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Invited Review

Paracrine control of steroid hormone secretion by chromaffin cells in the adrenal gland of lower vertebrates

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Summary. The adrenal glands of lower vertebrates display a notable intermingling between steroidogenic and chromaffin tissues, which increases from *Pisces* to *Aves*. As in mammals, adrenal chromaffin cells contain and release, in addition to catecholamines, serotonin and several peptides, which may affect the secretory activity of steroidogenic cells in a paracrine manner. Stimulatory molecules include serotonin, arginine-vasotocin, tachykinins, vasoactive intestinal peptide, pituitary adenylate cyclase-activating peptide and calcitonin gene-related peptide; inhibitory molecules are dopamine, somatotropic hormone-release inhibiting hormone and galanin. Epinephrine and norepinephrine appear to stimulate steroid secretion in *Aves* and to inhibit it in *Pisces*, while their action in *Amphibia* is controversial. Likewise, atrial natriuretic peptide exerts an anti-secretagogue action in *Amphibia* and a marked secretagogue effect in *Pisces* and *Aves*. The effects of opioids (enkephalins and endorphins) have scarcely been investigated and the findings obtained are highly questionable. Compared with the amazing mass of investigations carried out in mammals, studies in lower vertebrates are few, and in large part performed in *Amphibia* and *Aves*. It appears that much further work has to be done by comparative endocrinologists to fully clarify the physiological relevance of the functional interactions between chromaffin and steroidogenic cells in the adrenal glands of lower vertebrates.

Key words: Adrenal gland, Steroid secretion, Paracrine interactions, Chromaffin cells, Lower vertebrates

I. Introduction

The intimate morphological interrelationships between cortical and medullary chromaffin cells in the mammalian adrenal glands is today recognized to possess a great functional relevance. A large body of evidence indicates that not only steroid hormones may induce the activity of the enzymes involved in epinephrine synthesis (for review, see Cryer, 1992), but that also chromaffin cells, by secreting a lot of regulatory molecules, may modulate the function of cortical steroidogenic cells in a paracrine manner (for review, see Nussdorfer, 1996).

The peculiar arrangement of interrenal steroidogenic and chromaffin tissues in the adrenal glands of lower vertebrates may surely favour the paracrine interactions between the two cell types. In fact, the adrenal glands of lower vertebrates display a marked intermingling between steroidogenic and chromaffin cells, whose complexity increases from *Pisces* to *Aves* (for review, see Deane, 1962). Findings are available showing that adrenal chromaffin cells of lower vertebrates secrete, as those of mammals, not only catecholamines, but also serotonin and several regulatory peptides, which variously affect the function of steroidogenic cells.

As far as we are aware, no comprehensive review articles have been published on the possible paracrine interactions in the adrenal glands of non-mammalian vertebrates. Lesouhaitier et al. (1995) focused their attention on the neuroendocrine communications in the adrenals of *Anura*, and Hanke and Kloas (1995) provided a survey of the general mechanisms involved in the regulation of secretion of adrenal glands in *Pisces* and *Amphibia*.

In the following sections of this review, we shall summarize and discuss the morphological and functional background of the cortico-medullary interactions in the lower-vertebrate adrenals, and then the possible involvement of monoamines (catecholamines and serotonin) and other regulatory peptides secreted by chromaffin cells in the fine tuning of the secretory activity of steroidogenic cells.

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II. The morphological and functional background of the paracrine interactions between cortical and medullary cells in the adrenal glands of lower vertebrates

A. The morphological background

Several review articles are available dealing with the structure of the adrenal glands of lower vertebrates (Deane, 1962; Chester-Jones, 1976; Nussdorfer, 1986; Chester-Jones et al., 1987).

In *Cyclostomata*, presumptive steroidogenic cells are located in the pronephros and in the dorsal-vessel region; steroidogenic and chromaffin tissues are not mixed. In *Chondrichthyes* and *Osteichthyes*, the two tissues are in close contact, and adrenals lie near or inside the opisthonephroi and along the wall of the cardinal veins. In the *Amphibia*, a very conspicuous intermingling of steroidogenic and chromaffin cells occurs (Fig. 1A); adrenal complex in *Urodela* is discontinuous and located along the ventral aspect of mesonephroi, while in *Anura* it is aggregated into a pair of bodies frequently lying within the capsule of mesonephroi. In *Reptilia*, the adrenal complex consists of a pair of round or elongated bodies, located near the kidneys (*Chelonia* and *Crocodilia*), or dorsomedially to the cephalic pole of the gonads (*Lacertilia* and *Ophidia*); steroidogenic and chromaffin tissues are notably intermingled. In *Aves*, adrenal glands may be an impar median structure or two separate organs always in close contact with each other (interrenal gland); the gland is enclosed by a loose connective capsule, and contains radially arranged cords of steroidogenic cells among which clusters of chromaffin cells are mingled (Fig. 1B). As clearly results from the above survey, in the adrenal glands of lower vertebrates, with the exception of fishes, chromaffin cells may release their secretory products near steroidogenic cells, thereby affecting them in a paracrine manner.

A great deal of evidence indicates that the adrenal glands of lower vertebrates are richly innervated. In addition to catecholaminergic and cholinergic fibers, several peptidergic fibers have been identified by immunocytochemistry (see Section IV). In mammals, nerve fibers reaching the adrenal 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 has its cell body in the adrenal medulla (for review, see Vinson et al., 1994). Vizi et al. (1992, 1993) demonstrated the presence in the adrenal cortex of numerous varicose axon terminals ending free in close proximity to parenchymal cells, and releasing catecholamines in response to axonal firing. These features, which are reminiscent of the hypothalamo-pituitary neurosecretory process, strongly support the possibility of a paracrine non-synaptic modulatory role of catecholamine and other neuropeptides on adreno-cortical cells. Whether this mechanism is operative in adrenals of lower vertebrates is not known at present. However, evidence is available that at least in the frog splanchnic and vagus nerve destruction does not affect the morphology of some intradrenal peptidergic fibers, which would indicate their intraglandular origin.

B. Possible mechanisms involved in paracrine interactions

Theoretically, a regulatory molecule secreted by chromaffin cells or released by intra-adrenal neurons may act through a direct or an indirect mechanism. It can directly modulate the secretion of steroidogenic cells by binding to specific receptors located on their plasma membrane; if such a mechanism is operative, the regulatory molecule is able to affect *in vitro* steroid secretion of dispersed and purified steroidogenic cells. Alternatively, a regulatory molecule may act indirectly by eliciting, in a paracrine or autocrine manner, the release by chromaffin cells of other regulatory

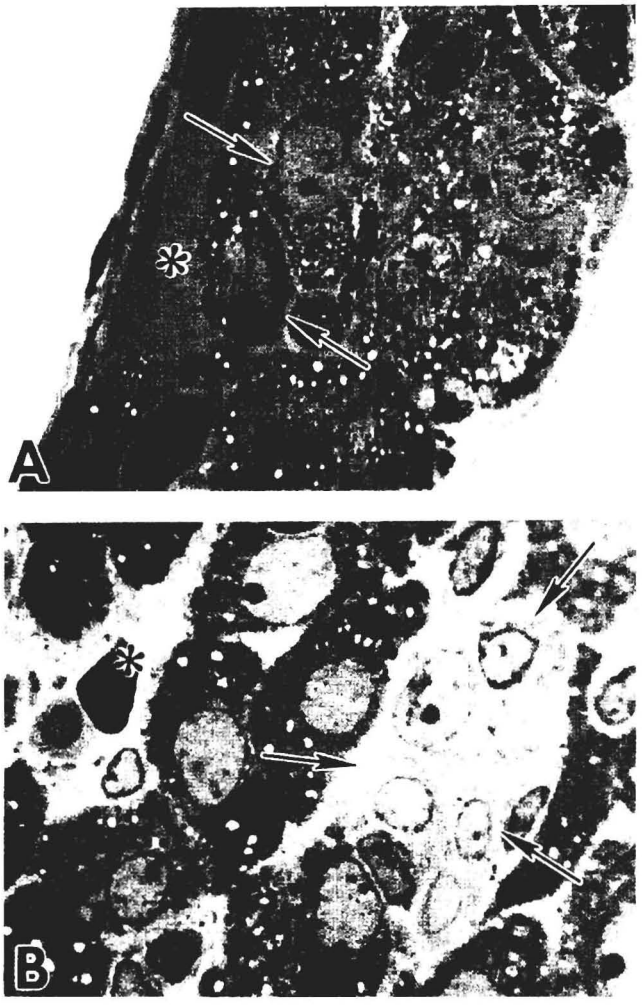

Fig. 1. Light micrographs of toluidine blue-stained 0.5 -thick epon sections of the adrenal gland of *Rana temporaria* (**A**) and *Gallus gallus domesticus* (**B**). The arrows indicate chromaffin cells intermingled with steroid secreting cells, which in the frog display a cytoplasm filled with lipid droplets. Asterisk: capillary lumen. x 1,250

molecules, which in turn may control the function of steroidogenic cells; if this mechanism is at play, the regulatory molecule can affect the function of steroidogenic cells only when the structural integrity of adrenal tissue is preserved (e.g. static or dynamic incubation of adrenal quarters).

Obviously, an intra-adrenal regulatory molecule can be assumed to exert a paracrine control of the function of adrenocortical cells of any physiological relevance only when it meets the following basic conditions. The content of the molecule must be sufficiently elevated in order to reach, upon appropriate stimulation of its release, a local concentration above its minimal effective one *in vitro*. Mazzocchi et al. (1993) provided a method to calculate this parameter, based on the demonstration that in the fresh mammalian adrenal tissue there is a strict correspondence between weight and volume (specific gravity, 1.039), and that the interstitial space (as calculated by morphometry) is about 3% of the total volume. Thus, if e.g. the adrenal content of a molecule is 100 pmol/g, its one-third release will produce a local concentration of 10^{-6} M. In fact, 30 pmol of the molecule will be released into about 30 l of interstitial space (3% of 1 ml, that is the volume of 1 g of fresh adrenal tissue), producing a local concentration of about 1 pmol/μl or 1 μmol/l. This method can be reasonably assumed to be valid also for non-mammalian adrenals.

III. The involvement of monoamines in the control of the functions of adrenal steroidogenic cells in lower vertebrates

A. Catecholamines

Nussdorfer (1996) has recently surveyed the investigations dealing with the effects of catecholamines (epinephrine, norepinephrine and dopamine) on the secretion of mammalian adrenocortical cells. To summarize: (i) epinephrine and norepinephrine, acting via adenylate cyclase-coupled ß-receptors, directly stimulate mineralocorticoid and probably glucocorticoid secretion of adrenocortical cells; and (ii) dopamine, acting via DA1 and DA2 receptors positively and negatively coupled with adenylate cyclase, exerts a prevalently inhibitory effect on the zona glomerulosa and aldosterone secretion.

1. Epinephrine and norepinephrine

Adrenal chromaffin cells of lower vertebrates synthesize and release catecholamines, epinephrine and norepinephrine being more abundant than dopamine (for review, see Hanke and Kloas, 1995; Lesouhaitier et al., 1995). However, the direct effect of the first two catecholamines on the secretion of adrenocortical cells has not been extensively investigated. Indirect findings indicate that in the teleostean fish *Cyprinus carpio*, epinephrine and norepinephrine inhibit cortisol production by adrenocortical cells (Gfell et al., 1995). Accordingly, in the frog *Rana ridibunda*, these catecholamines were found to exert a weak suppressive effect on corticosterone and aldosterone release by perifused adrenal tissue, their half-maximal effective concentrations being about $10^{-5}/10^{-4}$ M for corticosterone and 10^{-3} M for aldosterone (Morra et al., 1990). In contrast, Hanke and Kloas (1994, 1995) reported that in the urodele *Xenopus laevis* 10^{-6} M epinephrine reduced the stimulating action of ACTH on adrenal corticosterone secretion and enhanced that on aldosterone production. Epinephrine and the ß-adrenoceptor agonist isoprenaline, but not the ß-adrenoceptor agonist guanabenz, were found to raise aldosterone and corticosterone production by dispersed fowl interrenal cells; minimal and maximal effective concentrations being 10^{-8} M and 10^{-6} M, respectively (Mazzocchi et al., 1997b). Accordingly, these last investigators showed that the ß-adrenoceptor antagonist *l*-alprenolol, but not the α-receptor antagonist phentolamine, suppresses secretory responses to maximal effective concentrations of both epinephrine and isoprenaline. Mazzocchi and associates concluded that epinephrine enhances adrenal steroid secretion in the fowl, acting, as in mammals, via the activation of the ß-adrenoceptor subtype.

2. Dopamine

The studies dealing with the effect of dopamine give more consistent results. Morra et al. (1990, 1992) showed that dopamine causes a clear-cut inhibition of the basal release of both corticosterone and aldosterone by perifused frog adrenals; half-maximal effective concentration being about 10^{-6} M. The secretagogue effect of 10^{-9} M ACTH is not affected by dopamine, while that of 10^{-7} M angiotensin-II (ANG-II) is impaired (Morra et al., 1990). Using collagenase-dispersed adrenocortical cells, Morra and co-workers (1990) observed that after the second pulse dopamine induces a transient stimulatory effect. The infusion of repeated pulses of a DA1 receptor agonist mimiks the stimulating effect of dopamine, while the administration of a DA2 receptor agonist inhibits steroid secretion; neither dopamine nor the two dopamine-receptor agonists affect cyclic-AMP formation (Morra et al., 1992). The inhibitory effect of dopamine was found to be associated with a marked reduction in the release of both prostaglandin-E2 and 6-keto-prostaglandin-F1α by frog adrenal tissue (Morra et al., 1989). Morra et al. (1991) demonstrated that (i) a short pulse of dopamine evokes a biphasic effect on phosphatidylinositol breakdown, a transient increase being followed by a sustained inhibition; and (ii) the DA2-receptor agonist apomorphin elicits a marked inhibition of inositol phosphate production. Collectively, these findings suggest that dopamine controls steroid production by amphibian adrenocortical cells acting via the DA1- and DA2-receptor subtypes, which, at variance with those of mammals (see above), are positively and negatively coupled to both phospholipase A2 and phospholipase C. It is likely that under physiological conditions the inhibitory effect prevails.

B. Serotonin

Compelling evidence indicates that serotonin and serotoninergic-fibers are contained in the mammalian adrenals, where they directly stimulate mineralocorticoid and glucocorticoid secretion, acting via phospholipase C-coupled 5-HT_2 and adenylate cyclase-coupled 5-HT_4 receptors (for review, see Nussdorfer, 1996).

The occurrence of serotonin has been biochemically and immunocytochemically demonstrated in adrenal chromaffin cells of anurans (Delarue et al., 1988a,b). Serotonin is co-stored with epinephrine and is synthesized by chromaffin cells from L-tryptophan (Delarue et al., 1992).

The secreatagogue effect of serotonin on adrenocortical cells of lower vertebrates has been investigated almost exclusively in *Rana ridibunda*. Serotonin was found to raise basal corticosterone and aldosterone secretion of both perifused adrenal tissue and collagenase-dispersed adrenocortical cells in a concentration-dependent manner, minimal and maximal effective concentrations being 10^{-7} M and 10^{-6} M, respectively (Delarue et al., 1988a,b; Idres et al., 1989). Indomethacin, a cyclooxygenase inhibitor, and dantrolene, a blocker of intracellular calcium mobilization, do not impair the secretagogue action of serotonin (Delarue et al., 1988a,b), which therefore appears to be independent of phospholipase A2 and phospholipase C activation. Idres et al. (1991) and Contesse et al. (1994), using specific agonists and antagonists of serotonin-receptor subtypes 5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4, demonstrated that the adrenocortical secretagogue effect of serotonin is linked to the activation of the 5-HT_4 receptor. Zacopride, a benzamine derivative which is an agonist of this receptor subtype, evokes a concentration-related stimulation of steroid secretion, and, like serotonin, enhances cyclic AMP release by perifused adrenal tissue (Idres et al., 1991), as well as raises the cytosolic calcium concentration ($[Ca^{2+}]_i$) in cultured cells through the activation of a T-type calcium channel (Contesse et al., 1996). In conclusion, in contrast with mammals (see above), serotonin appears to enhance steroid secretion of frog adrenocortical cells by stimulating, exclusively via 5-HT_4 receptors, both adenylate cyclase and Ca^{2+} influx.

Before concluding, it must be recalled that serotonin was not shown to affect corticosterone production by chick adrenal quarters incubated *in vitro* (Cheung et al., 1987), thereby stressing that the effect of this monoamine on adrenocortical cells of lower-vertebrate species other than frog requires further investigation.

IV. The involvement of regulatory peptides in the control of the functions of adrenal steroidogenic cells of lower vertebrates

A. Hypothalamic peptides

Several hypothalamic peptides have been found in mammalian adrenal medulla: corticotropin-releasing hormone (CRH); arginine-vasopressin (AVP); oxytocin; somatotropic hormone-release inhibiting hormone (SRIF); and thyrotropin-releasing hormone (TRH) (for review, see Nussdorfer, 1996). The possible functions of these intramedullary peptides have been reviewed by Nussdorfer (1996), and can be summarized as follows. CRH appears to enhance the release by chromaffin cells of ACTH, the intramedullary CRH-ACTH system being mainly involved in the maintenance and stimulation of the secretion and growth of adrenal zonae fasciculata and reticularis. AVP, acting via phospholipase C-coupled V1 receptors, plays an important and direct role in the maintenance and stimulation of zona glomerulosa growth and aldosterone secretion. Oxytocin directly stimulates basal steroid secretion, but it inhibits ACTH-enhanced glucocorticoid secretion in rats. SRIF, acting via specific receptors that interfere with the intracellular mechanisms transducing ANG-II secretagogue signal, exerts a potent and specific inhibitory action on the zona glomerulosa. TRH seems to directly inhibit the late steps of glucocorticoid synthesis in the rat.

1. Arginine-vasotocin (AVT)

AVT, the amphibian counterpart of mammalian AVP, has been immunocytochemically and biochemically detected in the frog adrenal tissue (Larcher et al., 1989). AVT-immunoreactivity (ir) was found in chromaffin granules of both norepinephrine and epinephrine cells. Radioimmune assay (RIA) showed that adrenal content of AVT is about 2.6 pmol/g, which could produce a local concentration of about $2\text{-}3\times10^{-8}$ M (see Section II B). Mesotocin, the *amphibian* counterpart of mammalian oxytocin, was not cytochemically detected.

Larcher et al. (1989) showed that AVT concentration dependently raises aldosterone and corticosterone release by perifused frog adrenals, minimal and maximal effective concentrations being 10^{-10} M and 5×10^{-9} M, respectively; maximum rise is 4.8-fold for aldosterone and 3.4-fold for corticosterone. Kloas and Hanke (1990) obtained similar findings in *Xenopus laevis*, but minimal and maximal effective concentrations are 2×10^{-10} M and 2×10^{-8} M, and the maximum secretory response is far more elevated (47-fold and 26-fold rises for aldosterone and corticosterone, respectively). AVP, oxytocin and mesotocin were found to be about 400, 100 and 1500 times less potent than AVT (Larcher et al., 1990).

Further studies threw light on the mechanism underlying AVT secretagogue action in the frog. It has been observed that the V2-receptor antagonist [d(CH_2)$_5$D,Phe^2,Ile^4,Ala^9-NH_2]-AVP (10^{-6} M), but not the V1-receptor antagonist 1-(1-mercapto-4-phenyl-cyclohexane-acetic acid)-AVP, suppresses the secretagogue effect of 5×10^{-10} M AVT (Larcher et al., 1992a). These authors also showed that AVT does not change either cyclic AMP or prostaglandin-E2 production by the perifused frog adrenal tissue; accordingly, indomethacin, although per se decreasing basal secretion, does not significantly affect AVT-stimulated aldosterone and corticosterone production,

thereby confirming that prostaglandins are not involved in the secretagogue effect of AVT. In contrast, Larcher et al. (1992a) clearly demonstrated that AVT markedly raised inositolphosphate production, which suggests that this peptide acts via V2 receptors positively coupled to phospholipase C. This contention easily explains why AVT exerts additive effect only when combined with adrenocorticotropic factors stimulating adenylate cyclase (e.g. ACTH and serotonin), and why its action is inhibited by dopamine, which depresses phospholipase C activity (see Section III B) (Larcher et al., 1992b). Larcher et al. (1992c) showed that AVT causes an immediate increase in $[Ca^{2+}]_i$ in frog adrenocortical cells, followed by a sustained response. The V2-receptor antagonists block this effect of AVT, which cannot be observed in a calcium-deprived medium; the dihydropyridine Ca^{2+}-channel blocker nifedipine does not affect calcium response to AVT. On these grounds, Larcher and associates advanced the hypothesis that AVT exerts a two-fold action on $[Ca^{2+}]_i$ in frog adrenocortical cells: the initial rise is probably due to the immediate mobilization of intracellular calcium stores mediated by inositol triphosphate-gated channels, while the sustained increase has to be ascribed to the opening of nifedipine-insensitive plasma membrane channels.

2. SRIF

SRIF (up to 10^{-5} M) was not found to elicit any suppressory action on either basal or ANG-II-stimulated aldosterone release by perifused frog adrenals (Delarue et al., 1984). On the contrary, Cheung et al. (1988) observed that the *in vivo* administration to 1-day-old domestic cockerels of an anti-SRIF serum raised basal levels of circulating corticosterone, thereby making it likely that endogenous SRIF plays a role in the regulation of adrenocortical function in fowls. More recently, Mazzocchi et al. (1997a) showed that SRIF concentration dependently inhibits aldosterone and corticosterone response of dispersed turkey (*Meleagris gallopavo*) adrenocortical cells to 10^{-9} M ANG-II. Minimal and maximal effective concentrations are 10^{-8} M and 10^{-6} M, and elicit about 20% and 40% inhibition, respectively. As in mammals (see above), basal secretion is not affected, and the inhibitory action of SRIF is abolished by 10^{-6} M cyclo(7-aminoheptanonyl-Phe-D-Trp-Lys-Thr[Bzl]), which suggests the involvement of an SRIF receptor-mediated mechanism. SRIF was not found to affect steroid secretion of fowl adrenocortical cells, which, however, according to Kocsis et al. (1995a,b), are unresponsive to ANG-II.

Before concluding, it must be recalled that urotensin-II, a cyclic dodecapeptide originally isolated from the fish urophysis (the counterpart of the mammalian neurohypophysis) and then from the frog brain (Conlon et al., 1992a,b), was found to enhance steroid secretion of adrenocortical cells of the dogfish (Conlon et al., 1992b) and amphibians (Hanke and Kloas, 1994). Urotensin-II exhibits structural similarities with mammalian SRIF and appears to interact with SRIF receptors, whose activation would therefore mediate the opposite effect in mammal and lower-vertebrate adrenocortical cells (see above). However, Feuilloley et al. (1994) did not observe any appreciable effect of urotensin-II (from 10^{-10} to 10^{-6} M) on either basal or agonist-stimulated steroid release by perifused adrenals of *Rana ridibunda*.

B. Opioid peptides

Evidence is available that the three members of this family of peptides (enkephalins, endorphins and dynorphins) are synthesized and secreted by chromaffin cells of the mammalian adrenal medulla, and are able to variously affect the secretory activity of the cortex in a paracrine manner (for review, see Nussdorfer, 1996). To summarize, enkephalins exert a direct stimulatory action on both zona glomerulosa and zona fasciculata cells, while ß-endorphin appears to directly inhibit glucocorticoid secretion, at least under physiological conditions. Dynorphins suppress steroidogenesis in the rat, but the physiological relevance of this effect is questionable.

As far as we are aware, only enkephalins have been detected in adrenal chromaffin cells of lower vertebrates. Kondo and Jui (1984) and Reinecke et al. (1992) provided immunocytochemical evidence of the presence of enkephalin-ir in both chromaffin cells and nerve fibers of the frog adrenals. According to Leboulenger et al. (1983a,b), both met- and leu-enkephalins are present in about 40% of adrenomedullary cells of *Rana ridibunda* and are co-localized with vasoactive intestinal peptide in the chromaffin granules. The investigations on the direct effect of opioid peptides on adrenal steroidogenesis in lower vertebrates are very scarce and their rather controversial results may be summarized as follows.

1. Enkephalins

Leboulenger et al. (1983a) did not observe any appreciable effect of 10^{-5} M met-enkephalin and leu-enkephalin on either basal or ACTH-stimulated corticosterone production by amphibian adrenocortical cells. Conversely, Hanke and Kloas (1994) reported a clear-cut stimulatory action of 10^{-6} M met-enkephalin on both corticosterone and aldosterone responses to ACTH.

2. Endorphins

Hanke and Kloas (1994) found that 10^{-7} M α-endorphin enhanced ACTH-stimulated corticosterone and aldosterone secretion by amphibian adrenals, while Zerani and Gobbetti (1991,1992) described a net inhibitory action of ß-endorphin (about 4×10^{-8} M) on corticosterone and cortisol production of adrenocortical cells of *Rana esculenta* and *Triturus carnifex*. These last investigators also showed that naloxone blocked ß-endorphin secretagogue effect, and proposed that this peptide acts via specific μ-receptors.

C. Tachykinins

Tachykinins are a group of regulatory peptides belonging to the neuromedin family (for review, see Malendowicz and Markowska, 1994). Mammalian tachykinins include neurokinin A, neurokinin B, substance P (structurally related to neurokinin A and encoded by the same gene) and neuropeptide K (the N-terminally extended form of neurokinin A) (for review, see Maggio, 1988). Tachykinin-ir has been cytochemically and biochemically demonstrated in chromaffin cells and nerve fibers of adrenal glands of several mammalian species (for review, see Nussdorfer, 1996). According to Nussdorfer (1996), the direct effects of tachykinins on mammalian adrenocortical cells are probably pharmacological in nature. Substance P is likely to play a physiological role in the maintenance and stimulation of secretion and growth of rat zona glomerulosa, through an indirect mechanism involving the NK1 receptor-mediated enhancement of catecholamine release by medullary chromaffin cells. Neuropeptide K appears to indirectly stimulate glucocorticoid secretion of rat adrenal quarters by activating the CRH-ACTH system located in the medulla.

Frog interrenals have been found to contain three tachykinins: substance P, ranakinin (a peptide related to substance P); and [Leu^3,Ile^7]-neurokinin A (Leboulenger et al., 1993; Kodjo et al., 1995a). Immunocytochemistry showed the presence of tachykinin-positive nerve fibers preferentially apposed onto chromaffin cells. RIA demonstrated an intra-adrenal content of substance P and neurokinin A of about 8 and 4.6 pmol/g, which could give rise to local concentrations ranging from 5 to $9x10^{-8}$ M (see Section II B). Interestingly, the bilateral transection of splanchnic and vagus nerves does not apparently alter intra-adrenal tachykinin-ir, a finding ruling out the possibility of the extraglandular origin of tachykinin-positive fibers.

The effects of tachykinins on adrenal steroidogenesis have been studied exclusively in *Amphibia*. According to Leboulenger et al. (1993), substance P concentration dependently raises corticosterone and aldosterone release by perifused adrenal tissue of *Rana ridibunda*; minimal and maximal effective concentrations are 10^{-7} M and 10^{-5} M and elicit rises of 30-40% and 150-180%, respectively. In keeping with these findings, Hanke and Kloas (1994) demonstrated that substance P (from $5x10^{-7}$ to 10^{-6} M) enhances both corticosterone and aldosterone release from perifused adrenals of *Xenopus laevis*. All the other amphibian tachykinins, at a concentration of 10^{-5} M, were found to evoke increases in corticosterone output ranging from 150 to 200% (Leboulenger et al., 1993). These investigators also observed that substance P elicits the release of prostaglandin E2 and prostacyclin by frog adrenals, and that this response precedes by 10-15 min the rise in corticosteroid output. Accordingly, indomethacin abolishes the secretagogue effect of substance P, which indicates that the mechanism underlying this action of the tachykinin involves the activation of the arachidonic acid cascade.

More recently, Kodjo et al. (1995a) confirmed that ranakinin enhances steroidogenesis in perifused frog adrenal tissue. However, they showed that ranakinin does not affect basal secretion of dispersed adrenocortical cells, and hypothesized the involvement of an indirect mechanism of action requiring the integrity of adrenal tissue. Autoradiography demonstrated the presence of [^{3}H]-substance P binding sites exclusively in adrenal chromaffin cells, and microfluorimetry evidenced a ranakinin-induced rise in $[Ca^{2+}]_i$ in chromaffin, but not adrenocortical cells. Further investigations (Kodjo et al., 1995b) showed that neither the chelation of calcium in the perifusion medium nor nifedipine and ω-conotoxin alter calcium-response of chromaffin cells to ranakinin; conversely, the incubation of the cells with thapsigargin, an inhibitor of calcium-ATPase activity, abolishes ranakinin effect. Moreover, the phospholipase C antagonist U-73122 and pertuxis toxin completely block calcium response of chromaffin cells to ranakinin. In light of these findings, these authors suggested that (i) ranakinin-induced increase in $[Ca^{2+}]_i$ must be ascribed to mobilization of calcium from intracellular stores; and (ii) ranakinin stimulates frog adrenal chromaffin cells through the activation of phospholipase C via a pertuxin toxin-sensitive G protein.

Further studies were carried out to ascertain which receptor subtype is involved in the mediation of the indirect adrenocortical secretagogue effect of ranakinin (Kodjo et al., 1996). It has been found that the selective NK1-receptor antagonists [D-Pro^4,D-$Trp^{7,9}$]-substance P_{4-11} and CP-96,345 do not affect the corticosteroid stimulatory action of ranakinin, nor the selective NK1 agonist substance P_{6-11} and that the amphiphilic substance P analogue [D-Pro^2,D-Phe^7,D-Trp^9]-substance P enhance *per se* corticosteroid secretion. In contrast, the non-peptidic NK1 antagonist RP67580 and the NK1/NK2-receptor antagonist FK-224 significantly suppress the stimulatory effect of ranakinin on both corticosterone and aldosterone secretion. Kodjo and associates suggested that the effects of tachykinins on the frog adrenals are mediated by a novel type of receptor, which differs from the mammalian NK1 subtype.

D. Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP)

VIP and PACAP are 28- and 38-amino acid-residue peptides, widely distributed in the mammalian tissues. The N-terminal 28-amino acid sequence of PACAP has a 68% identity with VIP, and the two peptides share a number of physiological functions, including neurotransmission, stimulation of pituitary ACTH release, and regulation of vascular tone and gastrointestinal-tract activity (for review, see Fahrenkrug, 1989; Arimura and Shioda, 1995). The effects of VIP and PACAP, which are both synthesized and secreted by mammalian adrenal medulla, on the secretion and growth of the adrenal cortex have been recently reviewed by Nussdorfer

(1996). Intramedullary VIP is involved in the physiological regulation of zona glomerulosa secretion, the aldosterone secretagogue effect being both direct and indirectly mediated by the stimulation of catecholamine release. VIP may also aspecifically activate ACTH receptors located on both outer and inner adrenocortical cells, but under normal conditions this action is masked by circulating ACTH, that tonically activates such receptors. 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; however, the physiological relevance of this last effect of PACAP is very doubtful.

1. VIP

Immunocytochemical studies showed the co-localization of VIP-ir with enkephalin-ir in the chromaffin granules of adrenomedullary cells of *Rana ridibunda*, but no VIP-ergic fibers were observed (Leboulenger et al., 1983a,b).

Leboulenger et al. (1983a) reported that VIP concentration dependently raises both corticosterone and aldosterone release by perifused frog adrenal tissue. Minimal and maximal effective concentrations are 10^{-6} M and 10^{-5} M, and the maximal aldosterone response is much higher than the corticosterone one (2-fold versus 1.4-fold rise). The antimicrotubular agent cytochalasin B impairs both basal and VIP-stimulated steroid secretion of frog adrenals (Leboulenger et al., 1984). However, this finding may be conceivably interpreted as an aspecific effect of cytochalasin B consequent to the disruption of the cytoskeleton, which is well known to play an essential role in adrenocortical steroid synthesis and release (for review, see Nussdorfer, 1986; Hall, 1995; Feuilloley and Vaudry, 1996). Calcium chelation from perifusion medium and indomethacin, although lowering basal steroid-secretion rate, do not significantly alter corticosterone and aldosterone responses to VIP (Leboulenger et al., 1984). Collectively, these last findings would indicate that adrenocortical secretagogue effect of VIP in the frog does not involve either prostaglandin synthesis or calcium. It could be hypothesized that VIP acts by enhancing cyclic AMP production; however, a maximal effective concentration of VIP was found to exert potentiating, more than additive, effects when combined with a maximal effective concentration of serotonin (Leboulenger et al., 1988), which exerts its adrenocortical secretagogue effect by stimulating adenylate cyclase (see Section III B).

2. PACAP

Thick and thin PACAP-positive nerve fibers were detected in adrenal glands of *Rana ridibunda* (Yon et al., 1993, 1994): thick varicose fibers are located among adrenal cells, while thin fibers end in the walls of blood vessels. As demonstrated for tachykinins, bilateral splanchnotomy does not affect PACAP-ir distribution, which suggests the intra-adrenal origin of PACAP-positive fibers. According to Yon et al. (1993) PACAP content in fresh frog adrenals, as measured by RIA, attains 0.65 nmol/g, which could produce a local concentration of about 7×10^{-6} M (see Section II B).

Yon et al. (1993) reported that frog-PACAP38 concentration dependently increases corticosterone and aldosterone release by perifused frog adrenal glands. Minimal and maximal effective concentrations are 10^{-6} M and 10^{-5} M, and induce rises of about 1.5-fold and 2.5-fold, respectively. Dispersed frog adrenocortical cells display similar secretory responses to 10^{-6} M frog-PACAP38 (Yon et al., 1994). These last investigators provided the autoradiographic demonstration that both adrenocortical and chromaffin cells possess binding sites for [^{125}I]-PACAP27; moreover, they showed that frog-PACAP38 concentration dependently raises cyclic AMP release by frog adrenal quarters, and increases $[Ca^{2+}]_i$ in both adrenocortical and chromaffin cells. Yon and associates concluded that PACAP directly stimulates the secretory activity of frog adrenocortical cells by activating adenylate cyclase. However, it must be stressed that the above reviewed findings do not rule out the possibility of an additional indirect effect of PACAP, mediated by chromaffin cells. In keeping with this last contention, Mazzocchi et al. (1997c) observed that PACAP is able to enhance aldosterone and corticosterone production by fowl adrenal slices containing chromaffin cells, but not by dispersed adrenocortical cells, minimal and maximal effective concentrations being 10^{-8} M and 10^{-7} M, respectively. The secretory response to 10^{-7} M PACAP (about 3-fold rise) is suppressed by the PACAP-receptor selective antagonist $PACAP_{6-38}$ at a concentration of 10^{-6} M. Moreover, the PACAP (10^{-7} M)-induced increase in aldosterone and corticosterone secretion is annulled by 10^{-5} M *l*-alprenolol. In light of these findings, Mazzocchi and associates advanced the hypothesis that PACAP stimulates the secretory activity of fowl interrenals indirectly, by eliciting the release by chromaffin cells of catecholamines, which in turn enhance steroid production in a paracrine manner.

E. Galanin

Galanin is a 29-amino acid peptide widely distributed in the mammalian central and peripheral nervous systems (for review, see Bedecs et al., 1995). It is also contained in adrenal medulla and seems to be directly involved in the maintenance and stimulation of the secretion and growth of inner adrenocortical zones (for review, see Nussdorfer, 1996).

Zentel et al. (1990) detected by immunocytochemistry galanin-ir in virtually all adrenomedullary cells of ducks and chickens, but not pigeon adrenals, where in contrast galanin-positive nerve fibers are present. More recently, Gasman et al. (1996) observed the presence of an abundant network of nerve fibers immunoreactive to galanin in the adrenals of *Rana ridibunda*. Furthermore,

they demonstrated by coupled high performance liquid chromatography and RIA analysis, the existence of a single form of galanin exhibiting the same retention time of frog-galanin. Synthetic frog-galanin (from 10^{-9} to $3x10^{-6}$ M) was also found to concentration-dependently inhibit both basal and ACTH-, but not ANG-II-stimulated aldosterone and corticosterone release by perifused frog adrenal slices. These authors concluded that galanin released by nerve terminals in the amphibian adrenals exerts a negative modulation of steroid secretion, probably by inhibiting adenylate cyclase, but not phospholipase C or phospholipase A2 transduction pathway.

F. Calcitonin gene-related peptide (CGRP)

CGRP is a 37-amino acid peptide present in the mammalian central nervous system, as well as in nerve fibers to several peripheral organs (for review, see Owyang and Louie, 1989). CGRP-ir has been demonstrated in both nerve fibers and chromaffin cells of the mammalian adrenal cortex (for review, see Nussdorfer, 1996). According to the survey of Nussdorfer (1996), CGRP exerts a direct inhibitory effect on ANG-II-stimulated aldosterone secretion of zona glomerulosa cells, via a $CGRP_1$ receptor, whose activation interferes with the agonist-stimulated redistribution of intracellular calcium. However, the physiological relevance of this effect is questionable, since it can be observed only at a very high concentration of the peptide. Probably, CGRP enhances *in vivo* mineralocorticoid secretion by zona glomerulosa, via indirect mechanisms overcoming its direct inhibitory effect and probably involving the stimulation of catecholamine release by chromaffin cells.

CGRP-positive nerve fibers have been immunocytochemically demonstrated in the adrenal complex of the flat snake (Orezzoli et al., 1993, 1994), as well as in the chromaffin cells and nerve fibers of the adrenal glands of several anuran species (Kuramoto, 1987; Reinecke et al., 1992). Esneu et al. (1994) observed that in the *Rana ridibunda* adrenal gland CGRP-ir is exclusively located in the nerve fibers.

Esneu et al. (1994) reported that CGRP increases corticosterone and aldosterone secretion by both perifused frog adrenal tissue and dispersed adrenocortical cells. Frog CGRP is much more effective than rat and human peptides in the perifusion model, maximal effective concentration (about 10^{-6} M) eliciting a 2.75-fold increase in the release of both corticosterone and aldosterone. Further investigations (Esneu et al., 1996) characterized the CGRP-receptor subtype involved. $CGRP_1$-receptor subtype antagonists (human $CGRP_{8-37}$ and human $CGRP_{19-37}$) do not alter the secretory response to frog-CGRP, while a $CGRP_2$-receptor subtype agonist ([acetamidomethyl-Cis2,7]-human CGRP) evokes a concentration-related enhancement of corticosterone and aldosterone secretion (EC_{50} = $1.6x10^{-7}$ M). Likewise, adrenomedullin and amylin, two members of the CGRP family (for review, see Nussdorfer, 1996), induce a weak secretagogue effect. Frog-CGRP and $CGRP_2$-agonist significantly raise cyclic AMP release by frog tissue. These investigators maintain that CGRP directly stimulates adrenal steroid secretion in *Anura*, via the activation of $CGRP_2$ receptors positively coupled to adenylate cyclase.

G. Natriuretic peptide family

This group of regulatory peptides, originally isolated from atrial myocytes in the late 1970s, at present is known to include three members: atrial natriuretic peptide (ANP), brain or B-type natriuretic peptide (BNP) and C-type natriuretic peptide. An amazing mass of investigations indicates that these peptides exert a potent inhibitory action on aldosterone secretion in mammals, and are all contained and synthesized in adrenal medulla (for review, see Cantin and Genest, 1985; Rosenzweig and Seidman, 1991; Nussdorfer, 1996).

ANP-positive nerve fibers were found to run near adrenocortical cells in *Rana ridibunda* (Lihrmann et al., 1988). ANP-ir has been immunocytochemically detected in adrenal chromaffin cells of teleostean fishes (Kloas et al., 1994; Wolfensberger et al., 1995), lizards (Wolfensberger et al., 1995), anurans (Reinecke et al., 1992; Wolfensberger et al., 1995) and birds (Wolfensberger et al., 1995). According to these last investigators, ANP-ir is restricted to 35-40% of chromaffin cells, except in fishes where the number of positive cells attains 80-90%; about 30-55% of ANP-positive cells also contain neuropeptide Y-ir. Reinecke et al. (1992) also reported the presence of BNP-ir in the anuran adrenomedullary cells.

The effects of natriuretic peptides on adrenocortical-cell secretion in lower vertebrates have been quite extensively investigated, and the results have varied according to the class and species studied.

1. Pisces

Kloas et al. (1994) demonstrated by quantitative autoradiography [^{125}I]-rat ANP_{99-126} binding in the adrenal gland of *Cyprinus carpio* (EC_{50}, 87±15 pM; B_{max}, 56±11 amol/mm^2). Rat-ANP raises basal cortisol secretion (minimal effective concentration, 10^{-7} M), and more efficiently the acetylcholine-stimulated one (minimal effective concentration, 10^{-9} M). Similar results were obtained in the teleostean *Platichthys flesus* and *Salmo gairdneri*, where $2x10^{-7}$ M human ANP increases *in vitro* basal cortisol release by adrenal tissue of seawater-adapted animals (Arnold-Reed and Balment, 1991); since no effects were observed when adrenal from freshwater-adapted fishes were employed, these authors suggested that ANP may play a role in the animal survival in hypertonic media.

2. Amphibia

[^{125}I]-rat ANP_{99-126} binding sites were demonstrated by autoradiography especially in the outer layer

of adrenal glands of *Rana temporaria* (EC_{50}, 93±19 pM; B_{max}, 1.21±0.36 fmol/mm^2) (Kloas and Hanke, 1992a). Lihrmann et al. (1988) reported that ANP does not affect basal corticosterone and aldosterone yield by perifused adrenal tissue of *Rana ridibunda*; however, 10^{-6} M ANP significantly suppresses maximal ACTH- and ANG-II-stimulated secretion of both steroids (ACTH-stimulated corticosterone and aldosterone release by 25% and 56%, respectively; ANG-II-stimulated steroid secretion by about 26-28%). Kloas and Hanke (1992b) observed an inhibitory effect of ANP on corticosteroid release by *Xenopus laevis* adrenals.

3. Aves

Rosenberg et al. (1988, 1989) showed that 10^{-8} M ANP decreases both basal (-26%) and ACTH- or cholera toxin-stimulated (-84/-94%) aldosterone production by dispersed chicken adrenocortical cells. The loci of action of ANP appear to be both early (conversion of cholesterol to pregnenolone) and late steps (conversion of corticosterone to aldosterone) of aldosterone synthesis. ANP, at variance with ACTH, does not change cyclic AMP release by adrenocortical cells, and cyclic GMP and the guanylate-cyclase activator sodium nitroprusside exert an aldosterone antisecretagogue action similar to that of ANP. Opposite results were obtained by Kocsis et al. (1995) in the turkey *Meleagris gallopavo*. Turkey adrenocortical cells are provided with a single class of specific high affinity ANP receptors, whose concentration (89,400/cell) is 3.6-times higher than that of ANG-II receptors. ANP and BNP raise basal aldosterone, but not corticosterone secretion by dispersed adrenocortical cells, the minimal effective concentration being 10^{-8} M and the efficiency order being chicken-ANP > rat-ANP = human-ANP >> rat-BNP. ANP also enhances aldosterone response to a maximal effective concentration of ACTH (15%), ANG-II (49%) and K^+ (137%). Cyclic-GMP exerts similar effects as ANP, and ANP increases cyclic-GMP release by adrenal tissue, thereby suggesting that the mineralocorticoid secretagogue action of ANP involves the activation of guanylate cyclase. Kocsic and associates try to explain their conflicting results by stressing that chicken and turkey adrenocortical cells possess different physiological properties; the former being unresponsive and the latter responsive to ANG-II (Rosenberg et al., 1988; Kocsis et al., 1995).

H. Other regulatory peptides

Other regulatory peptides are contained in the adrenal glands of lower vertebrates, but at present no studies are available on their effects on *in vitro* steroid secretion of adrenocortical cells.

1. Pancreatic polypeptide family

This group of 36-amino acid peptides includes, in addition to pancreatic polypeptide, polypeptide YY and neuropeptide Y (for review, see Taylor et al., 1989). They are widely distributed in the mammalian tissues, including adrenal medulla, and exert multiple important physiological functions, among which is the regulation of adrenal secretion (for review, see Malendowicz et al., 1996; Nussdorfer, 1996). Polypeptide YY, at μM concentrations, directly depresses aldosterone secretion by rat zona glomerulosa cells. Intramedullary neuropeptide Y is surely involved in the fine tuning of zona glomerulosa secretion under physiological and pathological conditions requiring elevated release of aldosterone; the mechanisms are both direct and indirect, the latter involving the stimulation of catecholamine release. Polypeptide YY-ir has been immunocytochemically demonstrated in the adrenal complex of the teleostean eel (Reid et al., 1995). Likewise, neuropeptide Y-ir has been detected in adrenal chromaffin cells of the teleostean fishes (Reid et al., 1995; Wolfensberger et al., 1995), anurans (Reinecke et al., 1992; Wolfensberger et al., 1995), lizards (Wolfensberger et al., 1995) and birds (Garcia-Arrasas et al., 1992; Wolfensberger et al., 1995). According to Wolfensberger et al. (1995), about 30-40% of epinephrine cells are positive, and many of them also contain ANP-ir.

2. Neurotensin

This 13-amino acid peptide, widely distributed in the mammalian nervous system, exerts multiple physiological effects, including the regulation of the pituitary-adrenal axis. Neurotensin is also contained in the mammalian adrenal medulla and directly inhibits aldosterone secretion by zona glomerulosa cells, probably through its receptor-mediated interference with the transduction mechanisms of the agonists raising $[Ca^{2+}]_i$ (for review, see Ganguly and Davis, 1994; Nussdorfer, 1996). Neurotensin-ir has been immunocytochemically detected in both nor-epinephrine cells and nerve fibers of the adrenal gland of the flat snake *Waglerophis merremii* (Orezzoli et al., 1995).

V. Concluding Remarks

The preceding sections of this survey have shown that in the adrenal glands of lower vertebrates chromaffin cells may exert a stimulatory or inhibitory paracrine control of steroid-hormone secretion by releasing catecholamines and several regulatory peptides. Stimulatory molecules include serotonin, AVT, tachykinins, VIP, PACAP and CGRP; inhibitory molecules are dopamine, SRIF and galanin. Epinephrine and norepinephrine possess an inhibitory action in *Pisces* and a stimulatory one in *Aves*, their effect in *Amphibia* being controversial. Likewise, ANP exerts a stimulatory effect in *Pisces* and *Aves*, and an inhibitory action in *Amphibia*. The effect of opioids (enkephalins and endorphins) are questionable. In several instances the physiological relevance of all these secretagogue or antisecretagogue effects remains rather doubtful, since they have been observed by exposing steroidogenic cells

to very high concentrations of the regulatory molecules. At present, it seems that only AVT, tachykinins and PACAP may exert sizeable effects on steroidogenic cells at concentrations in the range of those they could locally attain upon their release from chromaffin cells.

Compared to the huge mass of data available on this topic in mammals (more than 700 papers) (Nussdorfer, 1996), studies carried out in lower vertebrates are very scarce (less than 60). Moreover, they are almost exclusively concerned with *Amphibia* and *Aves*, when the few presently available data indicate that a great variability occurs in the response of steroidogenic cells to the various regulatory molecules among the different classes of vertebrates.

In conclusion, it appears that much further work must be done to clarify the functional interactions between chromaffin and steroidogenic cells in the adrenal glands of lower vertebrates, and we shall now take the opportunity to stress some not yet addressed points, whose elucidation should be the task of future investigations.

First of all, the steroidogenic or antisteroidogenic effects of some regulatory molecules contained in adrenal chromaffin cells of lower vertebrates and which have been shown to play important roles in mammals (e.g. neuropeptide Y and neurotensin) have not yet been studied.

The greater part of studies dealing with the presence of regulatory peptides in lower-vertebrate adrenals have been done by immunocytochemical techniques. Up to now, RIA measurements of the intra-adrenal content are available only for few regulatory peptides (AVT, tachykinins and PACAP), and have been exclusively carried out in *Amphibia*. Moreover, the blood levels of the various regulatory molecules have not been assayed. This kind of investigation, coupled with those aimed at evaluating the *in vivo* effect of selective antagonists, could allow comparative endocrinologists to ascertain whether or not an intra-adrenal regulatory peptide plays a physiological role in the paracrine control of steroid hormone secretion.

Finally, molecular biology investigations of intra-adrenal mRNA expression of the various regulatory peptides, under both normal and experimental conditions, have not yet been performed. Parenthetically, it must be noted that analogous studies carried out in mammals have clearly demonstrated that some regulatory molecules play a relevant role only in the physiopathology of the adrenal gland, when e.g. an excess of steroid hormone production or a fluid and electrolyte imbalance have to be counteracted.

The settlement of these and many other basic topics will not only open new frontiers in our knowledge of adrenal cytophysiology in lower vertebrates, but surely will also improve our understanding of the significance of the morphological evolution of the adrenal gland among the classes of vertebrates.

References

Arimura A. and Shioda S. (1995). Pituitary adenylate cyclase activating polypeptide (PACAP) and its receptors: neuroendocrine and endocrine interaction. Front. Neuroendocrinol. 16, 53-88.

Arnold-Reed D.E. and Balment R.J. (1991). Atrial natriuretic factor stimulates *in vivo* and *in vitro* secretion of cortisol in teleosts. J. Endocrinol. 128, R17-R20.

Bedecs K., Berthold M. and Bartfai T. (1995). Galanin. 10 years with a neuroendocrine peptide. Int. J. Biochem. Cell Biol. 27, 337-349.

Cantin M. and Genest J. (1985). The heart and atrial natriuretic factor. Endocr. Rev. 6, 107-127.

Chester-Jones I. (1976). Evolutionary aspects of the adrenal cortex. J. Endocrinol. 71, 3P-31P.

Chester-Jones I., Ingleton P.M. and Phillips J.G. (1987). Fundamentals of comparative vertebrate endocrinology. Plenum Press. New York, London.

Cheung A., Hall T.R. and Harvey S. (1987). Serotoninergic regulation of corticosterone secretion in domestic fowl. J. Endocrinol. 113, 159-165.

Cheung A., Harvey S., Hall T.R., Lam S.K. and Spencer G.S.G. (1988). Effects of passive immunization with antisomatostatin serum on plasma corticosterone concentrations in young domestic cockerels. J. Endocrinol 116, 179-183.

Conlon J.M., O'Harte F., Smith D.D., Balment R.J. and Hazon N. (1992a). Purification and characterization of urotensin II and parvalbumin from an elasmobranch fish, *Scyliorhinus canicula* (common dogfish). Neuroendocrinology 55, 230-235.

Conlon J.M., O'Harte F., Smith D.D., Tonon M.C. and Vaudry H. (1992b). Isolation and primary structure of urotensin II from the brain of a tetrapod, the frog *Rana ridibunda*. Biochem. Biophys. Res. Commun. 188, 578-583.

Contesse V., Hamel C., Delarue C., Lefebvre H. and Vaudry H. (1994). Effect of a series of 5-HT4 receptor agonists and antagonists on steroid secretion by the adrenal gland *in vitro*. Eur. J. Pharmacol. 265, 27-33.

Contesse V., Hamel C., Lefebvre H., Dumuis A., Vaudry H. and Delarue C. (1996). Activation of 5-hydroxytryptamine$_4$ receptors causes calcium influx in adrenocortical cells: involvement of calcium in 5-hydroxytryptamine-induced steroid secretion. Mol. Pharmacol. 49, 481-493.

Cryer P.E. (1992). The adrenal medullae. In: The adrenal gland. 2nd ed. James V.T.H. (ed). Raven Press. New York. pp 465-489.

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

Delarue C., Netchitailo P., Leboulenger F., Perroteau I., Escher E. and Vaudry H. (1984). *In vitro* study of frog (*Rana ridibunda* Pallas) interrenal function by use of a simplified perifusion system. VII. Lack of effect of somatostatin on angiotensin-induced corticosteroid production. Gen. Comp. Endocrinol. 54, 333-338.

Delarue C., Leboulenger F., Morra M., Héry F., Verhofstad A.A.J., Bérod A., Denoroy L., Pelletier G. and Vaudry H. (1988a). Immunohistochemical and biochemical evidence for the presence of serotonin in amphibian adrenal chromaffin cells. Brain Res. 459, 17-26.

Delarue C., Lefebvre H., Idres S., Leboulenger F., Homo-Delarche G., Lihrmann I., Feuilloley M. and Vaudry H. (1988b). Serotonin stimulates corticosteroid secretion by frog adrenocortical tissue *in vitro*. J. Steroid Biochem. 29, 519-525.

Delarue C., Becquet D., Idres I., Héry F. and Vaudry H. (1992). Serotonin synthesis in adrenochromaffin cells. Neuroscience 46, 495-500.

Esneu M., Delarue C., Remy-Jouet I., Manzardo E., Fasolo A., Fournier

A., Saint-Pierre S., Conlon J.M. and Vaudry H. (1994). Localization, identification, and action of calcitonin gene-related peptide in the frog adrenal gland. Endocrinology 135, 423-430.

Esneu M., Delarue C., Fournier A. and Vaudry H. (1996). Characterization of the receptor mediating the effect of calcitonin gene-related peptide in the frog adrenal gland. Eur. J. Pharmacol. 308, 187-193.

Fahrenkrug G. (1989). Vasoactive intestinal peptide. Hdbook Physiol. Sect. 6: Gastrointest. Syst. 2, 691-702.

Feuilloley M. and Vaudry H. (1996). Role of the cytoskeleton in adrenocortical cells. Endocr. Rev. 17, 269-288.

Feuilloley M., Lesouhaitier O., Delarue C., De Marchis S., Conlon J.M., Bern H.A. and Vaudry H. (1994). *In vitro* study of the effect of urotensin II on corticosteroid secretion in the frog *Rana ridibunda*. J. Steroid Biochem. Mol. Biol. 48, 287-292.

Ganguly A. and Davis J.S. (1994). Role of calcium and other mediators in aldosterone secretion from the adrenal glomerulosa cells. Pharmacol. Rev. 46, 417-447.

Garcia-Arrasas J.E., Lugo-Chinchilla A.M. and Chevere-Colon I. (1992). The expression of neuropeptide Y immunoreactivity in the avian sympathoadrenal system conforms with two models of coexpression development for neurons and chromaffin cells. Development 115, 617-627.

Gasman S., Vaudry H., Cartier F., Tramu G., Fournier A., Conlon J.M. and Delarue C. (1996). Localization, identification, and action of galanin in the frog adrenal gland. Endocrinology 137, 5311-5318.

Gfell B., Kloas W. and Hanke W. (1995). Regulation of adrenal secretion of the common carp (*Cyprinus carpio*) by cholinergic receptors. Verh. Deuts. Zool. Gesellsch. 88, 102.

Hall P.F. (1995). The role of microfilaments and intermediate filaments in the regulation of steroid synthesis. J. Steroid Biochem. Mol. Biol. 55, 601-605.

Hanke W. and Kloas W. (1994). Hormonal regulation of osmomineral content in *amphibia*. Zool Sci. 11, 5-14.

Hanke W. and Kloas W. (1995). Comparative aspects of regulation and function of the adrenal complex in different groups of vertebrates. Horm. Metab. Res. 27, 389-397.

Idres S., Delarue C., Lefebvre H., Larcher A., Feuilloley M. and Vaudry H. (1989). Mechanism of action of serotonin on frog adrenal cortex. J. Steroid Biochem. 34, 547-550.

Idres S., Delarue C., Lefebvre H. and Vaudry H. (1991). Benzamide derivatives provide evidence for the involvement of a 5-HT_4 receptor type in the mechanism of action of serotonin in frog adrenocortical cells. Mol. Brain Res. 10, 251-258.

Kloas W. and Hanke W. (1990). Neurohypophysial hormones and steroidogenesis in the interrenals of *Xenopus laevis*. Gen. Comp. Endocrinol. 80, 321-330.

Kloas W. and Hanke W. (1992a). Atrial natriuretic factor (ANP) binding sites in frog kidney and adrenal. Paptides 13, 297-303.

Kloas W. and Hanke W. (1992b). Effects of atrial natriuretic factor (ANF) on corticosteroid and catecholamine secretion by the adrenal tissue of *Xenopus laevis*. Gen. Comp. Endocrinol. 85, 269-277.

Kloas W., Reinecke M. and Hanke W. (1994). Role of atrial natriuretic peptide for adrenal regulation in the teleost fish *Cyprinus carpio*. Am. J. Physiol. 267, R1034-R1042.

Kocsis J.F., Lamm E.T., McIlroy P.J., Scanes C.G. and Carsia R.V. (1995a). Evidence for a functionally distinct subpopulations of steroidogenic cells in the domestic turkey (*Meleagris gallopavo*) adrenal gland. Gen. Comp. Endocrinol. 98, 57-72.

Kocsis J.F., McIlroy P.J. and Carsia R.V. (1995b). Atrial natriuretic peptide stimulates aldosterone production by turkey (*Meleagris gallopavo*) adrenal steroidogenic cells. Gen. Comp. Endocrinol. 99, 364-372.

Kodjo M.K., Leboulenger F., Conlon J.M. and Vaudry H. (1995a). Effect of ranakinin, a novel tachykinin, on cytosolic free calcium in frog adrenochromaffin cells. Endocrinology 136, 4535-4542.

Kodjo M.K. Leboulenger F., Porcedda P., Lamacz M., Conlon J.M., Pelletier G. and Vaudry H. (1995b). Evidence for the involvement of chromaffin cells in the stimulatory effect of tachykinins on corticosteroid secretion by the frog adrenal gland. Endocrinology 136, 3253-3259.

Kodjo M.K., Leboulenger F., Morra M., Conlon J.M. and Vaudry H. (1996). Pharmacological profile of the tachykinin receptor involved in the stimulation of corticosteroid secretion in the frog *Rana ridibunda*. J. Steroid Biochem. Mol. Biol. 57, 329-335.

Kondo H. and Jui R. (1984). Co-existence of enkephalin and adrenalin in the frog adrenal gland. Histochemistry 80, 243-246.

Kuramoto H. (1987). An immunohistochemical study of chromaffin cells and nerve fibers in the adrenal gland of the bullfrog, *Rana catesbeiana*. Arch. Histol. Jpn. 50, 15-38.

Larcher A., Delarue C., Idres S., Lefebvre H., Feuilloley M., Vandesande F., Pelletier G. and Vaudry H. (1989). Identification of vasotocin-like immunoreactivity in chromaffin cells of the frog adrenal gland: effect of vasotocin on corticosteroid secretion. Endocrinology 125, 2691-2700.

Larcher A., Delarue C., Homo-Delarche F., Kikuyama S., Kupryszewsky G. and Vaudry H. (1992a). Pharmacological characterization of vasotocin stimulation of phosphoinositide turnover in frog adrenal gland. Endocrinology 130, 475-483.

Larcher A., Delarue C., Idres S. and Vaudry H. (1992b). Interactions between vasotocin and other corticotropic factors on the frog adrenal gland. J. Steroid Biochem. Mol. Biol. 41, 795-798.

Larcher A., Lamacz M., Delarue C. and Vaudry H. (1992c). Effect of vasotocin on cytosolic free calcium concentrations in frog adrenocortical cells in primary culture. Endocrinology 131, 1087-1093.

Leboulenger F., Leroux P., Delarue C., Tonon M.C., Charnay Y., Dubois P.M., Coy D.H. and Vaudry H. (1983a). Co-localization of vasoactive intestinal peptide (VIP) and enkephalins in chromaffin cells of the adrenal gland of amphibia. Stimulation of corticosteroid production by VIP. Life Sci. 32, 375-383.

Leboulenger F., Leroux P., Tonon M.C., Coy D.H., Vaudry H. and Pelletier G. (1983b). Coexistence of vasoactive intestinal peptide and enkephalins in the adrenal chromaffin granules of the frog. Neurosci. Lett. 37, 221-225.

Leboulenger F., Perroteau J., Netchitailo P., Lihrmann I., Leroux P., Delarue C., Coy H. and Vaudry H. (1984). Action of vasoactive intestinal peptide (VIP) on amphibian adrenocortical function *in vitro*. Peptides 5, 299-303.

Leboulenger F., Benyamina M., Delarue C., Netchitailo P., Saint Pierre S. and Vaudry H. (1988). Neuronal and paracrine regulation of adrenal steroidogenesis: interaction between acetylcholine, serotonin and vasoactive intestinal peptide (VIP) on corticosteroid production by frog interrenal tissues. Brain Res. 453, 103-109.

Leboulenger F., Vaglini L., Conlon J.M., Homo-Delarche F., Wang Y., Kerdelhue B., Pelletier G. and Vaudry H. (1993). Immunohistochemical distribution, biochemical characterization and biological action of tachykinins in the frog adrenal gland. Endocrinology 133, 1999-2008.

Lesouhaitier O., Esneu M., Kodjo M.K., Hamel C., Contesse V., Yon L., Remy-Jouet I., Fasolo A., Fournier A., Vandesande F., Pelletier G., Conlon J.M., Roubos E.W., Feuilloley M., Delarue C., Leboulenger

F. and Vaudry H. (1995). Neuroendocrine communication in the frog adrenal gland. Zool. Sci. 12, 255-264.

Lihrmann I., Netchitailo P., Feuilloley M., Cantin M., Delarue C., Leboulenger F., De Léan A. and Vaudry H. (1988). Effect of atrial natriuretic factor on corticosteroid production by perifused frog interrenal slices. Gen. Comp. Endocrinol. 71, 55-62.

Maggio J.E. (1988). Tachykinins. Annu. Rev. Neurosci. 11, 13-28.

Malendowicz L.K. and Markowska A. (1994). Neuromedins and their involvement in the regulation of growth, structure and function of the adrenal cortex. Histol. Histopathol. 9, 591-601.

Malendowicz L.K., Markowska A. and Zabel M. (1996). Neuropeptide Y-related peptides and hypothalamo-pituitary-adrenal function. Histol. Histopathol. 11, 485-494.

Mazzocchi G., Musajo F.G., Malendowicz L.K., Andreis P.G. and Nussdorfer G.G. (1993). Interleukin-1ß stimulates corticotropin-releasing hormone (CRH) and adrenocorticotropin (ACTH) release by rat adrenal gland *in vitro*. Mol. Cell. Neurosci. 4, 267-270.

Mazzocchi G., Gottardo G. and Nussdorfer G.G. (1997a). Effects of somatostatin on steroid production by interrenal cells of the domestic turkey and fowl. Zool. Sci. 4, 359-361.

Mazzocchi G., Gottardo G. and Nussdorfer G.G. (1997b). Catecholamines stimulate steroid secretion of dispersed fowl interrenal cells, acting through the ß-receptor subtype. Horm. Metab. Res. 29, 168-170.

Mazzocchi G., Gottardo G. and Nussdorfer G.G. (1997c). Pituitary adenylate cyclase-activating peptide enhances steroid production by fowl interrenal glands: evidence for an indirect mechanism probably involving the local release of catecholamines. Horm. Metab. Res. 29, 85-86.

Morra M., Leboulenger F., Homo-Delarche F., Netchitailo P. and Vaudry H. (1989). Dopamine inhibits corticosteroid secretion in frog adrenocortical cells: evidence for the involvement of prostaglandins in the mechanism of action of dopamine. Life Sci. 45, 175-181.

Morra M., Leboulenger F. and Vaudry H. (1990). Dopamine inhibits corticosteroid secretion from frog adrenal gland *in vitro*. Endocrinology 127, 218-226.

Morra M., Leboulenger F., Desrues L., Tonon M.C. and Vaudry H. (1991). Dopamine inhibits inositol phosphate production, arachidonic acid formation, and corticosteroid release by frog adrenal gland through a pertussis toxin-sensitive G-protein. Endocrinology 128, 2625-2632.

Morra M., Leboulenger F. and Vaudry H. (1992). Characterization of dopamine receptors associated with steroid secretion in frog adrenocortical cells. J. Mol. Endocrinol. 8, 43-52.

Nussdorfer G.G. (1986). Cytophysiology of the adrenal cortex. Int. Rev. Cytol. 98, 1-405.

Nussdorfer G.G. (1996). Paracrine control of adrenal cortical function by medullary chromaffin cells. Pharmacol. Rev. 48, 495-530.

Orezzoli A.A., González-Nicolini V., Villar M.J. and Tramezzani J.H. (1993). Histochemical characterization of nerve fibers in the adrenal gland of the flat snake (*Waglerophis merremii*). Biocell 17, 67-77.

Orezzoli A.A., González-Nicolini V., Villar M.J., Hökfelt T. and Tramezzani J.H. (1994). Histochemical study of chromaffin cells and nerve fibers in the adrenal gland of the flat snake (*Waglerophis merremii*). Gen. Comp. Endocrinol. 93, 411-423.

Orezzoli A.A., González-Nicolini V., Bilinski M., Villar M.J., Hökfelt T. and Tramezzani J.H. (1995). Immunohistochemical localization of neurotensin in a subpopulation of noradrenergic chromaffin cells of the adrenal gland of the flat snake (*Waglerophis merremii*). Gen. Comp. Endocrinol. 97, 179-187.

Owyang C. and Louie D. (1989). Newly discovered gut peptides. Hdbook Physiol. Sect. 6: Gastrointest. Syst. 2, 691-702.

Reid S.G., Fitsche R. and Jönsson A.C. (1995). Immunohistochemical localization of bioactive peptides and amines associated with chromaffin tissue of five species of fish. Cell Tissue Res. 280, 499-512.

Reinecke M., Heym C. and Forssman W.G. (1992). Distribution patterns and coexistence of neurohormonal peptides (ANP, BNP, NPY, SP, CGRP, enkephalins) in chromaffin cells and nerve fibers of the anuran adrenal organ. Cell Tissue Res. 268, 247-256.

Rosenberg J., Pines M. and Hurwitz S. (1988). Regulation of aldosterone secretion by avian adrenocortical cells. J. Endocrinol. 118, 447-453.

Rosenberg J., Pines M. and Hurwitz S. (1989). Inhibition of aldosterone secretion by atrial natriuretic peptide in chicken adrenocortical cells. Biochim. Biophys. Acta 1014, 189-194.

Rosenzweig A. and Seidman C.E. (1991). Atrial natriuretic factor and related peptide hormones. Annu. Rev. Biochem. 60, 229-255.

Taylor I.L. (1989). Pancreatic polypeptide family: pancreatic polypeptide, neuropeptide Y, and peptide YY. Handb. Physiol. Sect. 6: Gastrointest. Syst. 2, 475-543.

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

Vizi E.S., Tóth I.E., Szalay K.S., Windisch K., Orso E., Szabo D. and Vinson G.P. (1992). Catecholamines released from local adrenergic axon terminals are possibly involved in fine modulation of steroid secretion from zona glomerulosa cells: functional and morphological evidence. J. Endocrinol. 135, 551-561.

Vizi E.S., Tóth I.E., Orso E., Szalay K.S., Szabo D., Barayani M. and Vinson G.P. (1993). Dopamine is taken up from the circulation by, and released from, local noradrenergic varicose axon terminals in zona glomerulosa of the rat: a neurochemical and immunocytochemical study. J. Endocrinol. 139, 213-226.

Wolfensberger M., Forssmann W.G. and Reinecke M. (1995). Localization and coexistence of atrial natriuretic peptide (ANP) and neuropeptide Y (NPY) in vertebrate adrenal chromaffin cells immunoreactive to TH, DBH and PNMT. Cell Tissue Res. 280, 267-276.

Yon L., Feuilloley M., Chartrel N., Arimura A., Fournier A. and Vaudry H. (1993). Localization, characterization and activity of pituitary adenylate cyclase-activating polypeptide in the frog adrenal gland. J. Endocrinol. 139, 183-194.

Yon L., Chartrel N., Feuilloley M., De Marchis S., Fournier A., De Rijk E., Pelletier G., Roubos E. and Vaudry H. (1994). Pituitary adenylate cyclase-activating polypeptide stimulates both adrenocortical cells and chromaffin cells in the frog adrenal gland. Endocrinology 135, 2749-2758.

Zentel H.J., Nohr D., Müller S., Yanaihara N. and Weihe E. (1990). Differential occurence and distribution of galanin in adrenal nerve fibers and medullary cells in rodent and avian species. Neurosci. Lett. 120, 167-170.

Zerani M. and Gobbetti A. (1991). Effects of ß-endorphin and naloxone on corticosterone and cortisol release in the newt (Triturus carnifex): studies *in vivo* and *in vitro*. J. Endocrinol. 131, 295-302.

Zerani M. and Gobbetti A. (1992). *In vivo* and *in vitro* effects of ß-endorphin and naloxone on corticosterone and cortisol release in male and female water frog *Rana esculenta*. Comp. Biochem. Physiol. C 102, 537-542.