Histol Histopathol (1995) 10: 359-370

Histology and Histopathology

In-vitro and in-vivo studies of the effects of arginine-vasopressin on the secretion and growth of rat adrenal cortex

G. Mazzochi[1], L.K. Malendowicz[2], V. Meneghelli[1], G. Gottardo[1] and G.G. Nussdorfer[1]

[1]Department of Anatomy, University of Padua, Padua, Italy and

[2]Department of Histology and Embryology, School of Medicine, Poznan, Poland

Summary. Arginine-vasopressin (AVP) markedly increased basal aldosterone (ALDO) secretion by dispersed zona-glomerulosa (ZG) cells, and its effect was selectively reversed by V1-receptor antagonists (AVP-A1). Corticosterone (B) production by dispersed zona fasciculata (ZF) cells was not affected. The *bolus* intraperitoneal (i.p.) administration of AVP acutely raised the plasma concentrations of both ALDO and B in normal rats, but only that of ALDO in bilaterally adrenalectomized animals bearing regenerated adrenocortical autotransplants, which are deprived of medullary chromaffin cells. Accordingly, AVP raised ALDO and B secretions by adrenal slices (including both cortical and medullary tissues), and only ALDO production by autotransplant quarters. The B response of adrenal slices to AVP was blocked by α-helical-CRH and corticotropin-inhibiting peptide (two competitive inhibitors of CRH and ACTH, respectively), but not by l-alprenolol (a ß-adrenoreceptor antagonist); ALDO response was not affected by any of these antagonists. A 7-day i.p. infusion with AVP increased the volume of ZG cells and ZG-like cells of autotransplants, as well as their basal and maximally angiotensin-II-stimulated ALDO secretory capacity; it also raised the volume, and basal and maximally ACTH-stimulated B secretory capacity of ZF cells, but it did not affect ZF-like cells of autotransplants. The simultaneous administration of AVP-A1 annulled all these effects of AVP. When infused alone, AVP-A1 caused a marked atrophy of ZG cells, coupled with a net drop in their steroidogenic capacity; however, AVP-A1 infusion did not change the morphology and function of either ZF cells or ZG-like and ZF-like cells of autotransplants. Taken together, our findings allow us to draw the following conclusions: (i) AVP plays an important physiological role in the maintenance and stimulation of ZG growth and mineralocorticoid secretory activity in rats, the source of endogenous AVP exerting adrenoglomerulotropic action probably being adrenal chromaffin cells; and (ii) AVP indirectly stimulates the growth and glucocorticoid secretory activity of rat ZF cells, by activating intramedullary CRH/ACTH system; however, the physiological relevance of this effect of AVP appears to be doubtful.

Key words: Arginine vasopressin, Adrenal cortex, Steroidogenesis, Adrenal medulla, Rat

Offprint requests to: Prof. G.G. Nussdorfer, Department of Anatomy, Via Gabelli 65, I-35121 Padova, Italy

Introduction

A great deal of evidence indicates that arginine-vasopressin (AVP) exerts a specific mineralocorticoid secretagogue effect on mammalian adrenals *in vivo* and *in vitro* (Payet and Lehoux, 1982; Hinson et al., 1987; Porter et al., 1988; Schneider, 1988; Quinn et al., 1990; Quinn and Williams, 1992; Bähr et al., 1993). Moreover, proof is available that prolonged AVP administration enhances zona-glomerulosa (ZG) growth, by promoting both hypertrophy and hyperplasia of its parenchymal cells (Isler, 1973; Payet and Isler, 1976; Payet and Lehoux, 1980; Payet al., 1984; Lesniewska et al., 1991). More controversial are the effects of AVP on adrenal glucocorticoid secretion and growth of zonae fasciculata (ZF) and reticularis (ZR). The *in-vivo*-observed stimulatory action (Faucher et al., 1988; Hensen et al., 1988; Brooks and Blackemore, 1989; Brooks and White, 1990; Apostolakis et al., 1991; Bähr et al., 1991; Lesniewska et al., 1991) may be conceivably ascribed to the stimulation of the hypothalamo-pituitary axis. In fact, it is well known that AVP, like CRH, is a potent activator of pituitary ACTH-release (for review, see Antoni, 1986; Buckingham et al., 1992). The bulk of *in-vitro* investigations does not evidence any appreciable secretagogue action of AVP on dispersed inner (ZF/ZR) adrenocortical cells (Payet and Lehoux, 1982; Hinson et al., 1987); however, using superfused rat and human adrenocortical tissues or perfused intact dog adrenals, some researchers were able to observe a stimulatory effect of AVP on glucocorticoid secretion (Hinson et al.,

1987; Schneider, 1988; Perraudin et al., 1993).

It therefore seemed worthwhile to perform a combined *in-vitro* and *in-vivo* investigation on the acute and chronic effects of AVP on the mineralo- and glucocorticoid secretion, and on the growth and steroidogenic capacity of ZG and ZF/ZR of the rat adrenal cortex.

Materials and methods

In-vivo experiments

Adult male Wistar rats (220±20 g body weight) were purchased from Morini (Reggio Emilia, Italy). A group of rats was bilaterally adrenalectomized and 6 fragments of the capsular (ZG) tissue of their excised adrenals were implanted in the *musculus gracilis*; the animals were employed after 4 months of regeneration of autotransplants (Belloni et al., 1990). Another group of rats was sham-adrenalectomized.

Starting from the 5th day before each experiment, the animals were subcutaneously (s.c.) infused (Alzet osmotic pumps Mod 2001 or Mod. 2002; Alza, Palo Alto, CA, USA) with dexamethasone (10 µg/kg.h) plus ACTH (0.1 IU/kg.h) and captopril (0.5 mg/kg.h) plus angiotensin-II (ANG-II; 10 µg/kg.h), in order to interrupt their hypothalamo-pituitary-adrenal (HPA) axis and renin-angiotensin system (RAS) (Nussdorfer et al., 1988; Rebuffat et al., 1988). Dexamethasone, ACTH (human) and ANG-II were obtained from Sigma (St. Louis, MO, USA), and captopril (Capoten) from Squibb (Milan, Italy). This dose of dexamethasone induced a 70% decrease in the level of circulating (ACTH (21.0±3.2 vs 3.4±0.5 pM) and ACTH infusion reversed this effect (23.5±4.6 pM); the level of circulating ANG-II was not measured, but captopril infusion caused a 2-fold rise in plasma renin activity (PRA) (4.9±0.6 vs 9.5±1.1 fmol/ml.h) and ANG-II administration completely annulled this effect (5.4±0.8 fmol/ml.h). The rats were kept under a 12:12 light-dark cycle (illumination onset at 8:00 a.m.) at 23±1 °C, and maintained on a standard diet (Rat-Mouse Chow; Zoofarm, Padua, Italy) and tap water *ad libitum*.

The animals were treated as follows: (i) groups (n=6) of sham-operated and autotransplanted rats were intraperitoneally (i.p.) injected with increasing doses of AVP (from 10^{-14} to 10^{-6} mol/rat); other groups of rats (n= 6) were given, along with AVP, 10^{-7}mol/rat of Des-Gly- (Pha^1, D-$Tyr(Et)^2$, Lys^6, $Arg^{8)}$-AVP, a selective antagonist of V1 subtype of AVP receptors (AVP-A1) (Manning et al., 1990); the rats were sacrificed 60 min after the injection. (ii) Groups (n=8) of sham-operated and autotransplanted rats were i.p. infused (Alzet pumps) for 7 days with AVP (10 pmol.min), AVP-A1 (1 nmol.min) or AVP plus AVP-A1; the control group was infused with the saline vehicle. AVP and its antagonist were purchased from Peninsula (Merseyside, England).

The rats were decapitated between 10:00 and 11:00 a.m., their trunk blood was collected, and plasma was separated and stored at -20 °C. Adrenal and autotransplanted nodules were promptly removed.

Biochemical assays

PRA was assayed by RIA of angiotensin-I generated after incubation of plasma (ANG-I RIA-kit; Peninsula). ACTH was extracted from plasma (Rees et al., 1971) and its concentration determined by RIA (ACTH-RIA kit; IRE-Sorin, Vercelli, Italy). Aldosterone (ALDO) and corticosterone (B) were extracted and purified by HPLC (Neri et al., 1993), and their plasma concentrations (PAC and PBC, respectively) were measured by RIA (ALDO-CTK2; IRE-Sorin, and CTRX-RIA; Eurogenetic, Milan, Italy). Intra- and interassay variations were: ANG-I, 6.2% and 8.5%; ACTH, 5.5% and 7.4%; ALDO, 6.1% and 7.3%; and B, 7.5% and 8.8%

Morphology

The left adrenals were fixed in Bouin's solution, embedded in paraffin and serially cut at 6-7 µm. Sliced pieces of the right glands and of autotransplants were fixed in 3% glutaraldehyde, post-fixed in 1% osmium tetroxide and embedded in epon. Thick (0.5 µm) sections were cut with an LKB Supernova Ultratome (Reichert-Jung, Wien, Austria) at the level of the ZG and ZF of adrenals, and of subcapsular and inner zones of autotransplants. The volume of ZG and ZF, and the number of average volume of their parenchymal cells, as well as the average volume of ZG-like and ZF-like cells of autotransplants, were determined using conventional morphometric methods (Weibel, 1979), as detailed previously (Rebuffat et al., 1989; Belloni et al., 1990). Morphometry was performed using a semi-automatic procedure of analysis: images were obtained with a camera-connected Leitz microscope, randomly stored in a Hantares-80 computer, and then analyzed with a specific software purchased from Studio Casti Imaging (Venezia, Italy).

In-vitro experiments

Another 4 groups of sham-operated and autotransplanted rats were treated as in the 2nd *in-vivo* experiment. A number of animals were not infused (normal sham-operated and autotransplanted rats). The rats were decapitated, and adrenals and regenerated adrenocortical nodules were promptly removed. The adrenals of a number of normal rats were cut into slices containing both cortical and medullary tissues (Andreis et al., 1991b), and autotransplants were quartered (Belloni et al., 1990). The adrenals of the remaining normal rats, and those of infused animals were employed to obtain dispersed-cell preparations.

Preparation of dispersed cells

Adrenals were decapsulated, bisected and enucleated (to eliminate adrenal medulla). Dispersed capsular (ZG)

and inner (ZF/ZR) cells were obtained by collagenase/DNase 1 digestion and mechanical disaggregation (Szalay, 1981). The viability of isolated cells was checked by the Trypan-blue exclusion test and was found to be higher than 90%. Inner-cell contamination in capsular-cell preparations, as evaluated by phase microscopy, was always less than 8%. Dispersed cells obtained from 8 adrenals were pooled to obtain a single cell preparation, and 6 or 8 cell preparations for each incubation experiment were employed.

Incubation procedures

Dispersed cells, adrenal slices and autotransplant quarters were put in Medium 199 (DIFCO, Detroit, Mich., USA) and potassium-free Krebs-Ringer bicarbonate buffer with 0.2% glucose (2:1, v/v), containing 5 mg/ml human serum albumin. They were incubated ($3x10^5$ cells or 6-8 mg/ml) as follows: (i) dispersed cells from normal rats were incubated (n=6) with AVP (from 10^{-14} to 10^{-5} M); (ii) dispersed capsular cells from normal rats were incubated (n=6) with 10^{-7} M AVP, in the presence of increasing concentrations (from 10^{-11} to 10^{-4} M) of AVP-A1 or [d(CH_2)$_5$, D-Phe^2, Ile^4, Ala^9-NH_2]-AVP, a selective antagonist of V2 subtype of AVP receptors (AVP-A2; Peninsula) (Perraudin et al., 1993); (iii) capsular cells from normal rats were incubated (n=6) with 10^{-12} M, 10^{-10} M or 10^{-8} M ANG-II, in the presence of increasing concentrations (from 10^{-14} to 10^{-6} M) of AVP; (iv) adrenal slices and autotransplant quarters from normal rats were incubated (n=6) with increasing concentrations (from 10^{-14} to 10^{-6} M) of AVP, in the presence or absence of 10^{-5} M AVP-A1; (v) adrenal slices from normal rats were incubated (n=8) with 10^{-7}M AVP, in the presence or absence of 10^{-7} l-alprenolol (AL; Sigma), a blocker of ß-adrenoreceptors (De Léan et al., 1984), 10^{-6} M α-helical-CRH (α-CRH; Peninsula), a selective antagonist of CRH (Rivier et al., 1984), or 10^{-6} M corticotropin-inhibiting peptide (CIP; Peninsula), an antagonist of ACTH (Li et al., 1978); and (vi) dispersed adrenocortical cells and autotransplant quarters from infused rats were incubated (n=8) with 10^{-9} M ANG-II, 10^{-9} M ACTH or without any agonist. The incubation was carried out in shaking bath at 37 °C for 90 min, in an atmosphere of 95% O_2 and 5% CO_2.

Biochemical assays

ALDO and B concentrations in the incubation medium were determined by RIA, as described above. Intra- and interassay variations were: ALDO, 7.5% and 8.6%; and B, 6.8% and 9.1%.

Statistics

The data obtained were averaged per experimental group, and SE was calculated. The statistical comparison of results was done by one-way ANOVA, followed by the Multiple Range Test of Duncan.

Results

Acute experiments

AVP concentration-dependently increased ALDO

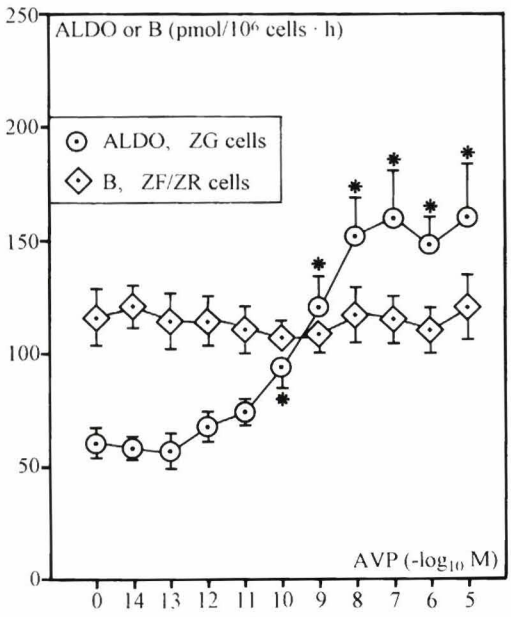

Fig. 1. Acute concentration-dependent effect of AVP on ALDO and B secretions of dispersed rat adrenocortical cells. Data are means±SE (n= 6). *:p<0.01 from the respective 0-group.

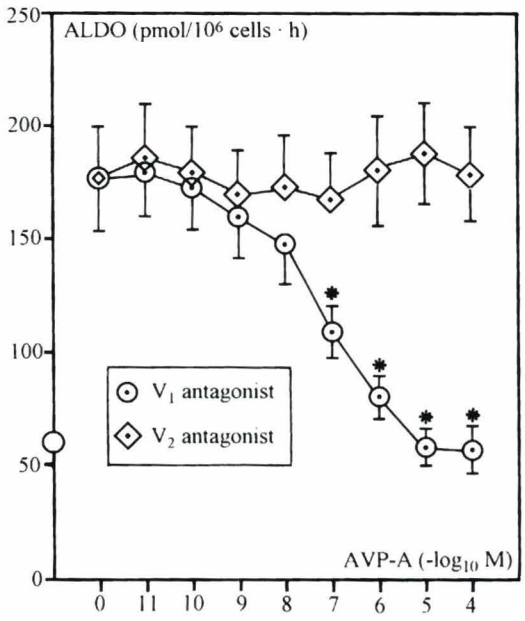

Fig. 2. Effect of AVP-A's on AVP (10^{-7} M)-stimulated ALDO secretion of dispersed rat ZG cells. Basal value is shown on the ordinate. Data are means±SE (n= 6). *: p<0.01 from 0-group.

secretion by dispersed ZG cells: minimal and maximal effective concentrations were 10^{-10} M (53%) and $10^{-8}/10^{-7}$ M (2.5-fold), respectively; B production by dispersed ZF/ZR cells was not affected (Fig. 1). The ALDO secretagogue effect of 10^{-7} M AVP was concentration-dependently counteracted by AVP-A1, but not AVP-A2; 10^{-5} M AVP-A1 completely suppressed the effect of the maximal effective concentration of AVP (Fig. 2) AVP concentration-dependently raised 10^{-12} M and 10^{-10} M, but not 10^{-8} M ANG-II-stimulated ALDO secretion of dispersed capsular cells; the increases were significant at an AVP concentration of 10^{-8} M (2-fold and 50% respectively) (Fig. 3).

The pharmacological interruption of the HPA axis and RAS in both sham-operated and autotransplanted rats did not induce any significant alteration in the basal values of both biochemical and morphological parameters examined (Figs. 4, 5, 9-12). The *bolus* i.p. administration of AVP acutely enhanced PAC in both sham-operated (Fig. 4) and autotransplanted rats (Fig. 5); minimal and maximal effective doses were 10^{-12} mol (47% and 33%, respectively) and 10^{-10} mol (72% and 67%, respectively). This effect of AVP was suppressed by the simultaneous administration of 10^{-7} mol of AVP-A1 (Figs. 4, 5). AVP also induced a moderate rise in PBC in sham-operated rats, which was significant (50%) at a dose of 10^{-9} mol/rat and was annulled by AVP-A1 (Fig. 4). PBC did not undergo any appreciable change in autotransplanted animals (Fig. 5).

AVP concentration-dependently increased ALDO secretion of both adrenal slices (Fig. 6) and auto-transplant quarters (Fig. 7); minimal and maximal effective concentrations were 10^{-10} M (63% and 44%, respectively) and 10^{-8} M (2-fold and 2.5-fold, respectively). Again this effect of AVP was completely annulled by 10^{-5} M AVP-A1 (Figs. 6, 7). 10^{-8} M AVP evoked a marked rise in B yield by adrenal slices (2-fold) (Fig. 6), but not by autotransplant quarters (Fig. 7).

As expected, 10^{-7} M AVP elicited notable increases in ALDO and B secretions by adrenal slices (2.7-fold and 88%, respectively). ALDO response was not affected by either 10^{-7} M AL or 10^{-6} M α-CRH and CIP; B response was not changed by AL, but it was completely annulled by both α-CRH and CIP (Fig. 8).

Chronic experiments

The prolonged infusion with AVP significantly increased basal PAC level in both sham-operated (33%) and autotransplanted rats (44%); PBC increased only in sham-operated animals (25%); all these effects of AVP were annulled by the simultaneous administration of AVP-A1 (Fig. 9). The infusion with AVP-A1 alone evoked a decrease in PAC in sham-operated (-25%), but not in autotransplanted animals, and had no effect on

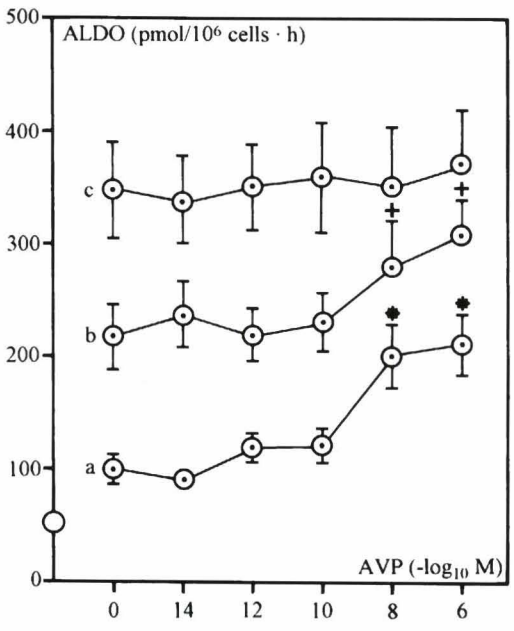

Fig. 3. Effect of AVP on 10^{-12} M (a), 10^{-10} M (b) and 10^{-8} M (c) ANG-II-stimulated ALDO secretion of dispersed rat ZG cells. Basal value is shown on the ordinate. Data are means±SE (N= 6). +: p<0.05 and *: p<0.01 from the respective 0-group.

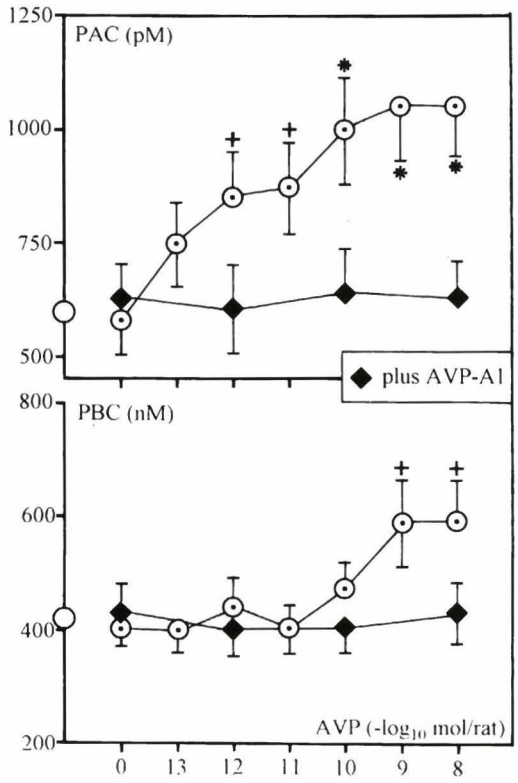

Fig. 4. Acute dose-dependent effect of AVP on PAC and PBC in sham-operated rats with pharmacologically-interrupted HPA axis and RAS, and its inhibition by the simultaneous administration of 10^{-7} mol/rat AVP-A1. Basal values in sham-operated rats with intact HPA axis and RAS are shown on the ordinates. Data are means±SE (n= 6). +: p<0.05 and *: p<0.01 from the respective 0-group.

PBC (Fig. 9).

Chronic AVP administration markedly increased the volume of ZG (32%), and the number (27%) and average volume (30%) of its parenchymal cells (Fig. 10), as well as the average volume of ZG-like cells of autotransplants (38%) (Fig. 11). Again, the simultaneous infusion with AVP-A1 annulled these effects of AVP (Figs. 10, 11). The treatment with AVP-A1 alone induced a small but significant decrease in the volume of ZG and in the number and volume of its cells (-38%, -16%, and 22%, respectively) (Fig. 10), but not in the average volume of ZG-like cells (Fig. 11). AVP caused a moderate hypertrophy of ZF cells (21%), that was annulled by AVP-A1 (Fig. 10), but not of ZF-like cells of autotransplants (Fig. 11). The infusion with AVP-A1 alone did not affect ZF-cell and ZF-like cell morphology (Figs. 10, 11).

ANG-II and ACTH (10^{-9} M) acute exposure notably enhanced ALDO and B outputs by dispersed capsular and inner cells, respectively (5.7-fold and 8.8-fold), as well as by autotransplant quarters of saline-infused rats (4.2-fold and 6.9-fold, respectively) (Fig. 12). AVP infusion significantly raised both basal and stimulated ALDO secretion of both kinds of preparations (32% and 31%, and 60% and 51%, respectively), and the simultaneous administration of AVP-A1 blocked this effect (Fig. 12). The infusion

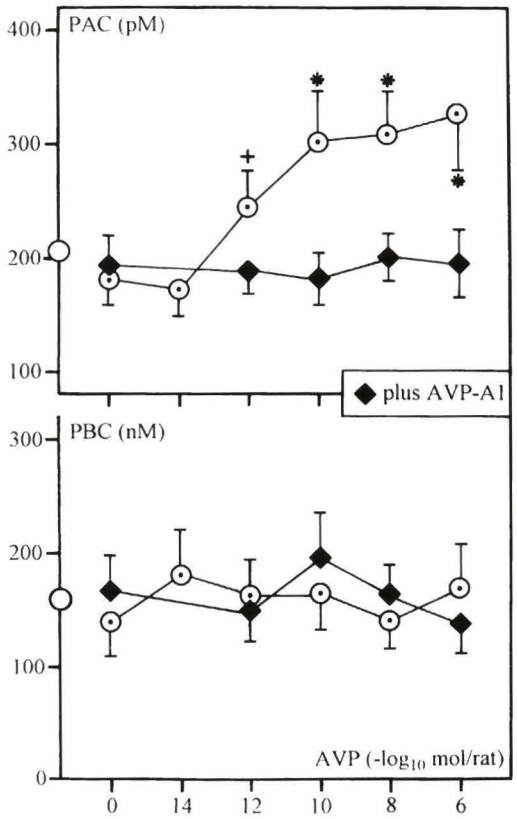

Fig. 5. Acute dose-dependent effect of AVP on PAC and PBC in autotransplanted rats with pharmacologically-interrupted HPA axis and RAS, and its inhibition by the simultaneous administration of 10^{-7} mol/rat AVP-A1. Basal values in autotransplanted rats with intact HPA axis and RAS are shown on the ordinates. Data are means±SE (n= 6). +: p<0.05 and *: p<0.01 from the respective 0-group.

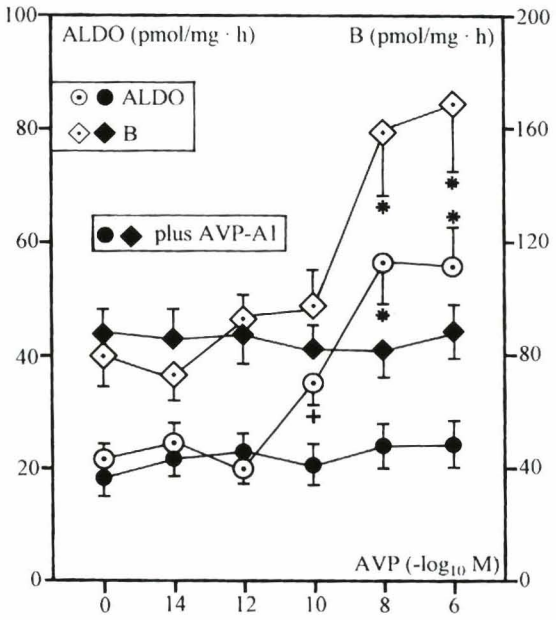

Fig. 6. Acute concentration-dependent effect of AVP on ALDO and B secretions of adrenal slices, and its inhibition by 10^{-5} M AVP-A1. Data are means±SE (n= 6). +: p<0.05 and *: p<0.01 from 0-group.

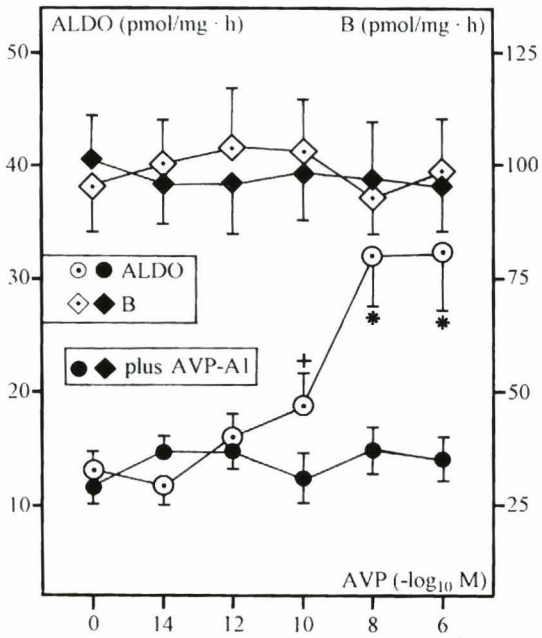

Fig. 7. Acute concentration-dependent effect of AVP on ALDO and B secretions of adrenocortical-autotransplant quarters, and its inhibition by 10^{-5} M AVP-A1. Data are means±SE. (n= 6). +: p<0.05 and *: p<0.01 from 0-group.

with AVP-A1 alone decreased basal and stimulated ALDO yield by dispersed ZG cells (-29% and -38%, respectively), but not by autotransplant quarters (Fig. 12). AVP chronic administration induced a rise in basal and stimulated B secretion of dispersed ZF/ZR cells (30% and 25%, respectively), but not of autotransplant quarters (Fig. 12); again, AVP-A1 annulled the effect of AVP, but when infused alone did not affect B secretion of either kind of preparations (Fig. 12).

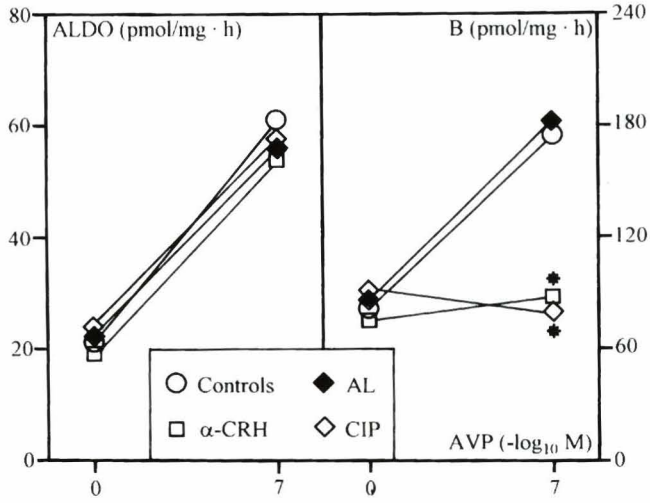

Fig. 8. Effect of 10^{-7} M AL, 10^{-6} M α-CRH and 10^{-6} M CIP on basal and AVP (10^{-7} M)-stimulated ALDO and B secretions by adrenal slices. Data are means (n= 8), and SE are not shown. *: p<0.01 from the respective control group.

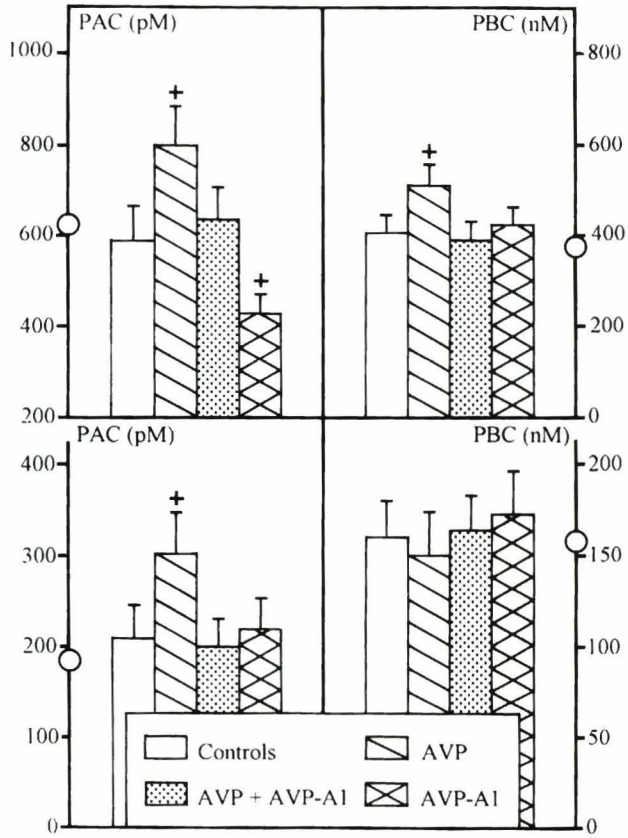

Fig. 9. Effect of 7-day infusion with AVP, AVP plus AVP-A1 and AVP-A1 on PAC and PBC in sham-operated (upper panels) and auto-transplanted rats (lower panels) with pharmacologically-interrupted HPA axis and RAS. Basal values in animals with intact HPA axis and RAS are shown on the ordinates. Data are means±SE (n= 8). +: p<0.05 from the respective control group.

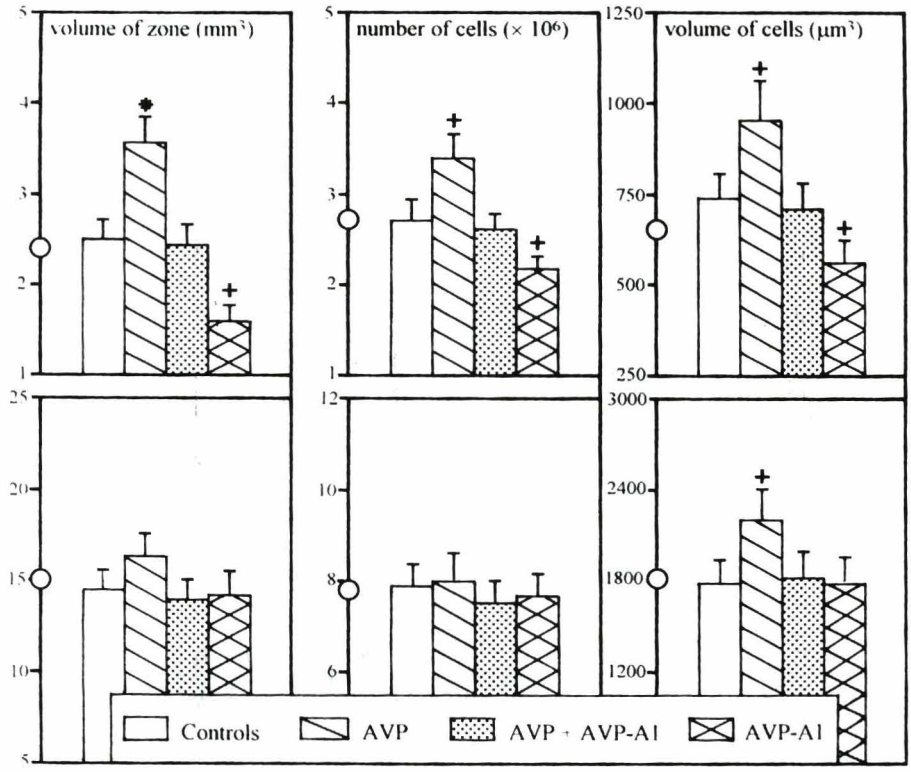

Fig. 10. Effect of 7-day infusion with AVP, AVP plus AVP-A1 and AVP-A1 on the morphometric parameters of ZG (upper panels) and ZF (lower panels) of sham-operated rats with pharmacologically-interrupted HPA axis and RAS. Basal values in animals with intact HPA axis and RAS are shown on the ordinates. Data are means±SE (n= 8). +: p<0.05 and *: p<0.01 from the respective control group.

Discussion

Mineralocorticoid secretion and ZG growth

Our present *in-vivo* and *in-vitro* findings clearly indicate that, according to previous investigations (see Introduction), AVP exerts a potent and direct acute ALDO secretagogue effect on rat ZG cells. It must be noted that a good agreement exists between the *in-vitro* and *in-vivo* minimal and maximal effective concentrations of AVP. In fact, given that the peritoneal absorption rate of peptides in rats is very high, and assuming 10-12 ml of blood per rat (Reaven et al., 1988), it may be calculated that the *bolus* i.p. injection of the minimal (10^{-12} mol) and maximal (10^{-10} mol) doses of AVP produce blood concentrations of the peptide of about 10^{-10} M and 10^{-8} M, respectively. The possibility that the *in-vivo* effect of AVP may be at least partly due to the activation of pituitary ACTH release (see Introduction) or to the interference of the AVP vasopressor and/or antidiuretic actions with kidney renin release (for review, see Brooks and Keil, 1992) may be excluded, inasmuch as we employed rats whose HPA axis and RAS had been pharmacologically interrupted (see Materials and methods).

Our results indicate that AVP-A1, but not AVP-A2, blocks ALDO secretagogue effect of AVP, and this is in keeping with the demonstration that specific AVP receptors of the V1 subtype are located on ZG cells (Antoni, 1984; Balla et al., 1985; Guillon and Gallo-Payet, 1986; Gallo-Payet et al., 1991; Lutz et al., 1993). AVP potentiates submaximally, but not maximally, ANG-II-stimulated ALDO production by dispersed ZG cells, a finding suggesting that AVP and ANG-II share the same mechanism of action. Accordingly, evidence indicates that the transduction of the ALDO secretagogue signal of AVP (like that of ANG-II) involves the activation of phosphoinositidase-C (Balla et al., 1985; Gallo-Payet et al., 1986, 1991; Guillon and Gallo-Payet, 1986; Woodcock et al., 1986, 1990a,b; Enyedi et al., 1988; Guillon et al., 1990).

As it has been previously demonstrated (see Introduction), prolonged treatment with AVP enhances the growth of rat ZG, by inducing hypertrophy and hyperplasia of its parenchymal cells. Compelling evidence indicates that the hypertrophy of adrenocortical cells is the morphological counterpart of the enhanced gene expression of the enzymes of steroid synthesis (for review, see Nussdorfer, 1986). This is confirmed by the fact that after prolonged AVP infusion basal PAC is increased and both basal and maximally ANG-II-stimulated ALDO secretion of hypertrophied ZG cells is markedly raised. AVP-A1 infusion blocked these effects of AVP, which also demonstrates that the adreno-glomerulotrophic action of AVP involves the activation of V1 receptors.

According to what has been previously shown

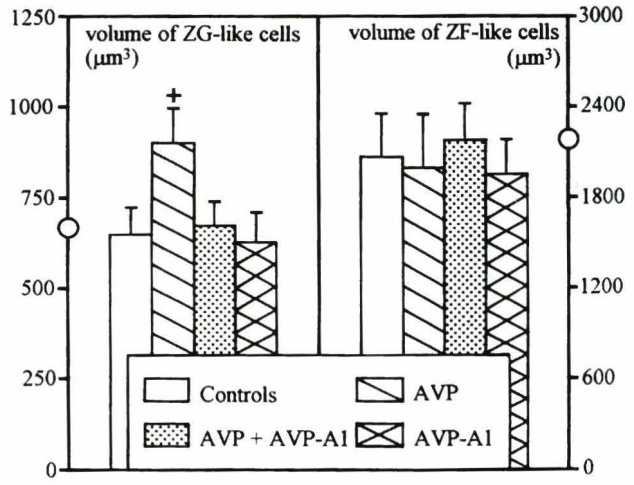

Fig. 11. Effect of 7-day infusion with AVP, AVP plus AVP-A1 and AVP-A1 on the average volume of ZG-like and ZF-like cells of adrenocortical autotransplants in rats with pharmacologically-interrupted HPA axis and RAS. Basal values in animals with intact HPA axis and RAS are shown on the ordinates. Data are means±SE (n= 8). +: $p<0.05$ from the respective control group.

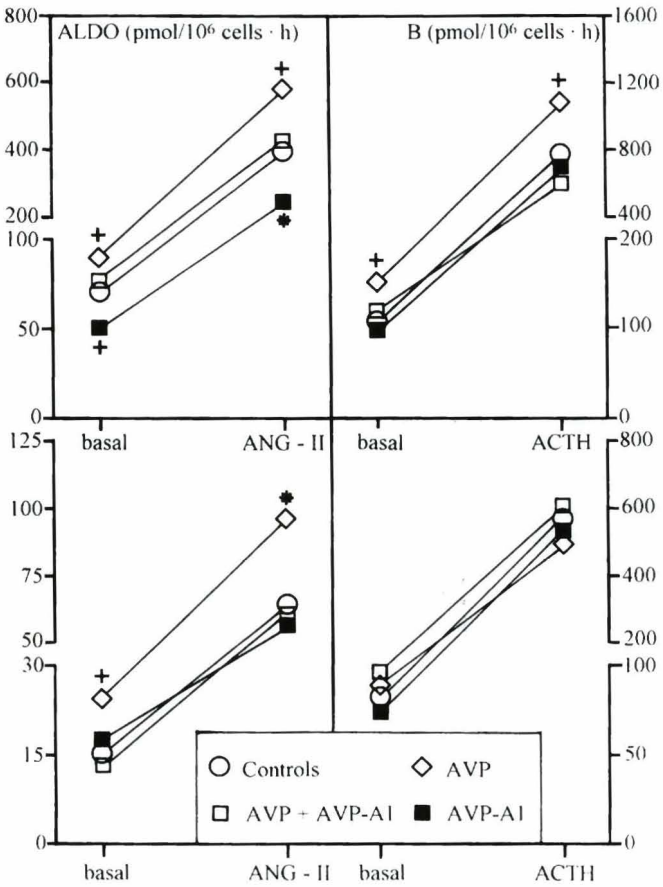

Fig. 12. Effect of 7-day infusion with AVP, AVP plus AVP-A1 and AVP-A1 on basal and agonist (10^{-9} M)-stimulated ALDO and B secretions of dispersed adrenocortical cells of sham-operated rats (upper panels) and adrenocortical nodules of autotransplanted rats (lower panels). Data are means (n= 8), and SE are not shown. +: $p<0.05$ and *: $p<0.01$ from the respective control group.

(Mazzocchi et al., 1993b), a 1-week infusion with AVP-A1 alone produces in sham-operated rats a notable atrophy of ZG and a significant decrease of its ALDO secretory capacity. This finding strongly suggests that endogenous AVP plays a role in the physiological control of ZG function in rats. However, this contention apparently conflicts with the fact that the minimal effective concentrations of AVP exerting an acute ALDO secretagogue action are about 100 pM, while under basal conditions AVP blood level in mammals is about 2-3 pM. Also, in the case of extreme stimulation of pituitary AVP release (e.g. severe haemorrhage or prolonged water restriction), plasma AVP does not exceed a concentration of 50-100 pM (Dunn et al., 1973; Baylis, 1989). This consideration suggests that posterior pituitary is not the source of AVP involved in the maintenance and stimulation of ZG function, and forces us to admit the existence of a local intra-adrenal source of AVP. Our present data seem to throw light onto this topic.

Regenerated adrenocortical nodules secrete both mineralo- and glucocorticoids, and are responsive to ACTH and ANG-II; their subcapsular and juxta-septal ZG-like cells have ANG-II receptors (Belloni et al., 1990). However, due to the lower weight of regenerated adrenocortical tissue in comparison to that of normal adrenals, PAC and PBC are markedly lower than in sham-operated rats (Belloni et al., 1990, 1991). Autotransplants regenerated from adrenal capsular fragments are completely devoid of chromaffin cells, a contention based on both morphological (serial sectioning) and biochemical findings (HPLC evaluation of catecholamine content) (Belloni et al., 1990).

Autotransplanted rats respond to acute AVP administration by a marked increase in PAC, and autotransplant quarters display a clearcut ALDO response to AVP; prolonged AVP infusion enhances the growth and steroidogenic capacity of ZG-like cells. All these AVP effects are blocked by AVP-A1. Thus, it appears evident that ZG-like cells of autotransplants, like ZG cells of normal adrenals, possess V1-receptors and intracellular mechanism(s) transducing the mineralocorticoid secretagogue signal of AVP. However, at variance with sham-operated rats, autotransplanted animals do not show any response to the infusion with AVP-A1 alone, a finding which reasonably suggests that the local production of endogenous AVP exerting adrenoglomerulotropic action is not operative in them.

Taken together, these results allow us to hypothesize that adrenal medulla may be the local source of endogenous AVP paracrinally controlling ZG function in rats. Two lines of evidence lend support to this view: (i) it is well demonstrated that adrenal medulla, by secreting catecholamines and many regulatory peptides, exerts a paracrine control of the cortex function (Bornstein et al., 1990b, Hinson, 1990; Hinson et al., 1992; Mazzocchi et al., 1993a; Malendowicz et al., 1994a,b; Rebuffat et al., 1994), the morphological background of which may be the presence of abundant interdigitations between medullary and cortical tissues occurring in the adrenal gland (Gallo-Payet et al., 1987; Bornstein et al., 1991, 1994; Bornstein and Ehrhart-Bornstein, 1992); and (ii) adrenal chromaffin cells of several mammalian species contain AVP immunoreactivity (Ang and Jenkins, 1984; Nicholson et al., 1984; Nussey et al., 1984, 1987; Ivell et al., 1986; Ravid et al., 1986; Hawthorn et al., 1987). In this connection, it must also be recalled that arginine-vasotocin, the amphibian counterpart of mammalian AVP, is contained in the chromaffin cells of the frog interrenals and is able to paracrinally stimulate adrenocortical-cell secretion (Larcher et al., 1989, 1992).

Glucocorticoid secretion and ZF growth

Our present investigation confirms the view (see Introduction) that quite high concentrations of AVP exert a stimulatory effect on the secretory activity and growth of inner zones of rat adrenal cortex. Also, these effects of AVP appear to be mediated by V1-receptors, since they are blocked by AVP-A1. Bird et al. (1990) reported that AVP elicits cortisol secretion of cultured bovine ZF/ZR cells, by stimulating phosphoinositidase-C; however, it must be recalled that bovine ZF cells, at variance with those of rats, are sensitive to ANG-II and possess an active phosphatidylinositol mechanism of transduction (Rainey et al., 1991; Bird et al., 1992a,b; Luong et al., 1992; Quali et al., 1992). Our *in-vitro* findings cast doubts on the possibility that AVP acts directly on ZF cells, inasmuch as dispersed inner cells do not evidence a B response to AVP.

Nevertheless, high concentrations of AVP evoke a sizable increase in B output by adrenal slices, containing both cortical and medullary tissue. This rather intriguing finding may be explained in the light of several investigations demonstrating that the integrity of adrenal tissue is frequently needed for many neuropeptides may exert their steroidogenic action (for review, see Vinson et al., 1985, 1992). This may be due to the fact that, as has been recently demonstrated for oxytocin (Warchol et al., 1993), the integrity of cell-to-cell contacts allows the spreading of the secretagogue signal from the few responsive cells to the bulk of the unresponsive-cell population. However, this does not seem to be the case as far as AVP is concerned, since AVP does not elicit any appreciable glucocorticoid secretory response by either enucleated (i.e. demedullated) adrenal fragments (data not shown) or autotransplant quarters. Moreover, the acute and chronic administrations of AVP do not affect *in-vivo* B secretion in autotransplanted rats, nor do they stimulate ZF-like cell growth. On the basis of these data, the hypothesis that adrenal chromaffin cells play a pivotal role in the mediation of AVP effect on inner adrenocortical zones of the rat adrenal gland can be advanced.

AVP receptors of the V1 subtype are present in adrenal medullary cells (Taylor et al., 1989). Hence, it may be conceived that AVP stimulates the secretion by chromaffin cells of an agonist, which, in turn,

paracrinally enhances glucocorticoid secretion and the growth of inner adrenocortical cells (see above). Evidence indicates that catecholamines are able to enhance steroidogenesis *in vitro* (De Léan et al., 1984; Pratt et al., 1985; Horiuchi et al., 1987; Racz et al., 1987; Bertin et al., 1991; Walker et al., 1991; Vizi et al., 1992). However, AVP does not seem to enhance catecholamine secretion (Porter et al., 1988; Taylor et al., 1989), and AL, a potent ß-adrenoreceptor antagonist, does not block either ALDO or B responses of adrenal slices to AVP. Many lines of evidence suggest the existence of a CRH/ACTH system in the rat adrenal medulla, which may affect the cortex function in a paracrine manner (Bornstein et al., 1990a, Andreis et al., 1991a, 1992; Markowska et al., 1993), and whose activity is enhanced when hypothalamo-pituitary CRH/ACTH system is suppressed by hypophysectomy (Mazzocchi et al., 1994b). It has also been shown that interleukin-1ß (Andreis et al., 1991b; Mazzocchi et al., 1993c), neuromedin U-8 (Malendowicz et al., 1994a,b) and neuropeptide K (Mazzocchi et al., 1994a) exert a direct secretagogue effect on the rat adrenal cortex by stimulating such a local intramedullary CRH/ACTH system. In light of these considerations, it appears reasonable to conceive that AVP, in addition to stimulating the central branch of the CRH/ACTH system (see Introduction), may also activate the intramedullary peripheral one. Our present findings lend a strong support to this contention, inasmuch as the AVP-induced B (but not ALDO) response of adrenal slices is completely abolished by both α-CRH and CIP, two competitive inhibitors of CRH and ACTH, respectively.

The stimulatory effect of AVP on ZF growth and secretory activity does not appear to play, on the contrary to that on ZG and ALDO secretion, an important physiological role. In fact, minimal effective concentrations of AVP are very high (at least 10 nM), and the treatment with AVP-A1 alone does not affect the growth and glucocorticoid secretory activity of adrenal ZF in sham-operated rats.

Conclusions

Our investigations clearly demonstrate that AVP plays a physiological role in the maintenance, and probably in the stimulation of rat ZG growth and mineralocorticoid secretory activity. The source of endogenous AVP exerting this effect seems to be adrenal medulla, and AVP released by chromaffin cells paracrinally acts on ZG cells by binding to V1-receptors. AVP also stimulates the growth and glucocorticoid secretory activity of rat adrenal ZF, probably by activating the intramedullary CRH/ACTH system via V1-receptor located in chromaffin cells. However, this effect of AVP does not appear to have a physiological relevance, at least under basal conditions.

References

Andreis P.G., Neri G. and Nussdorfer G.G. (1991a). Corticotropin-releasing hormone (CRH) directly stimulates corticosterone secretion by the rat adrenal gland. Endocrinology 128, 1198-1200.

Andreis P.G., Neri G., Belloni A.S., Mazzocchi G. and Nussdorfer G.G. (1991b). Interleukin-1ß enhances corticosterone secretion by acting directly on the rat adrenal gland. Endocrinology 129, 53-57.

Andreis P.G., Neri G., Mazzocchi G., Musajo F.G. and Nussdorfer G.G. (1992). Direct secretagogue effect of corticotropin-releasing factor on the rat adrenal cortex: the involvement of the zona medullaris. Endocrinology 131, 69-72.

Ang V.T.Y. and Jenkins J.S. (1984). Neurohypophysial hormones in the adrenal medulla. J. Clin. Endocrinol. Metab. 58, 688-691.

Antoni F.A. (1984). Novel ligand specificity of pituitary vasopressin receptors in the rat. Neuroendocrinology 39, 186-188.

Antoni F.A. (1986). Hypothalamic control of adrenocorticotropin secretion: advances since the discovery of 41-residue corticotropin-releasing factor. Endocr. Rev. 7, 351-378.

Apostolakis E.M., Longo L.D. and Yellon S.M. (1991). Regulation of basal adrenocorticotropin and cortisol secretion by arginine vasopressin in the fetal sheep during late gestation. Endocrinology 129, 295-300.

Bähr V., Hensen J., Hader O., Bolke T. and Oelkers W. (1991). Stimulation of steroid secretion by adrenocorticotropin injections and by arginine vasopressin infusions. No evidence for a direct stimulation of the human adrenal by arginine vasopressin. Acta Endocrinol. 125, 348-353.

Bähr V., Sander-Bähr C., Hensen J. and Oelkers W. (1993). Influence of sodium and potassium diets on adrenal vasopressin content and direct effects of vasopressin on aldosterone synthesis in adrenocortical cells. Horm. Metab. Res. 25, 411-416.

Balla T., Enyedi P., Spät A. and Antoni F.A. (1985). Pressor-type vasopressin receptors in the adrenal cortex: properties of binding effects on phosphoinositide metabolism and aldosterone secretion. Endocrinology 117, 421-423.

Baylis P.H. (1989). Vasopressin and its neurophysin. In: Endocrinology. 1st ed. Vol. 1. De Droot L.J. (ed) W.B. Saunders Company. Philadelphia,. London. pp 213-219.

Belloni A.S., Neri G., Musajo F.G., Andreis P.G., Boscaro M., D'Agostino D., Rebuffat P., Boshier D.P., Gottardo G., Mazzocchi G. and Nussdorfer G.G. (1990). Investigations on the morphology and function of adrenocortical tissue regenerated from gland capsular fragments autotransplanted in the *musculus gracilis* of the rat. Endocrinology 126, 3251-3262.

Belloni A.S., Neri G., Andreis P.G., Musajo F.G., Boscaro M., Mazzocchi G. and Nussdorfer G.G. (1991). Effects of prolonged sodium restriction on the morphology and function of rat adrenocortical autotransplants. Cell Tissue Res. 265, 35-41.

Bertin R., De Marco F., Laury M.C. and Portet R. (1991). Are adrenal catecholamines involved in the enhancement of aldosterone production in cold acclimated rats? Arch. Int. Physiol. Biochim. Biophys. 99, 479-488.

Bird I.M., Nicol M., Williams B.C. and Walker S.W. (1990). Vasopressin stimulates cortisol secretion and phosphoinositide catabolism in cultured bovine adrenal fasciculata/reticularis cells. J. Mol. Endocrinol. 5, 109-116.

Bird I.M., Clyne C.D., Lyghtly E.R.T., Williams B.C. and Walker S.W. (1992a). Further characterization of the steroidogenic

responsiveness of purified zona fasciculata/reticularis cells from bovine adrenal cortex before and after primary culture changing responsiveness to phosphoinositidase-C agonists. J. Endocrinol. 133, 21-28.

Bird I.M., Williams B.C. and Walker S.W. (1992b). Identification and metabolism of phosphoinositol species formed on angiotensin-II stimulation of zona fasciculata/reticularis cells from the bovine adrenal cortex. Mol. Cell. Endocrinol. 83, 29-38.

Bornstein S.R. and Ehrhart-Bornstein M. (1992). Ultrastructural evidence for a paracrine regulation of the rat adrenal cortex mediated by the local release of catecholamines from chromaffin cells. Endocrinology 131, 3126-3128.

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

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

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

Bornstein S.R., Gonzales-Hernandez J.A., Ehrhart-Bornstein M., Adler G. and Scherbaum W.A. (1994). Intimate contact of chromaffin and cortical cells within the human adrenal gland forms the cellular basis for important intraadrenal interactions. J. Clin. Endocrinol. Metab. 78, 225-233.

Brooks V.L. and Blakemore L.J. (1989). Vasopressin: a regulator of adrenal glucocorticoid production. Am. J. Physiol. 256, E566-E572.

Brooks V.L. and Keil L.C. (1992). Vasopressin and angiotensin-II in reflex regulation of ACTH, glucocorticoids and renin. Effect of water deprivation. Am. J. Physiol. 263, R762-R769.

Brooks A.N. and White A. (1990). Activation of pituitary-adrenal function in fetal sheep by corticotrophin-releasing factor and arginine vasopressin. J. Endocrinol. 124, 27-35.

Buckingham J.C., Smith T. and Loxley H.D. (1992). The control of ACTH secretion. In: The adrenal gland. 2nd ed. James V.H.T. (ed). Raven Press. New York. pp 131-158.

De Léan A., Racz K., Mc Nicoll N. and Desrosier M.L. (1984). Direct ß-adrenergic stimulation of aldosterone secretion in cultured bovine adrenal subcapsular cells. Endocrinology 115, 485-492.

Dunn F.L., Brennan T.J., Nelson A.E. and Robertson G.L. (1973). The role of blood osmolality and volume in regulating vasopressin secretion in the rat. J. Clin. Invest. 52, 3212-3219.

Enyedi P., Balla T., Antoni F.A. and Spät A. (1988). Effect of angiotensin II and arginine vasopressin on aldosterone production and phosphoinositide turnover in rat adrenal glomerulosa cells: a comparative study. J. Mol. Endocrinol. 1, 117-124.

Faucher D.J., Laptook A.R., Parker C.R., Porter J.C. and Rosenfeld C.R. (1988). Increased fetal secretion of ACTH and cortisol by arginine vasopressin. Am. J. Physiol. 254, R410-R416.

Gallo-Payet N., Guillon G., Balestre M.N. and Jard S. (1986). Vasopressin induces breakdown of membrane phosphoinositides in adrenal glomerulosa and fasciculata cells. Endocrinology 119, 1042-1047.

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

Gallo-Payet N., Chouinard L., Balestre M.N. and Guillon G. (1991). Involvement of protein kinase C in the coupling between the V_1 vasopressin receptor and phospholipase C in rat glomerulosa cells. Effects on aldosterone secretion. Endocrinology 129, 623-634.

Guillon G. and Gallo-Payet N. (1986). Specific vasopressin binding to rat adrenal glomerulosa cells. Relationship to inositol lipid breakdown. Biochem. J. 235, 209-214.

Guillon G., Balestre M.N., Chouinard L. and Gallo-Payet N. (1990). Involvement of distinct G-proteins in the action of vasopressin on rat glomerulosa cells. Endocrinology 126, 1699-1708.

Hawthorn J., Nussey S.S., Henderson J.R. and Jenkins J.S. (1987). Immunohistochemical localization of oxytocin and vasopressin in the adrenal glands of rat, cow, hamster and guinea pig. Cell Tissue Res. 250, 1-6.

Hensen J., Hader O., Bähr V. and Oelkers W. (1988). Effects of incremental infusions of arginine vasopressin on adrenocorticotropin and cortisol secretion in man. J. Clin. Endocrinol. Metab. 66, 668-671.

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

Hinson J.P., Vinson G.P., Porter I.D. and Whitehouse B.J. (1987). Oxytocin and arginine vasopressin stimulate steroid secretion by the isolated perfused rat adrenal gland. Neuropeptides 10, 1-7.

Hinson J.P., Kapas S., Orford C.D. and Vinson G.P. (1992). Vasoactive intestinal peptide stimulation of aldosterone secretion by the rat adrenal cortex may be mediated by the local release of catecholamines. J. Endocrinol. 133, 253-258.

Horiuchi T., Tanaka K. and Shimizu N. (1987). Effect of catecholamines on aldosterone release in isolated rat glomerulosa cell suspension. Life Sci. 40, 2421-2428.

Isler H. (1973). Effect of posterior pituitary powder on the mitotic activity of the zona glomerulosa of the adrenal gland in intact and hypophysectomized rats: a preliminary observation. Anat. Rec. 177, 321-324.

Ivell R., Schmale H., Krisch B., Nahke P. and Richter D. (1986). Expression of a mutant vasopressin gene: differential polyadenylation and read-trough of the mRNA 3' end in a frame-shift mutant. EMBO J. 5, 971-977.

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

Lesniewska B., Nowak M., Miskowiak B., Nussdorfer G.G. and Malendowicz L.K. (1991). Effects of arginine-vasopressin on the pituitary-adrenocortical axis of intact and dexamethasone-suppressed rats. Exp. Pathol. 43, 181-188.

Li C.H., Chung D., Yamashiro D. and Lee C.Y. (1978). Isolation, characterization and synthesis of a corticotropin-inhibiting peptide from human pituitary glands. Proc. Nat. Acad. Sci. USA 75, 4306-4309.

Luong T.T., Boulay G. and Guillemette G. (1992). Study of the stereoselectivity of inositol 1, 4, 5-triphosphate recognition sites of bovine adrenal cortex. Can. J. Physiol. Pharmacol. 70, 434-441.

Lutz R.A., Tomasz G., Luem S., Blum P. and Pliska V. (1993).

Vasopressin receptors in the adrenal cortex of sheep. Does autoradiography indicate an irreversible binding of the ligand? J. Receptor Res. 13, 283-294.

Malendowicz L.K., Andreis P.G., Markowska A., Nowak M., Warchol J.B., Neri G. and Nussdorfer G.G. (1994a). Effects of neuromedin U-8 on the secretory activity of the rat adrenal cortex: evidence for an indirect action requiring the presence of the zona medullaris. Res. Exp. Med. 194, 69-79.

Malendowicz L.K., Nussdorfer G.G., Markowska A., Tortorella C., Nowak M. and Warchol J.B. (1994b). Effects of neuromedin U (NMU)-8 on the rat hypothalamo-pituitary-adrenal axis. Evidence of a direct effect of NMU-8 on the adrenal gland. Neuropeptides 26, 47-53.

Manning M., Stoev S., Kolodziejczyk A., Klis W.A., Kruszynski M., Misicka A., Olma A., Wo N.C. and Sawyer W.H. (1990). Design of potent and selective linear antagonists of vasopressor (V1-receptor) responses to vasopressin. J. Med. Chem. 33, 3079-3085.

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

Mazzocchi G., Malendowicz L.K., Meneghelli V., Gottardo G. and Nussdorfer G.G. (1993a). Vasoactive intestinal polypeptide (VIP) stimulates hormonal secretion of the rat adrenal cortex *in vitro*: evidence that adrenal chromaffin cells are involved in the mediation of the mineralocorticoid, but not glucocorticoid secretagogue action of VIP. Biomed. Res. 14, 435-440.

Mazzocchi G., Markowska A., Malendowicz L.K., Musajo F., Meneghelli V. and Nussdorfer G.G. (1993b). Evidence that endogenous arginine-vasopressin (AVP) is involved in the maintenance of the growth and steroidogenic capacity of rat adrenal zona glomerulosa. J. Steroid Biochem. Mol. Biol. 45, 251-256.

Mazzocchi G., Musajo F.G., Malendowicz L.K., Andreis P.G. and Nussdorfer G.G. (1993c). 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., Malendowicz L.K., Andreis P.G., Meneghelli V., Markowska A., Belloni A.S. and Nussdorfer G.G. (1994a). Neuropeptide K enhances glucocorticoid release by acting directly on the rat adrenal gland: the possible involvement of zona medullaris. Brain Res. 661, 91-96.

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

Neri G., Malendowicz L.K., Andreis P.G. and Nussdorfer G.G. (1993). Thyrotropin-releasing hormone inhibits glucocorticoid secretion of rat adrenal cortex: *in vivo* and *in vitro* studies. Endocrinology 133, 511-514.

Nicholson H.D., Swann R.W., Burford G.D., Wathes D.C., Porter D.G. and Pickering B.T. (1984). Identification of oxytocin and vasopressin in the testis and in adrenal tissue. Regul. Pept. 8, 141-146.

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

Nussdorfer G.G., Malendowicz L.K., Belloni A.S., Mazzocchi G. and Rebuffat P. (1988). Effects of substance P on the rat adrenal zona glomerulosa *in vivo*. Peptides 9, 1145-1149.

Nussey S.S., Ang V.T.Y. and Jenkins J.S. (1984). Blattleboro rat adrenal contains vasopressin. Nature 310, 64-66.

Nussey S.S., Prysor-Jones R.A., Ang V.T.Y. and Jenkins J.S. (1987). Arginine vasopressin and oxytocin in the bovine adrenal gland. J. Endocrinol. 115, 141-149.

Payet N. and Isler H. (1976). Adrenal glomerulosa mitotic stimulation by posterior pituitary hormones. Cell Tissue Res. 172, 93-101.

Payet N. and Lehoux J.G. (1980). A comparative study of the role of vasopressin and ACTH in the regulation of growth and function of rat adrenal glands. J. Steroid Biochem. 12, 461-467.

Payet N. and Lehoux J.G. (1982). Aldosterone and corticosterone stimulation by ACTH in isolated rat adrenal glomerulosa cells. Interaction with vasopressin. J. Physiol. (Paris) 78, 317-321.

Payet N., Deziel Y. and Lehoux J.G. (1984). Vasopressin: a potent growth factor in adrenal glomerulosa cells in culture. J. Steroid Biochem. 20, 449-454.

Perraudin V., Delarue C., Lefebvre H., Contesse V., Kuhn J.M. and Vaudry H. (1993). Vasopressin stimulates cortisol secretion from human adrenocortical tissue through activation of V1 receptors. J. Clin. Endocrinol. Metab. 76, 1522-1528.

Porter I.D., Whitehouse B.J., Taylor A.H. and Nussey S.S. (1988). Effect of arginine vasopressin and oxytocin on acetylcholine-stimulation of corticosteroid and catecholamine secretion from the rat adrenal gland perfused *in situ*. Neuropeptides 12, 265-271.

Pratt J.H., Turner D.A., McAteer J.A. and Henry D.P. (1985). Beta-adrenergic stimulation of aldosterone production by rat adrenal capsular explants. Endocrinology 117, 1189-1194.

Quali R., Poulette S., Penhoat A. and Saez J.M. (1992). Characterization and coupling of angiotensin-II receptor subtypes in cultured bovine adrenal fasciculata cells. J. Steroid Biochem. Mol. Biol. 43, 271-280.

Quinn S.J. and Williams G.H. (1992). Regulation of aldosterone secretion. In: The adrenal gland. 2nd ed. James V.T.H. (ed). Raven Press. New York. pp 159-189.

Quinn S.J., Enyedi P., Tillotson D.L. and Williams G.H. (1990). Cytosolic calcium and aldosterone response patterns of rat adrenal glomerulosa cells stimulated by vasopressin: comparison with angiotensin II. Endocrinology 127, 541-548.

Racz K., De Léan A., Kuchel O. and Buu N.T. (1987). Adrenomedullary mechanisms in aldosterone regulation. In: Corticosteroids and peptide hormones in hypertension. Mantero F. and Vecsei P. (eds). Raven Press. New York. pp 77-90.

Rainey W.E., Byrd E.W., Sinnokrot R.A. and Carr B.R. (1991). Angiotensin-II activation of cAMP and corticosterone production in bovine adrenocortical cells. Effects of nonpeptide angiotensin-II antagonists. Mol. Cell. Endocrinol. 81, 33-42.

Ravid R., Oosterbaan H.P., Hausen B.L. and Swaab D.F. (1986). Localisation of oxytocin, vasopressin and parts of precursors in the human neonatal adrenal. Histochemistry 84, 401-407.

Reaven E., Kostrna M., Ramachandran J. and Azhar S. (1988). Structure and function changes in rat adrenal glands during aging. Am. J. Physiol. 255, E903-E911.

Rebuffat P., Belloni A.S., Malendowicz L.K., Mazzocchi G., Gottardo G. and Nussdorfer G.G. (1988). Zona glomerulosa morphology and function in streptozotocin-induced diabetic rats. Endocrinology 123, 949-955.

Rebuffat P., Kasprzak A., Andreis P.G., Mazzocchi G., Gottardo G., Coi A. and Nussdorfer G.G. (1989). Effects of prolonged cyclosporine-A treatment on the morphology and function of the rat adrenal cortex. Endocrinology 125, 1407-1413.

Rebuffat P., Belloni A.S., Musajo F.G., Rocco S., Markowska A., Mazzocchi G. and Nussdorfer G.G. (1994). Evidence that endogenous somatostatin (SRIF) exerts an inhibitory control on the function and growth of rat adrenal zona glomerulosa. The possible involvement of zona medullaris as a source of endogenous SRIF. J. Steroid Biochem. Mol. Biol. 48., 353-360.

Rees L.H., Cook D.M., Kendall J.W., Allen C.F., Kramer R.M., Ratcliffe J.G. and Knight R.A. (1971). A radioimunoassay for rat plasma ACTH. Endocrinology 89, 254-261.

Rivier J., Rivier C. and Vale W. (1984). Synthetic competitive antagonists of corticotropin-releasing factor: effect on ACTH secretion in the rat. Science 224, 889-890.

Schneider E.G. (1988). Effect of vasopressin on adrenal steroidogenesis. Am. J. Physiol. 255, R806-R811.

Szalay K.S. (1981). Effect of pituitary intermediate lobe extract on steroid production by isolated zona glomerulosa and fasciculata cells. Acta Physiol. Hung. 57, 225-231.

Taylor A.H., Whitley G. St. J. and Nussey S.S. (1989). The interaction of arginine vasopressin and oxytocin with bovine adrenal medulla cells. J. Endocrinol. 121, 133-139.

Vinson G.P., Hinson J.P. and Raven P.W. (1985). The relationship between tissue preparation and function; methods for the study of control of aldosterone secretion: a review. Cell Biochem. Funct. 3, 235-253.

Vinson G.P., Whitehouse B.J. and Hinson J.P. (1992). The adrenal cortex. Prentice Hall. Englewood Cliffs. New Jersey.

Vizi E.S., Toth 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 the fine tuning of steroid secretion from zona glomerulosa cells: functional and morphological evidence. J. Endocrinol. 135, 551-561.

Walker S.W., Lightly E.R.T., Williams B.C. and Bird I.M. (1991). Adrenergic and cholinergic regulation of steroidogenesis from cultured bovine adrenal zona fasciculata/reticularis cells. Endocrine Res. 17, 237-265.

Warchol J.B., Filipiak K., Ignaszak E., Nussdorfer G.G. and Malendowicz L.K. (1993). Oxytocin directly stimulates corticosterone secretion by dispersed rat adrenal zonae fasciculata and reticularis cells: evidence for the spreading of the oxytocin-evoked signal from responsive to unresponsive cells. Biomed. Res. 14, 261-264.

Weibel E.R. (1979). Stereological methods. 1. Practical methods for biological morphometry. Academic Press. London.

Woodcock E.A., McLeod J.K. and Johnson C.I. (1986). Vasopressin stimulates phosphatidylinositol turnover and aldosterone synthesis in rat adrenal glomerulosa cells: comparison with angiotensin II. Endocrinology 118, 2432-2436.

Woodcock E.A., Little P.J. and Tanner J.K. (1990a). Inositol phosphate release and steroidogenesis in rat adrenal glomerulosa cells. Comparison of the effects of endothelin, angiotensin II and vasopressin. Biochem. J. 271, 791-796.

Woodcock E.A., Tanner J.K., Caroccia L.M. and Little P.J. (1990b). Mechanisms involved in the stimulation of aldosterone production by angiotensin II, vasopressin and endothelin. Clin. Exp. Pharmacol. Physiol. 17, 263-268.

Accepted November 30, 1994