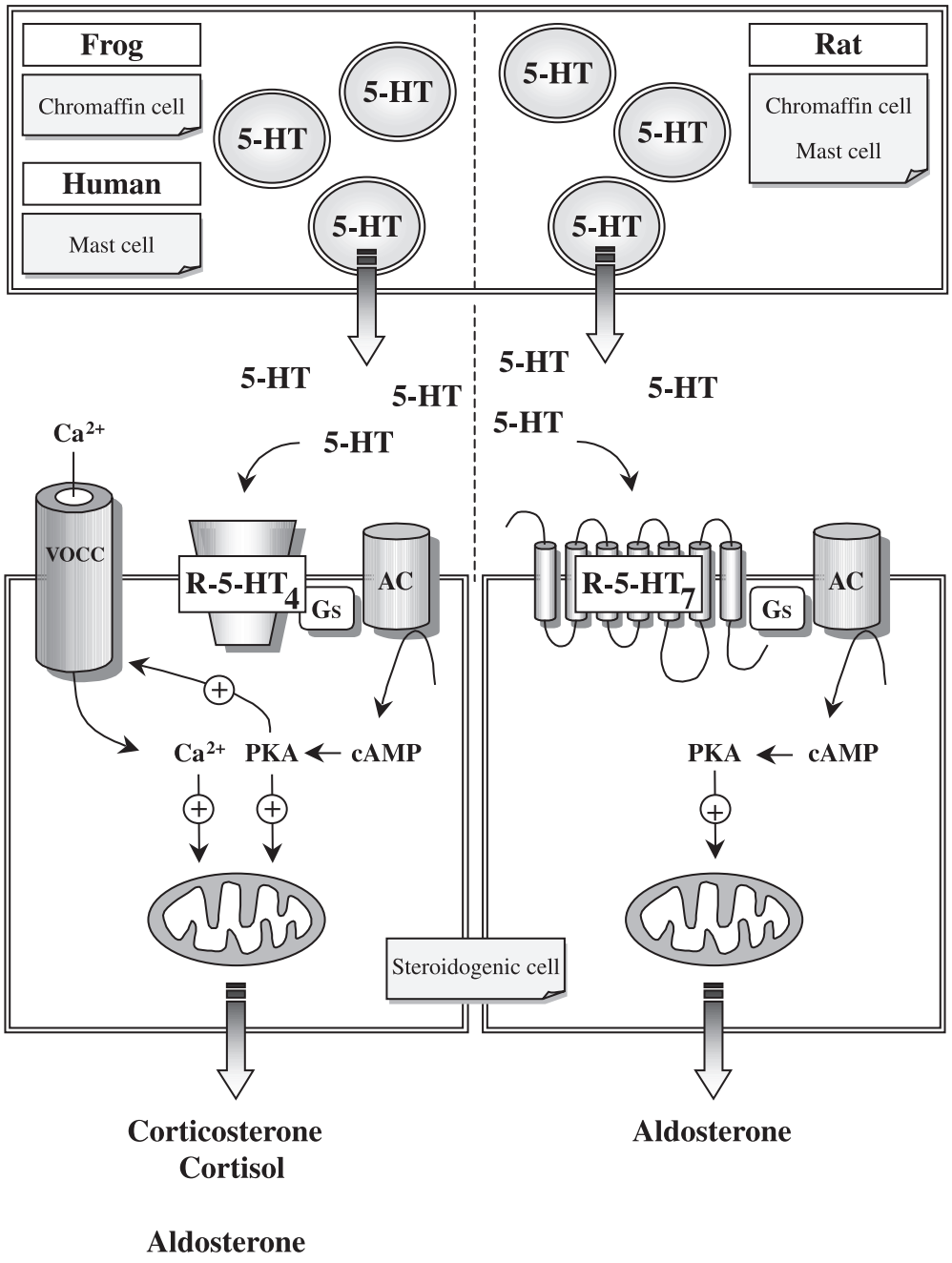
cAMP formation is mimicked by the 5-HT_4 receptor agonist zacopride in frog (Idres et al. 1991) and human adrenal cells (Lefebvre et al. 1992). Since both 5-HT_4 receptors and 5-HT_7 receptors are positively coupled to the adenylyl cyclase/PKA pathway (Table 1), these data provide further evidence for the involvement of these two subtypes in the corticotropic action of 5-HT. In rat, it has been shown that 5-HT stimulates $^{45}Ca^{2+}$ uptake by glomerulosa cells (Davies et al. 1991). Measurement of cytosolic calcium concentration ($[Ca^{2+}]_i$) by the microfluorimetric technique revealed that 5-HT induces a dose-dependent increase in $[Ca^{2+}]_i$ in cultured frog (Contesse et al. 1996) and human adrenocortical cells (Hamel et al. 1996; V. Contesse, C. Hamel, H. Lefebvre, C. Delarue, and H. Vaudry 1996, in preparation). The stimulatory effect of 5-HT on $[Ca^{2+}]_i$ is mimicked by the 5-HT_4 receptor agonist zacopride (Fig. 7) and abolished by the selective 5-HT_4 receptor antagonist GR113808 (Contesse et al. 1996). Suppression of calcium in the incubation medium abolishes the increase in $[Ca^{2+}]_i$ associated with 5-HT_4 receptor activation in frog (Contesse et al. 1996) and human adrenocortical cells (Hamel et al. 1996), indicating that 5-HT causes calcium influx through the plasma membrane. The type of calcium channel coupled to 5-HT_4 receptor activation has been characterized in frog adrenocortical cells (Contesse et al. 1996). The increase in $[Ca^{2+}]_i$ evoked by zacopride is completely abolished by the T-type calcium channel blocker pimozide (Fig. 7A) but is not affected by the L-type channel blocker nifedipine nor by the N-type channel blocker ω-conotoxin GVIA, indicating that the 5-HT-induced calcium influx can be accounted for by activation of T-type calcium channels (Contesse et al. 1996).

Since, in adrenocortical cells, activation of 5-HT_4 receptors is associated with an increase of both cAMP formation and $[Ca^{2+}]_i$, the question arises as to the sequence of these intracellular events. The effect of zacopride on $[Ca^{2+}]_i$ in frog adrenocortical cells is potentiated by the phophodiesterase inhibitor IBMX and markedly attenuated in the presence of the PKA inhibitor Rp-cAMPS (Contesse et al. 1996), indicating that calcium influx is secondary to activation of the cAMP/PKA pathway. In addition, the stimulatory effect of 5-HT_4 receptor agonists on corticosteroid secretion by perifused frog adrenal slices (Contesse et al. 1996) or cultured human adrenocortical cells (V. Contesse, S. Lenglet, H. Lefebvre, and H. Vaudry 1998, unpublished data) is significantly reduced in the presence of the PKA inhibitors Rp-cAMPS or H89, or after suppression of calcium in the incubation medium. These data indicate that both the activation of PKA and the rise in $[Ca^{2+}]_i$ evoked by 5-HT receptor stimulation contribute to the stimulatory effect of 5-HT on corticosteroid secretion.

Physiological and physiopathological relevance of the intra-adrenal serotonergic control of steroid secretion

Intravenous injection of 5-HT or oral administration of the 5-HT precursors tryptophan or 5-HTP to normal volunteers causes a significant increase in plasma corticosteroid levels (Modlinger et al. 1979; Mantero et al. 1982; Shenker et al. 1985*b*). However, the fact that 5-HT exerts a stimulatory effect at different levels of the HPA axis as well as on renin secretion (see above) raises the question as to the site of action of 5-HT or its precursors. To answer this question, the effect of 5-HT_4 receptor agonists on corticosteroid secretion

Fig. 9. Schematic representation summarizing the localization, effect, and mechanism of action of serotonin (5-HT) in frog, rat, and human adrenal glands. 5-HT is produced by chromaffin cells in frogs, mast cells in humans, and chromaffin cells and (or) mast cells in rats. Therefore, in all 3 species, 5-HT can be released in the vicinity of steroidogenic cells. Activation of the 5-HT_4 receptor in frog or human steroidogenic cells increases adenylyl cyclase (AC) activity, which in turn stimulates calcium influx through voltage-operated calcium channels (VOCC). Both the increase in cAMP formation and the $[Ca^{2+}]_i$ rise contribute to stimulation of corticosterone/cortisol and aldosterone secretion. In rat glomerulosa cells, activation of the 5-HT_7 receptor increases AC activity and stimulates aldosterone secretion through activation of the protein kinase A (PKA) pathway.



has been investigated in healthy volunteers pretreated with dexamethasone. Oral administration of a single dose of cisapride (10 mg) or zacopride (0.4 mg) induces a robust increase in plasma aldosterone levels, but does not affect renin (Fig. 8A), potassium, ACTH, nor cortisol concentration (Lefebvre et al. 1993, 1995). These data indicate that the stimulatory effect of 5-HT_4 receptor agonists on aldosterone secretion cannot be accounted for by activation of the hypothalamo-pituitary complex or the RAS, nor by elevation of kalemia, but can be likely ascribed to a direct action on the adrenal cortex (Fig. 8B).

The observation that ACTH and AII can exert homologous and heterologous modulation of ACTH receptors (Lebrethon et al. 1994) suggests that ACTH and (or) AII could also modulate the expression of adrenal serotonergic receptors. To address this issue, the effect of cisapride has

been studied in patients with suppressed plasma ACTH, i.e., corticotropic insufficiency (CI) and in patients with suppressed renin-AII activity, i.e., primary hyperaldosteronism (PH). In PH patients, the abnormal zona glomerulosa tissue (adenoma or bilateral adrenocortical hyperplasia) secretes large amounts of aldosterone, which in turn increases sodium retention, extracellular fluid volume, and causes hypertension. Consequently, in these PH patients, the renin-AII activity is suppressed (Weinberger et al. 1979). In both CI and PH patients, the 5-HT_4 receptor agonist cisapride increases plasma aldosterone in very much the same manner as in healthy volunteers (Lefebvre et al. 1997). These observations suggest that prolonged suppression of ACTH or AII does not affect the sensitivity of glomerulosa cells to 5-HT_4 receptor agonists. These data also indicate that hyperplasic and adenomatous glomerulosa tissue, like normal glomerulosa cells, express a functional 5-HT_4 receptor. In agreement with this finding, it has been found that 5-HT stimulates aldosterone secretion in vitro from aldosterone-producing adenoma cells (Shenker et al. 1985*a*).

Several clinical studies indicate that aldosterone secretion is partially independent from the RAS, suggesting that the secretory activity of glomerulosa cells may be regulated by intra-adrenal factors such as 5-HT. Therefore, 5-HT may also contribute to the pathogeny of aldosterone overproduction. To test this hypothesis, the effect of cisapride has been evaluated in vivo and in vitro in a series of patients with Conn's adenoma, and the occurrence of 5-HT_4 receptor mRNA has been concurrently investigated in the adenomatous tissues (Lefebvre et al. 1999). In all patients studied, oral administration of cisapride induced a robust and significant increase in aldosterone levels. The stimulatory effect of cisapride on aldosterone secretion was confirmed in vitro on perifused Conn's adenoma and the secretory response evoked by cisapride was abolished by the 5-HT_4 receptor antagonist GR 113808. RT-PCR amplification using specific primers for the 5-HT_4 receptor demonstrated the occurrence of several isoforms of 5-HT_4 receptor mRNA, supporting the hypothesis that serotonin may be implicated in the overproduction of aldosterone. In addition, the demonstration of the presence of numerous mast-like cells in aldosteronoma tissue (Aiba et al. 1985) suggests that 5-HT, released by intratumoral mast cells, may play a role in the pathophysiology of these tumors by exerting tonic paracrine stimulation of aldosterone secretion.

5-HT may also play a significant role in the physiopathology of cortisol secretion disorders. ACTH-independent Cushing's syndrome is usually caused by cortisol-secreting adrenal adenomas, carcinomas, or bilateral hyperplasia. It has been occasionally demonstrated that the excess secretion of cortisol is a consequence of an abnormal (ectopic) expression and (or) eutopic overexpression of receptors for gastric inhibitory polypeptide, vasopressin, catecholamines, or interleukin-1 (Lacroix et al. 1998). Recently, the case of a patient with an ACTH-independent Cushing's syndrome was reported in which cortisol secretion was stimulated by luteinizing hormone (LH) and by cisapride (Lacroix et al. 1999). In this patient, suppression of endogenous LH by the GnRH receptor agonist leuprolide led to a complete reversal of the Cushing's syndrome. However, the fact that the patient did not develop cortisol insufficiency suggests that 5-HT, acting through overexpressed eutopic 5-HT_4 receptors, exerted a tonique stimulation of the fasciculata cells (Lacroix et al. 1999). Identification of overexpression of adrenal 5-HT_4 receptors in certain types of Cushing's syndrome opens novel pharmacological opportunities for the treatment of hypercortisolism by means of specific 5-HT_4 receptor antagonists.

Conclusion

The data summarized in the present review underline the multiple facets of the effects of 5-HT in the regulation of corticosteroid secretion. Central serotonergic neurons have been shown to stimulate CRF and ACTH secretion both at the hypothalamic and pituitary levels, through activation of 5-HT_{1A} and 5-$HT_{2A/2C}$ receptors. In particular, 5-HT plays a crucial role in the regulation of stress-induced stimulation of CRF neurons (Saphier et al. 1995) and in the circadian rhythmicity of plasma ACTH (Szafarczyk et al. 1983). There is also strong evidence that centrally acting 5-HT is involved in the derangements of the HPA axis in depressed patients (Delbende et al. 1992). The observation that 5-HT can activate the renin-angiotensin system through activation of 5-HT_2 receptors also suggests that 5-HT may play a significant role in the regulation of mineralocorticoid secretion in normal and pathological conditions.

At the adrenal level, it is now well established that various intra-adrenal factors contribute to adapt the activity of corticosteroid-secreting cells to different physiological situations. 5-HT that is localized within the adrenal gland fulfills all the criteria of a paracrine corticotropic factor. Indeed, 5-HT has been shown to stimulate the secretory activity of adrenocortical cells through activation of the 5-HT_4 or 5-HT_7 receptor subtypes positively coupled to adenylyl cyclase. The fact that 5-HT is produced by chromaffin cells and (or) intra-adrenal mast cells suggests that 5-HT may play a pivotal role in the modulation of corticosteroid secretion during acute stress, inflammation, and (or) allergic response. 5-HT may also be involved in the pathogenesis of adrenal diseases. The identification of functional 5-HT_4 receptors in Conn's adenoma strongly suggests that a serotonergic mechanism may contribute to abnormal aldosterone production. Eutopic overexpression of adrenal 5-HT_4 receptors may also play a role in certain cases of ACTH-independent Cushing's syndrome.

While the effect and the mechanism of action of 5-HT on adrenocortical cells are now largely elucidated, the physiological and pathophysiological relevance of the serotonergic control of the adrenal cortex remains to be established, notably in humans. In this respect, clinically safe 5-HT_4 receptor antagonists are urgently needed to ascertain that 5-HT actually acts as a paracrine factor regulating corticosteroid secretion. The development of such compounds may also prove to be extremely useful for the treatment of certain forms of adrenal disorders.

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