Vol. 96, No. 2, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 30, 1980 Pages 626-631

SYNTHESIS AND RENAL EFFECTS OF ALA-GLY-[ARG^8]-VASOPRESSIN

Yun L. Chan and Clark W. Smith

Department of Physiology and Biophysics
University of Illinois Medical Center
Chicago, Illinois 60680, USA

Received August 8,1980

SUMMARY

A new neurohypophyseal hormone analogue, Ala-Gly-[Arg^8]-vasopressin, was synthesized by the stepwise solution techniques and its effect on systemic blood pressure and renal function was examined in nondiuretic Sprague-Dawley rats. Clearance of inulin was used to study glomerular filtration rate. Intravenous administration of 50 pmole/100 g. b. wt. Ala-Gly-[Arg^8]-vasopressin caused diuresis and natriuresis without significant change of mean arterial blood pressure. The fractional excretion of sodium was increased by 225% within 10 min after the analogue administration. The present study suggests that this analogue has a direct effect on renal tubular transport of electrolyte independent of affecting systemic circulation.

INTRODUCTION

Neurohypophyseal hormones have been postulated to act as a natriuretic hormone responsible for the natriuresis produced by the expansion of extracellular volume [1]. Several neurohypophyseal hormones and their analogues have been reported to exert natriuretic effect by mechanisms unrelated to their antidiuretic action [2,3]. However, those studies could not exclude the possibility that the hemodynamic effects may play a role in eliciting the natriuretic effects of these hormones [4]. Recently Ala-Gly-[Arg^8] vasopressin has been isolated from bovine neurohypophysis and demonstrated to have a strong natriuretic effect without a pressor effect in the dog [5]. In view of the possibility of this newly discovered peptide being the "natriuretic hormone," we decided to synthesize this compound and to study its effect on renal function.

Abbreviation: AVP, arginine vasopressin; DCC, dicyclohexylcarbodiimide; DMF, dimethylformamide; EtOH, ethanol; Et_2O, ethylether; HOAc, acetic acid, HBT, 1-hydroxybenzotriazole.

0006-291X/80/180626-06$01.00/0

Ala-Gly-[Arg^8]-vasopressin was synthesized by stepwise solution techniques and its effect on renal electrolyte excretion, glomerular filtration, renal plasma flow and systemic blood pressure was examined in non-diuretic Sprague-Dawley rats. The present results indicate that this new peptide hormone can produce diuresis and natriuresis without alterating systemic blood pressure and glomerular filtration rate.

MATERIALS AND METHODS

(I) Materials

Tert-Butyloxycarbonyl (Boc) amino acids were purchased from Bachem. All other chemicals were reagent grade. Thin layer chromatography plates were precoated silica gel 60 F-254 from E. Merck.

(II) General Procedures

Melting points were determined in open capillary tubes and are reported uncorrected. Optical rotations were measured in a Zeiss Circle polarimeter (0.01°). For thin layer chromatograpny (TLC), loads of 50 g were applied and chromatograms were developed for 10-15 cm in the following solvent systems (all by volume): (A) 1-butanol-pyridine-acetic acid- H_2O (15:10:3:6); (B) 1-butanol-acetic acid -H_2O (4:1:1); (C) ethyl acetate-pyridine-acetic acid-H_2O (5:5:1:3). Visualization was made by observation with short wave uv light and treatment with Cl_2 gas followed by starch-KI (1%:1%) spray. Amino acid analyzes were performed on a Durrum-Dionex D-500 amino acid analyzer, following hydrolysis for 22h in constant boiling HCl at 110°C *in vacuo*.

(III) Synthesis of Boc-Gly-Cys(Bzl)-Tyr-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2

Z-Cys(Bzl)-Tyr(Bzl)-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2 [6] (0.94 g, 0.57 mmol) was dissolved in 2M HBr/HOAc (25ml). After 60 min the HBr salt was precipitated by addition of Et_2O, and the precipitate filtered and washed with Et_2O. While the HBr salt of the 9-peptide dried *in vacuo*, a solution of Boc-Gly (0.13 g, 0.72 mmol) in 1,2-dimethoxyethane (5ml) was preactivated [7] by DCC (0.15 g, 0.72 mmol) in the presence of HBT [8] (0.16 g, 1.08 mmol). After 10 min the HBr salt of the 9-peptide [9] was dissolved in DMF (20ml) and the pH adjusted to 7.5-8 with N-methylmorpholine. The preactivation mixture was then filtered into the DMF solution. The coupling reaction was complete after 3 h as measured by a semiquantitative ninhydrin test [10]. The product was precipitated by addition of H_2O, filtered, washed three times each with H_2O, 5% $NaHCO_3$, H_2O, EtOH, and Et_2O and dried *in vacuo*: 0.85 g (95% yield); mp 218°C decomposed; $[\alpha]_D^{24}$ -39° (*c* 1, DMF); TLC (A) R_f 0.67, (B) R_f 0.46. Amino acid analysis: Asp, 1.05; Glu, 1.06; Pro, 1.04; Gly, 2.00; Tyr, 0.97; Phe, 0.97; Cys(Bzl), 1.85; Arg, 1.01; NH_3, 2.94.

(IV) Synthesis of Z-Ala-Gly-Cys(Bzl)-Tyr-Phe-Gln-Asn-Cys(Bzl)-Pro-Arg(Tos)-Gly-NH_2

The Boc group was removed from the 10-peptide (Section III) by 1h treatment in CF_3CO_2H, and the resulting salt precipitated by

addition of Et_2O and dried in vacuo. Z-Ala was then coupled to the 10-peptide and the product isolated as described in Section 2.3: 0.81 g (95%); mp 215°C decomposed; $[\alpha]_D^{24}$ -15° (c 1, DMF); TLC (A) R_f 0.66; (B) 0.46; (C) 0.86. amino acid analysis: Asp, 1.02; Glu, 1.02; Pro, 1.02; Gly, 2.00; Ala, 0.98, Tyr, 0.90; Phe, 1.00; Cys(Bzl), 1.80; Arg, 1.00; NH_3, 3.30.

(V) Synthesis of Ala-Gly-Arginine Vasopressin

The protecting groups were removed from the 11-peptide (0.35g, 0.21 mmol) prepared in Section 2.4 by treatment with Na in anhydrous liquid ammonia [11]. The cyclic disulfide bond was formed by oxidation of the sulfhydryl intermediate with ICH_2CH_2I [12] (59 mg, 0.21 mmol) in MeOH-H_2O (200:200 v/v) as described for a synthesis of AVP [6]. The product was purified by passage down a 2.15 x 118 cm column of Sephadex G-15 (fine) equilibrated with 50% HOAc [13] followed by partition chromatography [14] on a 2.82 x 68 cm column of Sephadex G-25 (black polymerizate, 100-200 mesh) in the system 1-butanol-ethanol-aqueous 3.5% HoAc and 1.5% pyridine (4:1:5 v/v/v) as described for AVP [15]. Final purification was effected by passage down a 2.5 x 91 cm column of Sephadex G-15 (fine) equilibrated with 0.2M HOAc and isolated by lyophilization: 125 mg (43% yield); $[\alpha]_D^{24}$ -52.8° 1° (c 0.55 1M HOAc); TLC (A) R_f 0.32, (C) R_f 0.67. Amino acid analysis: Cys(O_3H), 1.86; Asp, 0.98; Glu, 1.00; Pro, 0.99; Gly, 1.95; Ala, 1.01; Tyr, 0.92; Phe, 0.97; Arg, 0.96; NH_3, 3.11.

(VI) Renal Function Study

Experiments were performed in male Sprague-Dawley rats weighing 180-250g. The rats were allowed free access to food and water prior to the experiment. They were anesthetized by intraperitoneal injection of Inactin (100 mg/kg b.wt.). After tracheotomy, and carotid artery and and jugular vein were cannulated. Blood pressure was measured continuously with the aid of a transducer and polygraph through the carotid artery catheter. The bladder was catheterized through an abdominal incision. A 3% solution of inulin in physiological saline was infused at a rate of 1.8 ml/hr through the jugular vein. An equilibration period of 60 min was allowed before any urine collection was started. Urine samples were collected for 10 min under mineral oil in preweighed glass vials and the urine volume was determined gravimetrically. Blood samples were collected from the carotid artery during the mid-portion of each clearance period. Blood and urine samples were analyzed for inulin, Na^+ and K^+. Inulin was assayed by the method of Fuhr et al, [16] Na^+ and K^+ were determined by flame photometry. Clearance of inulin was used to determine glomerular filtration rate (GFR). Electrolyte excretion was expressed in both absoluted terms and as fractional excretion (clearance of the electrolyte corrected for GFR and expressed as percentage).

To test the effect of the hormone, a single dose ($5x10^{-11}$M/100-g.b.wt) was injected intravenously after two control clearance period. Six more clearance periods of 10 min duration were carried out after starting the hormone administration.

RESULTS AND DISCUSSION

Control experiments on renal function were done with intravenous injection of saline without Ala-Gly-[Arg^8]-vasopressin (AGAVP). There

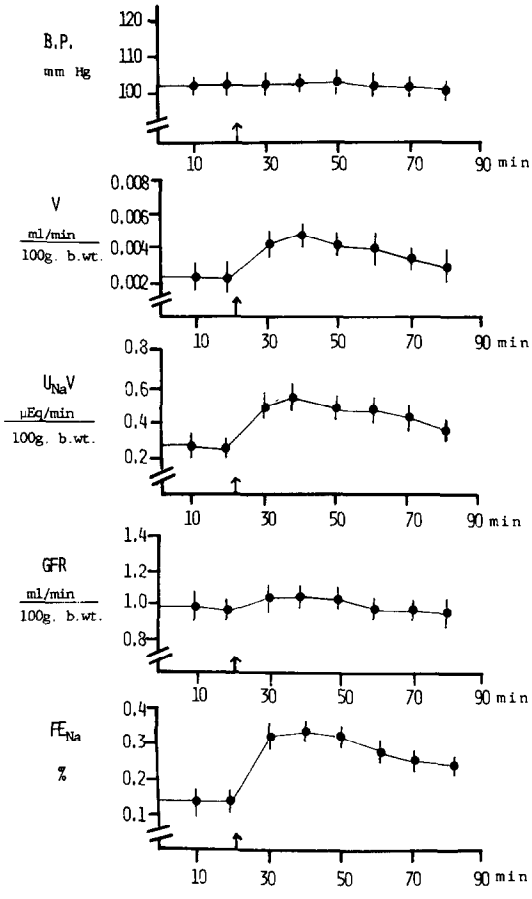

Figure I *Effects of Ala-Gly-[Arg^8]-vasopressin on systemic blood pressure and renal function of non-diuretic rats. Ala-Gly-[Arg^8]-vasopressin was administered intravenously (5.0×10^{-11} mol/100g b.wt) after two control periods as indicated by arrows. B. P.: mean arterial blood pressure. V: urine flow rate. $U_{Na}V$: urinary excretion of sodium. GFR: glomerular filtration rate. FE_{Na}: fractional excretion of sodium ($U_{Na}V/GFR \times P_{Na}$) Abscissa: urine collection period for every 10 min in sequence after equilibration period for inulin infusion. Data are presented as mean standard errors for 6 rats.*

were no significant changes in mean arterial blood pressure (B.P.), urinary flow rate (V), urinary excretion of sodium ($U_{Na}V$), effective renal plasma flow (ERPF), GFR, fraction excretion of sodium (FE_{Na}) and potassium (FE_k) during the clearance period after injection. However, there were changes in V, $U_{Na}V$ and FE_{Na} after AGAVP administration. The results were presented in Figure 1. During the first 10 min clearance period, V was significantly increased by 102% ($P < 0.001$), $U_{Na}V$ by 192% ($P < 0.001$) and FE_{Na} by 225% ($P < 0.001$) while B.P. remained unchanged. These effects

maintained for another two clearance periods, then gradually fell off suggesting degradation of AGAVP. Although GFR was slightly increased, the change was not statistically significant ($P > 0.05$). These results indicated that AGAVP could produce diuresis and natriuresis in the rat independent from alteration in systemic and renal hemodynamics. Although similar dose of arginine-vasopressin would cause natriuresis, it could drastically increase the B.P. [6]. "Pressure diuresis" which partially explained the mechanisms of the natriuretic effect of arginine vasopressin [4] was excluded from AGAVP effect, since AGAVP did not increase B.P. The possibility that the effect was due to the release of another hormone by AGAVP was excluded, since direct administration of the hormone into renal artery had the similar effect, as noted by our preliminary study and a previous study [5],. It appears unlikely that a shift of renal blood flow could account for the effect of AGAVP, since both the effective renal plasma flow and GFR were unaltered. Thus it seems appropriate to suggest that AGAVP has a direct effect on renal tubular transport of electrolyte. Although details of the mechanism require further study, this hormone might be a promissing candidate for the "natriuretic hormone" released during extracellular volume expansion. It will, at least, provide a potential tool to study the mechanism of the natriuretic action of neurohypophyseal hormones.

Acknowledgements

We thank Dr. R. Walter and Dr. H. Getelman for stimulating our interest in this study and Ms. Y. Ting for her excellent technical assistance. Preliminary report has appeared in the abstract Clinical Research 28(2) 441, 1980.

REFERENCES

1. Peters, G. and Roch-Ramel, F. (1970) International encyclopedia of Pharmacology and therapeutics, section 41, Vol. 1. pp 229-278, Pergamon Press, New York.
2. Chan, W.Y. and Vigneaud, V. du. (1970) J. Pharmacol. Exp. Ther. 174, 541-549.
3. Kurtzman, N.A., Rogers, P.W., Boonjarern, S. and Arruda, J.A.L. (1975) Am. J. Physiol. 228, 890-894.
4. Metha, P.K., Arruda, J.A.L., Kurtzman, N.A., Smith, C.W. and Walter, R. (1980) Mineral Electrolyte Metab. 3, 10-20.

5. Gitelman, H.J., Klapper, D.G., Alderman, F.R. and Blythe, W.B. (1980) Science 207, 893-896.
6. Botos, C.R., Smith, C.W., Chan, Y.-L. and Walter, R. (1979) J. Med. Chem. 22, 926-931.
7. Jager, G., Konig, W., Wissman, H. and Geiger, R. (1974) Chem. Ber. 107, 215-231; Nestor, J.J., Jr., Ferger, M.F. and Chan, W.Y. (1975). J. Med Chem. 18, 1022-1024.
8. Konig, W. and Geiger, R. (1970) Chem. Ber. 103, 788-798.
9. Bodanszky, M. (1977) in Peptides: Proceedings of the Fifth American Peptide Symposium (Goodman, M. and Meienhofer, J. eds) pp. 1-13, Wiley, N.Y.
10. Dyckes, D.F., Nestor, J.J., Jr., Ferger, M.F. and du Vigneaud, V. (1974) J. Med. Chem. 17, 250-252.
11. Sifferd, R.H. and du Vigneaud, V. (1935) J. Biol. Chem. 108, 753-761.
12. Weygand, F. and Zumach, G. (1962) Z. Naturfosch. B. 17, 807-810.
13. Manning, M., Wuu, T.-C. and Baxter, J.W.M. (1968) J. Chromatog. 38, 396-398.
14. Yamashiro, D., Gillessen, D. and du Vigneaud, V. (1966) J. Am. Chem. Soc. 88, 1310-1313.
15. Yamamoto, D.M., Upson, D.A., Linn, D.K. and Hruby, V.J. (1977) J. Am. Chem. Soc. 99, 1564-1570.
16. Fuhr, J., Kacmarczyk, J., and Kruttgen, C.D. (1955) Klin. Wochschr. 33, 729-730.