Br. J. Pharmac. (1982), **76**, 579–585

RELEASE OF β-LIPOTROPIN- AND β-ENDORPHIN-LIKE MATERIAL INDUCED BY ANGIOTENSIN IN THE CONSCIOUS RAT

U. BEUERS, G. HERTTING & W. KNEPEL

Pharmakologisches Institut der Universität Freiburg i.Br., Hermann-Herder-Str. 5, D-7800 Freiburg i. Br., GFR

1 The influence of the renin-angiotensin system on plasma β-endorphin-like immunoreactivity (β-EI) was investigated in the conscious rat by use of a radioimmunoassay for β-endorphin without prior extraction.

2 Intravenous infusion of angiotensin I, II or (des-1-Asp)angiotensin II (angiotensin III) caused a dose-dependent increase in plasma β-EI, angiotensin III infusion being less effective than angiotensin I or II. The plasma adrenocorticotrophin (ACTH) levels too were elevated by angiotensin II. The receptor antagonist, saralasin, prevented the angiotensin II-induced β-EI release as did dexamethasone pretreatment.

3 Both the release of β-EI and the pressor response to angiotensin I were abolished by the converting enzyme inhibitor, captopril (SQ 14225). In contrast, captopril did not affect the action of angiotensin II.

4 In view of the appreciable cross-reactivity of β-lipotropin (β-LPH) in our assay, plasma β-EI was analysed by Sephadex G-50 chromatography. In plasma extracts of angiotensin II-infused rats, immunoreactivity corresponding to human β-endorphin comprised about 49% of the total immunoreactivity, whereas 51% co-migrated with human β-LPH.

5 The increase in plasma levels of β-EI elicited by angiotensin II was diminished by about 35% in rats with a hereditary absolute lack of vasopressin (Brattleboro rats), when compared to normal rats.

6 These results suggest that the renin-angiotensin system can stimulate the secretion of β-LPH and β-endorphin with ACTH from rat anterior pituitary. One link in mediating the response appears to be vasopressin. The physiological function remains to be defined.

Introduction

Several lines of evidence suggest that the renin-angiotensin system may be involved in the control of β-endorphin release from the anterior lobe of the pituitary gland. (1) Specific angiotensin binding sites are distributed throughout the hypothalamus including the median eminence and are found in the adenohypophysis in especially high concentration (Sirett, McLean, Bray & Hubbard, 1977; Harding, Stone & Wright, 1981). (2) In these brain structures as well as in the pituitary, angiotensin-like immunoreactivity is stained by immunocytochemistry (Changaris, Keil & Severs, 1978; Quinlan & Phillips, 1981). The staining in fibres projecting to the zona externa of the median eminence is increased after bilateral adrenalectomy (Kilcoyne, Hoffman & Zimmerman, 1980), when plasma β-endorphin-like immunoreactivity (β-EI) is markedly elevated (Guillemin, Vargo, Rossier, Minick, Ling, Rivier, Vale & Bloom, 1977). However, no angiotensin II was found in brain extracts by radioimmunoassay after high pressure liquid chromatography (Meyer, Phillips & Eiden, 1982). (3) Blood-borne angiotensin has access to binding sites of the anterior pituitary or of several circumventricular organs including the median eminence (Gregg & Malvin, 1977; van-Houten, Schiffrin, Mann, Posner & Boucher, 1980). (4) In dogs, angiotensin II infusion was shown to raise plasma adrenocorticotrophin (ACTH) concentration (Ramsay, Keil, Sharpe & Shinsako, 1978). Since ACTH and β-endorphin are derived from a common precursor molecule (Mains, Eipper & Ling, 1977) and are released concomitantly in response to several stimuli (Guillemin *et al.*, 1977), this suggests that angiotensin may also affect β-endorphin release.

Therefore, we investigated in the conscious rat the effect of agiotensin I, II or (des-1-Asp)angiotensin II (angiotensin III) infusion on plasma β-EI.

0007-1188/82/080579-07 $01.00

Methods

The experiments were generally performed on conscious male Wistar rats (200–250 g). When indicated, normal male Long Evans rats and male rats with hereditary hypothalamic diabetes insipidus from the Brattleboro strain of Long Evans rats (280–330 g) were used. The Brattleboro rats are totally unable to synthesize or secrete vasopressin (Valtin, 1977). The animals were allowed food and water *ad libitum* until the experiments began and were placed in a room with constant temperature (23°C) and humidity (55%), with lights on from 06 h 00 min to 18 h 00 min.

Experimental procedures

Experiments were performed between 10 h 00 min and 12 h 00 min. Drugs were given by the intravenous or intraperitoneal route. Except for saralasin, doses quoted are in terms of the base. For intravenous administration, rats were restrained in cylindrical wire cages and the tail vein cannulated (PE 50). The angiotensins or the vehicle (0.1 M phosphate buffer, pH 7.4) were infused for 20 min by means of an 'infusor'-pump (Braun-Melsungen) (infusion rate, 42 µl/min) and blood was collected immediately thereafter. In studies including angiotensin receptor blockade by saralasin, infusion of the antagonist began 10 min before the onset of angiotensin infusion and continued until the end of the experiment. Captopril (SQ 14225) (15 mg/kg) or the vehicle (0.1 M phosphate buffer, pH 7.4) was injected intravenously in a volume of 0.1 ml per 100 g body weight 10 min before the start of the angiotensin I or II infusion. The dose of captopril was chosen to ensure almost complete inhibition of angiotensin-converting enzyme for a sufficient length of time (Katovich, Barney, Fregly & McCaa, 1979). Dexamethasone (0.5 mg/kg in a volume of 5 ml/kg) or saline (0.9% w/v NaCl solution) was given intraperitoneally the day before the experiment and once more (same dose) 1 h 30 min before the start of the infusion.

Rats were killed by rapid decapitation and trunk blood was collected in polypropylene tubes containing 0.25 ml of a solution of disodium edetate (0.18 mol/l), control and experimental animals being killed alternately. The plasma was separated by centrifugation (2,000 *g* for 10 min at 4°C) and centrifuged once more (10,000 *g* for 10 min at 4°C).

Measurements

An aliquot of 0.5 or 1.0 ml plasma was analysed for β-endorphin-like immunoreactivity (β-EI) by radioimmunoassay without prior extraction, as has been described recently (Anhut, Knepel, Nutto & Hertting, 1981). The antiserum was raised against human β-endorphin and human ^{125}I-β-endorphin was used as tracer. Detection limit of the assay was about 7 fmol per tube (10% tracer displacement). The cross-reactivity of human β-lipotropin (β-LPH) or camel β-endorphin was about 47% or 31%, respectively, on a molar basis. ACTH, angiotensin II or arginine vasopressin did not cross-react. All quoted values of β-EI are calculated as human β-endorphin equivalents.

In some plasma samples, β-EI was extracted by use of a cation exchange resin and characterized by gel chromatography on Sephadex G-50 column. The extraction and gel filtration procedure has been described (Knepel, Anhut, Nutto & Hertting, 1981).

In some plasma samples ACTH concentrations were also measured by means of a radioimmunoassay of unextracted plasma (unpublished). The antiserum was purchased from Ferring GmbH (Kiel, GFR). Porcine ACTH(1-39) was labelled with ^{125}Iodine by the chloramin-T method and was also used as standard. Detection limit of the assay was about 20 fmol per tube. Angiotensin II, β-endorphin or arginine vasopressin did not cross-react.

Arterial blood pressure was recorded by means of a Statham transducer and a Watanabe recorder with the cannula (PE 50) placed in the ventral tail artery under ether anaesthesia at least 2 h before the experiments.

Statistical evaluation

All values are expressed as mean ± s.e.mean. The significance of the difference between mean values was evaluated by Student's *t* test.

Drugs

The following drugs were used: captopril (SQ 14225) (Squibb); (1-Asp, 5-Ile)angiotensin II and (des-1-Asp)angiotensin II (Serva); (1-Asp, 5-Ile) angiotensin I and dexamethasone acetate (Sigma); saralasin acetate (Röhm Pharma); porcine ACTH(1-39) (Ferring).

Results

Intravenous infusion of angiotensin I, II or III

Intravenous infusion of angiotensin II caused dose-dependent increases in plasma β-EI (Figure 1). Plasma ACTH levels following angiotensin II (1000 ng kg^{-1}min^{-1}) infusion were also measured: they increased from 46.9 ± 20.8 fmol/ml ($n = 6$) in vehicle-infused rats to 246.0 ± 16.1 fmol/ml ($n = 6$)

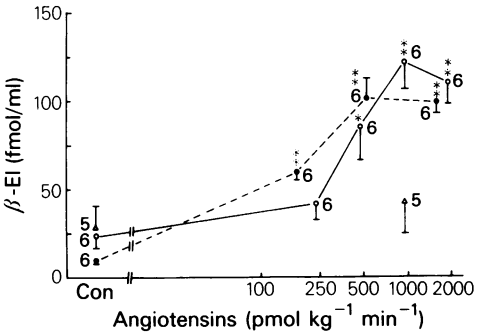

Figure 1 Dose-response relationship for the effect of angiotensin I (●) or angiotensin II (○) infusions on plasma β-endorphin-like immunoreactivity (β-EI). Controls (Con) received the vehicle; (△) indicate that either saralasin (in controls) or saralasin plus angiotensin II was infused. Mean values are given; vertical lines show s.e.mean ; figures denote number of animals used. Significant differences from the respective controls: $*P<0.02$; $**P<0.001$.

in angiotensin II-treated animals ($P<0.001$). All doses of angiotensin II used were sufficient to produce maximum blood pressure response. Angiotensin II 250, 500, 1000 or 2000 ng kg^{-1}min^{-1} elevated mean arterial blood pressure from 113 ± 1 mmHg ($n=6$) in controls to 169 ± 2, 171 ± 2, 175 ± 3 or 174 ± 2 mmHg, respectively ($n=6$ and $P<0.001$, each).

Like angiotensin II, angiotensin I or III infusions also produced an increase in plasma β-EI. Angiotensin I was similar in potency to angiotensin II (Figure 1) while angiotensin III was less effective. Plasma β-EI was 59.0 ± 13.3 fmol/ml ($n=6$) following vehicle infusion but was 41.5 ± 13.6 (NS), 141.3 ± 31.6 ($P<0.05$), 132.4 ± 30.6 ($P<0.05$) or 183.0 ± 29.0 fmol/ml ($P<0.01$) after infusion of angiotensin III 1.25, 2.5, 5.0 or 10.0 μg kg^{-1}min^{-1} ($n=6$ each), respectively (molecular weight of angiotensin III: 930.9 daltons).

Effect of saralasin or dexamethasone on β-endorphin release induced by angiotensin II

Intravenous infusion of saralasin (25 μg kg^{-1}min^{-1}) did not change plasma β-EI when given alone (Figure 1). However, angiotensin II (1000 ng kg^{-1} min^{-1}) failed to raise plasma β-EI in the presence of saralasin (Figure 1).

Pretreatment of the animals with dexamethasone (0.5 mg/kg i.p. twice) prevented an increase in plasma β-EI by angiotensin II. Plasma levels of β-EI were not detectable either in dexamethasone- or in dexamethasone plus angiotensin II (500 ng kg^{-1}min^{-1})-treated rats ($n=6$ or 12, respectively). However, in vehicle-treated rats, angiotensin II (500 ng kg^{-1} min^{-1}) elevated plasma β-EI from 30.5 ± 8.1 fmol/ml ($n=6$) in controls to 102.1 ± 12.2 fmol/ml ($n=12$) ($P<0.01$).

Effect of captopril on β-endorphin release induced by angiotensin I or II

As shown in Figure 2, captopril (15 mg/kg i.v.) slightly elevated plasma β-EI when given alone, although this effect was not statistically significant ($P>0.05$). The increase in plasma β-EI induced by angiotensin I (750 ng kg^{-1}min^{-1}) was completely abolished by captopril, whereas the increase in plasma β-EI, when elicited by angiotensin II (1000 ng kg^{-1} min^{-1}), was left unchanged by captopril pretreatment (Figure 2).

Similarly, captopril alone did not change mean arterial blood pressure; it was 114 ± 3 or 114 ± 5 mmHg, respectively, in the absence or presence of captopril ($n=5$, each). Infusion of angiotensin I (750 ng kg^{-1}min^{-1}) elevated mean arterial blood pressure to 171 ± 3 mmHg ($n=5$, $P<0.001$), but failed to do so, when captopril was also injected

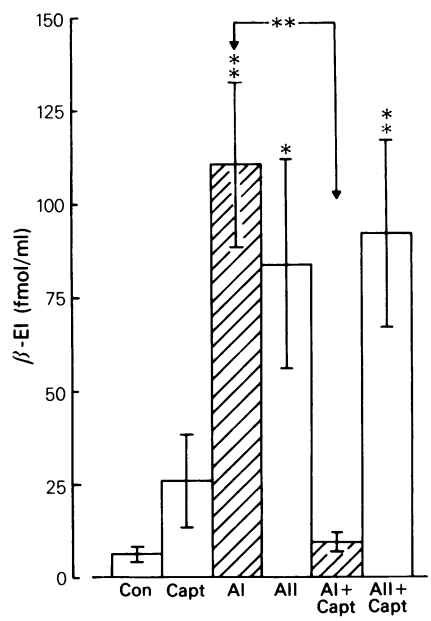

Figure 2 Effect of captopril on the increase in plasma β-endorphin-like immunoreactivity (β-EI) elicited by angiotensin I (AI) or angiotensin II (AII). Captopril (Capt, 15 mg/kg i.v.) was given 10 min before intravenous infusion of AI (750 ng kg^{-1}min^{-1}) or angiotensin II (1000 ng kg^{-1}min^{-1}). Blood was collected 20 min after the start of angiotensin infusion. Controls (Con) received the vehicle. Values are mean of $n=6$ each; vertical lines show s.e.mean. Significant differences from controls: $*P<0.025$; $**P<0.01$.

(116 ± 2 mmHg, n =5). In contrast, captopril had no effect on blood pressure following angiotensin II (1000 ng kg^{-1}min^{-1}) infusion; it was 172 ± 2 or 170 ± 2 mmHg, respectively, with or without captopril pretreatment (n = 5 each).

Gel filtration on Sephadex G-50 column

In view of the appreciable cross-reactivity of β-LPH in our β-endorphin assay, plasma β-EI was analysed by Sephadex G-50 chromatography. The elution profile of β-EI in plasma extracts of angiotensin II (1000 ng kg^{-1}min^{-1})- or vehicle-infused rats is shown in Figure 3. No immunoreactive material was found eluting in a manner different from human β-LPH or human β-endorphin. In vehicle-treated rats, most of the β-EI behaved similar to human β-LPH. On the other hand, immunoreactivity corresponding to β-endorphin comprised about 49% of the total immunoreactivity in the plasma extract of angiotensin II-infused animals, whereas 51% co-migrated with human β-LPH.

Effect of angiotensin II on plasma β-EI in Long Evans or Brattleboro rats

The results are shown in Figure 4. As in Wistar rats, intravenous infusion of angiotensin II (1000 ng kg^{-1}min^{-1}) raised plasma β-EI both in normal Long Evans rats and in Brattleboro rats suffering from hereditary hypothalamic diabetes insipidus ($P < 0.01$ each). However, the plasma levels of β-EI reached after angiotensin II infusion were significantly lower in Brattleboro rats than in Long Evans rats ($P < 0.05$).

Discussion

An increase in ACTH release in response to an angiotensin II infusion has been found in dogs (Ramsay *et al.*, 1978), whereas the reports on the effect in man are conflicting (Rayyis & Horton, 1971; Semple, Buckingham, Mason & Fraser, 1979). The results of the present study show that angiotensin II elevates the plasma levels of ACTH in the rat and that it also enhances the release of immunoreactive β-endorphin. The concomitant release of ACTH and β-endorphin supports the hypothesis that the regulatory mechanisms involved in the secretion of both hormones are common and identical (Mains *et al.*, 1977; Guillemin *et al.*, 1977). The action of angiotensin II on plasma β-EI appears to be a specific one, since it was dose-dependent and could be blocked by the angiotensin receptor antagonist, saralasin.

Like angiotensin II, angiotensin I or III also elicited release of β-EI. Angiotensin II is generally considered to be that part of the renin-angiotensin system, which is biologically active (see: Peach, 1977). Consistent with this view, angiotensin III was found to be less effective in elevating plasma β-EI than angiotensin II, when infused intravenously. Nevertheless, a physiological role for angiotensin III in β-endorphin release, for instance after local gener-

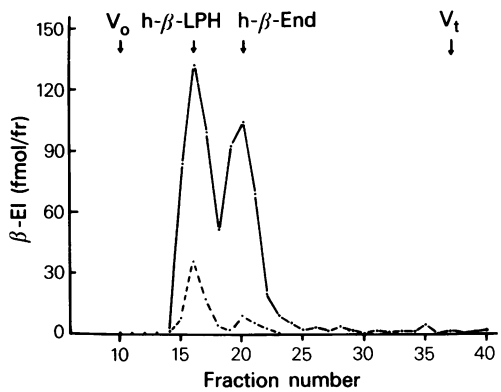

Figure 3 Elution profile of β-endorphin-like immunoreactivity (β-EI) after gel filtration of plasma extracts on Sephadex G-50 column. Plasma pools (10 ml) of vehicle- (broken line) or angiotensin II (1000 ng kg^{-1}min^{-1})- infused (solid line) rats were extracted and subjected to gel chromatography. Arrows indicate the void volume (V_0), the elution volume of human β-lipotropin (h-β-LPH), human β-endorphin (h-β-End) and the total volume (V_t).

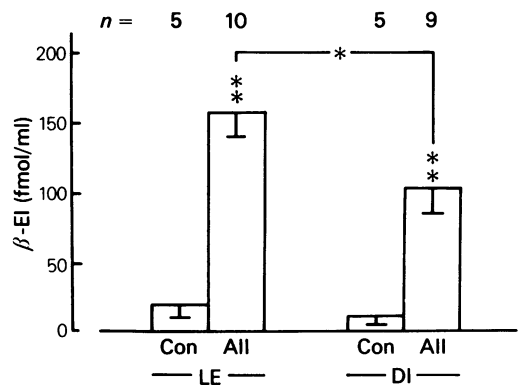

Figure 4 Effect of angiotensin II on plasma β-endorphin-like immunoreactivity (β-EI) in normal Long Evans rats (LE) or in rats suffering from diabetes insipidus (DI) with an absolute lack of vasopressin. Angiotensin II (AII, 1000 ng kg^{-1}min^{-1}) was infused for 20 min; controls (Con) received the vehicle. Mean values are given; vertical lines show s.e.mean; n = number of animals used. Significant differences from the respective controls: $^*P < 0.05$; $^{**}P < 0.005$.

ation, cannot be entirely ruled out. Furthermore, experiments using the converting enzyme inhibitor, captopril, revealed that the action of angiotensin I on β-EI release depends on its conversion to angiotensin II. Both the release of β-EI and the pressor response to angiotensin I were abolished by captopril, suggesting inhibition of conversion, since angiotensin I possesses virtually no pressor effect of its own (see Peach, 1977). An unspecific mechanism of action of captopril appears unlikely, as it did not influence the increase in blood pressure or plasma β-EI induced by angiotensin II. Analogous to these findings, are observations that captopril prevents the increase in water consumption (Katovich *et al.*, 1979) or vasopressin release (Knepel, Beuers, Nutto & Hertting, 1982) in response to angiotensin I.

Following hypophysectomy plasma levels of β-EI are no longer detectable either under 'basal' conditions or after stress (Guillemin *et al.*, 1977), suggesting that β-EI found in plasma is of pituitary origin. The potent glucocorticoid, dexamethasone, is known to inhibit the release of ACTH/β-LPH-related peptides from the anterior but not from the intermediate lobe of the pituitary gland (see: Rosa, Policastro & Herbert, 1980). Thus, the inhibition by dexamethasone of angiotensin II-induced increase in plasma β-EI may indicate that the β-EI is released from the adenohypophysis. Gel filtration chromatography revealed an elevation not only of β-endorphin-like but also of β-LPH-like material. Since β-LPH occurs predominantly in the pars distalis of the pituitary (Fratta, Yang, Majane & Costa, 1979; Gramsch, Kleber, Höllt, Pasi, Mehraein & Herz, 1980), this further supports the assumption that the β-EI, released by angiotensin II, mainly originates in the anterior pituitary.

The mechanism of action of angiotensin II on β-endorphin release is unknown. It is unlikely that the secretion of β-endorphin is just an unspecific stress response of the organism to the marked blood pressure increase produced by angiotensin II, because infusion of angiotensin II ($250\,ng\,kg^{-1}min^{-1}$) was maximally effective in elevating blood pressure but did not raise plasma β-EI. Buckingham & Hodges (1977) found an increased release by angiotensin of corticotrophin releasing hormone from rat hypothalami incubated *in vitro*, whereas Jones & Hillhouse (1977) did not. Gann (1969) used dogs in which the brain had been removed to leave an island of either pituitary and median eminence or anterior pituitary only, taking secretion rates of cortisol as an index of ACTH release. He found infusions of angiotensin II much more effective in dogs with the median eminence than in dogs without. In contrast, Maran & Yates (1977) infused angiotensin II in dogs through cannulae in the jugular vein, anterior pituitary and cerebral third ventricle and reached the conclusion that angiotensin II stimulates ACTH release by an action at the anterior pituitary. Our results cannot reconcile these conflicting reports but indicate that vasopressin may be involved in angiotensin-induced ACTH/β-endorphin release. When compared to normal Long Evans rats, the increase in plasma β-EI following angiotensin II infusion was diminished by about 35% in rats with hereditary hypothalamic diabetes insipidus from the Brattleboro strain of Long Evans rats, which are totally unable to synthesize or secrete vasopressin. β-EI is slightly elevated in the pituitaries of Brattleboro rats (Rossier, Battenberg, Pittman, Bayon, Koda, Miller, Guillemin & Bloom, 1979) and its secretion can be stimulated by exogenous vasopressin as well as it can in normal Long Evans rats (Knepel, Anhut, Nutto & Hertting, 1980), excluding unspecific influences. Under the same experimental conditions as applied in the present study, angiotensin II in doses capable of releasing β-endorphin has recently been shown to cause vasopressin release (Knepel & Meyer, 1980). Although vasopressin is clearly not the most potent ACTH/β-endorphin releasing factor (Vale, Spiess, Rivier & Rivier, 1981), it could act synergistically (Gillies & Lowry, 1979). The precise role of vasopressin in angiotensin-induced β-endorphin release remains to be defined.

The doses of angiotensin II necessary to induce β-endorphin release presumably produce plasma levels of angiotensin II which are far higher than those reached under physiological conditions (Knepel & Meyer, 1980). Nevertheless, this does not exclude a physiological function, since the simultaneous increase in blood pressure might have rendered smaller doses of angiotensin II ineffective (Gann, 1966; Baertschi, Ward & Gann, 1976). Furthermore, angiotensin II may be generated within the anterior pituitary, since the adenohypophysis contains high concentrations of renin (Slater, Defendini & Zimmerman, 1980; Hirose, Yokosawa, Inagami & Workman, 1980). There is some evidence that the renin-angiotensin system may be involved in the mediation of the adrenocortical response to hypovolaemia (Gann, 1969).

β-LPH stimulates the production of aldosterone in dispersed rat adrenal capsular cells (Matsuoka, Mulrow, Franco-Saenz & Li, 1981). β-Endorphin and other endogenous opioid peptides may modulate drinking behaviour (Brown & Holtzman, 1981; Summy-Long, Keil, Deen, Rosella & Severs, 1981), vasopressin release (Knepel, Nutto & Hertting, 1982) and urine or electrolyte excretion (Huidobro-Toro & Huidobro, 1981). Thus, release by angiotensin of β-LPH and β-endorphin may add to the multiple actions of angiotensin, which can be considered as concerned with the control of blood volume.

References

ANHUT, H., KNEPEL, W., NUTTO, D. & HERTTING, G. (1981). Vasopressin stimulates release of β-lipotropin and β-endorphin in conscious rats as measured by radioimmunoassay of unextracted plasma. *Naunyn-Schmiedebergs Arch. Pharmac.*, **316**, 59–63.

BAERTSCHI, A.J., WARD, D.G. & GANN, D.S. (1976). Role of atrial receptors in the control of ACTH. *Am. J. Physiol.*, **231**, 692–699.

BROWN, D.R. & HOLTZMAN, S.G. (1981). Suppression of drinking by naloxone in the rat: a further characterization. *Eur. J. Pharmac.*, **69**, 331–340.

BUCKINGHAM, J.C. & HODGES, J.R. (1977). Production of corticotrophin releasing hormone by the isolated hypothalamus of the rat. *J. Physiol.*, **272**, 469–479.

CHANGARIS, D.G., KEIL, L.C. & SEVERS, W.B. (1978). Angiotensin II immuno-histochemistry of the rat brain. *Neuroendocrinology*, **25**, 257–274.

FRATTA, W., YANG, H.-Y.T., MAJANE, B. & COSTA, E. (1979). Distribution of β-endorphin and related peptides in the hypothalamus and pituitary. *Neuroscience*, **4**, 1903–1908.

GANN, D.S. (1966). Carotid vascular receptors and control of adrenal corticosteroid secretion. *Am. J. Physiol.*, **211**, 193–197.

GANN, D.S. (1969). Parameters of the stimulus initiating the adrenocortical response to hemorrhage. *Ann. N. Y. Acad. Sci.*, **156**, 740–755.

GILLIES, G. & LOWRY, P. (1979). Corticotrophin releasing factor may be modulated vasopressin. *Nature*, **278**, 463–464.

GRAMSCH, C., KLEBER, G., HÖLLT, V., PASI, A., MEHRAEIN, P. & HERZ, A. (1980). Pro-opiocortin fragments in human and rat brain: β-endorphin and α-MSH are the predominant peptides. *Brain Res.*, **192**, 109–119.

GREGG, C.M. & MALVIN, R.L. (1977). Binding of angiotensin by neurohypophysis and adenohypophysis *in vivo* and *in vitro*. *Proc. Soc. exp. Biol. Med.*, **155**, 400–404.

GUILLEMIN, R., VARGO, T., ROSSIER, J., MINICK, S., LING, N., RIVIER, C., VALE, W. & BLOOM, F. (1977). β-Endorphin and adrenocorticotropin are secreted concomitantly by the pituitary gland. *Science*, **197**, 1367–1369.

HARDING, J.W., STONE, L.P. & WRIGHT, J.W. (1981). The distribution of angiotensin II binding sites in rodent brain. *Brain Res.*, **205**, 265–274.

HIROSE, S., YOKOSAWA, H., INAGAMI, T. & WORKMAN, R.J. (1980). Renin and prorenin in hog brain: ubiquitous distribution and high concentrations in the pituitary and pineal. *Brain Res.*, **191**, 489–499.

HUIDOBRO-TORO, J.P. & HUIDOBRO, F. (1981). Central effects of morphine, levorphanol, (—)-methadone and the opioid-like peptides β-endorphin and D-alanine$_2$-methionine enkephalinamide on urine volume outflow and electrolytes. *J. Pharmac. exp. Ther.*, **217**, 579–585.

JONES, M.T. & HILLHOUSE, E.W. (1977). Neurotransmitter regulation of corticotropin-releasing factor *in vitro*. *Ann. N. Y. Acad. Sci.*, **297**, 536–560.

KATOVICH, M.J., BARNEY, C.C., FREGLY, M.J. & McCAA, R.E. (1979). Effect of an angiotensin converting enzyme inhibitor (SQ 14,225) on β-adrenergic and angiotensin-induced thirsts. *Eur. J. Pharmac.*, **56**, 123–130.

KILCOYNE, M.M., HOFFMAN, D.L. & ZIMMERMAN, E.A. (1980). Immunocytochemical localization of angiotensin II and vasopressin in rat hypothalamus: evidence for production in the same neuron. *Clin. Sci.*, **59**, 57s–60s.

KNEPEL, W., ANHUT, H., NUTTO, D. & HERTTING, G. (1980). Evidence that vasopressin is involved in the isoprenaline-induced β-endorphin release. *Eur. J. Pharmac.*, **68**, 359–363.

KNEPEL, W., ANHUT, H., NUTTO, D. & HERTTING, G. (1981). The effect of isoprenaline on plasma concentrations of immunoreactive β-endorphin and β-lipotropin in the conscious rat. *Naunyn-Schmiedebergs Arch. Pharmac.*, **317**, 154–158.

KNEPEL, W., BEUERS, U., NUTTO, D. & HERTTING, G. (1982). Effect of captopril (SQ14225) on vasopressin release induced by angiotensin in the conscious rat. *Clin. Sci.*, **62**, 341–342.

KNEPEL, W. & MEYER, D.K. (1980). Role of the renin-angiotensin system in isoprenaline-induced vasopressin release. *J. cardiovasc. Pharmac.*, **2**, 815–824.

KNEPEL, W., NUTTO, D. & HERTTING, G. (1982). Evidence for inhibition by β-endorphin of vasopressin release during foot-shock induced stress in the rat. *Neuroendocrinology*, **34**, 353–356.

MAINS, R.E., EIPPER, B.A. & LING, N. (1977). Common precursor to cortico-tropins and endorphins. *Proc. natn. Acad. Sci., U.S.A.*, **74**, 3014–3018.

MARAN, J.W. & YATES, F.E. (1977). Cortisol secretion during intrapituitary infusion of angiotensin II in conscious dogs. *Am. J. Physiol.*, **233**, E273–E285.

MATSUOKA, H., MULROW, P.J., FRANCO-SAENZ, R. & LI, C.H. (1981). Effects of β-lipotropin and β-lipotropin-derived peptides on aldosterone production in the rat adrenal gland. *J. clin. Invest.*, **68**, 752–759.

MEYER, D.K., PHILLIPS, M.I. & EIDEN, L. (1982). Studies on the presence of angiotensin II in rat brain. *J. Neurochem.*, **38**, 816–820.

PEACH, M.J. (1977). Renin-angiotensin system: biochemistry and mechanisms of action. *Physiol. Rev.*, **57**, 313–370.

QUINLAN, J.T. & PHILLIPS, M.I. (1981). Immunoreactivity for an angiotensin II-like peptide in the human brain. *Brain Res.*, **205**, 212–218.

RAMSAY, D.J., KEIL, L.C., SHARPE, M.C. & SHINSAKO, J. (1978). Angiotensin II infusion increases vasopressin, ACTH, and 11-hydroxycorticosteroid secretion. *Am. J. Physiol.*, **234**, R66–R71.

RAYYIS, S.S & HORTON, R. (1971). Effect of angiotensin II on adrenal and pituitary function in man. *J. clin. Endocrinol.*, **32**, 539–546.

ROSA, P.A., POLICASTRO, P. & HERBERT, E. (1980). A cellular basis for the differences in regulation of synthesis and secretion of ACTH/endorphin peptides in anterior and intermediate lobes of the pituitary. *J. exp. Biol.*, **89**, 215–237.

ROSSIER, J., BATTENBERG, E., PITTMAN, Q., BAYON, A., KODA, L., MILLER, R., GUILLEMIN, R. & BLOOM, F. (1979). Hypothalamic enkephalin neurones may regulate the neurohypophysis. *Nature*, **277**, 653–655.

SEMPLE, P.F., BUCKINGHAM, J.C., MASON, P.A. & FRASER, R. (1979). Suppression of plasma ACTH concentration by angiotensin II infusion in normal humans and in a subject with a steroid 17α-hydroxylase defect. *Clin. Endocrinol.*, **10**, 137–144.

SIRETT, N.E., McLEAN, A.S., BRAY, J.J. & HUBBARD, J.I. (1977). Distribution of angiotensin II receptors in rat brain. *Brain Res.*, **122**, 299–312.

SLATER, E.E., DEFENDINI, R. & ZIMMERMAN, E.A. (1980). Wide distribution of immunoreactive renin in nerve cells of human brain. *Proc. natn. Acad. Sci., U.S.A.*, **77**, 5458–5460.

SUMMY-LONG, J.Y., KEIL, L.C., DEEN, K., ROSELLA, L. & SEVERS, W.B. (1981). Endogenous opioid peptide inhibition of the central actions of angiotensin. *J. Pharmac. exp. Ther.*, **217**, 619–629.

VALE, W., SPIESS, J., RIVIER, C. & RIVIER, J. (1981). Characterization of a 41-residue ovine hypothalamus peptide that stimulates secretion of corticotropin and β-endorphin. *Science*, **213**, 1394–1397.

VALTIN, H. (1977). Genetic models for hypothalamic and nephrogenic diabetes insipidus. In *Disturbances in Body Fluid Osmolality*. ed. Andreoli, T.E., Grantham, J.J. & Rector, F.C. pp. 197–215. Bethesda: American Physiological Society.

VANHOUTEN, M., SCHIFFRIN, E.L., MANN, J.F.E., POSNER, B.I. & BOUCHER, R. (1980). Radioautographic localization of specific binding sites for blood-borne angiotensin II in the rat brain. *Brain Res.*, **186**, 480–485.

(*Received December 10, 1981.*)