Vol. 99, No. 1, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 16, 1981 Pages 65-72

SPECIFIC STIMULATION OF STEROIDOGENESIS IN RAT ADRENAL ZONA GLOMERULOSA CELLS BY PITUITARY PEPTIDES.

G.P. Vinson*, B.J. Whitehouse**, A. Dell***,
A.T. Etienne*** and H.R. Morris***.

* Department of Biochemistry, St. Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ.
** Department of Physiology, Queen Elizabeth College, London W8 7AH.
*** Department of Biochemistry, Imperial College, London SW7.

Received January 5,1981

SUMMARY: The capacity of the following peptides to stimulate steroidogenesis in suspensions of capsule (largely glomerulosa) and fasciculata/reticularis cells from rat adrenals was studied: $ACTH_{1-24}$, $ACTH_{1-13}$-amide, α-MSH, γ_1-MSH, γ-MSH precursor, $ACTH_{4-10}$, CLIP, and ovine and human β-lipotropin. Only α-MSH and $ACTH_{1-13}$-amide stimulated glomerulosa cells alone, without effect on fasciculata/reticularis cells. Like $ACTH_{1-24}$ the two samples of β-lipotropin stimulated both capsule and inner zone cell types in a similar manner. Their activity is attributable to slight $ACTH_{1-39}$ contamination, as shown by HPLC fractionation. The other peptides lacked any activity. It is likely that the predicted specific glomerulosa stimulant from the pituitary closely resembles α-MSH.

INTRODUCTION

The control of zona glomerulosa function and aldosterone secretion is multifactorial, and the effects of ACTH, angiotensin II and III, potassium ions and other factors have been described (1-8). Much evidence suggests that further pituitary factors, presumably peptides, also exist which contribute to the overall control of aldosterone production, particularly during conditions of sodium depletion (7, 9-18). In recent publications we have shown that one component of posterior pituitary extracts which specifically stimulates the zona glomerulosa of the rat adrenal is α-MSH (14,15). This peptide which has no effect on inner adrenocortical zones, accounted for perhaps 30% of the total glomerulosa stimulating activity in the commercially available posterior

0006-291X/81/050065-08$01.00/0

pituitary preparation Pitressin. Two others, designated B and C, await complete characterisation (14). Meanwhile it is interesting to note that in the rat at least, glomerulosa cells from animals subjected to restriction of dietary sodium respond with increased aldosterone secretion to concentrations of α-MSH as low as 10^{-10} moles per l, falling within the normal range of circulating concentrations of α-MSH in this species, and it therefore seems likely that α-MSH has a physiological role (15). The question arises whether the capacity to stimulate zona glomerulosa cells specifically is limited only to α-MSH, or is found more widely in the known range of pituitary peptides. In this respect Matsuoka et al.(16) have recently claimed that β-lipotropin is another such specific glomerulosa stimulant. Furthermore, Bravo and co-workers have isolated an aldosterone stimulating glycoprotein from human urine (17), although it is not clear whether this substance originates from the pituitary.

This paper describes further experiments which examine peptide structure/function relationships in regard to the control of the zona glomerulosa.

MATERIALS AND METHODS

Animals.

Rats were taken from the Wistar strain colony maintained at the Medical College of St. Bartholomew's Hospital. Females weighing about 220 g were used throughout. Suspensions of adrenal capsule (largely zona glomerulosa) and fasciculata/reticularis cells were prepared and incubated as previously described (18). Final incubations were in 5 ml Krebs bicarbonate Ringer (3.6 mM K^+), with glucose (200 mg%) and BSA (Sigma, fraction V, 1%), for two hours at 37° under 95% O_2/5% CO_2. Serial dilutions of the peptides to be tested were made in the same medium, and added to incubation flasks in 0.1 ml volumes.

Peptides.

The peptides tested were α-MSH (Sigma), $ACTH_{1-13}$-amide, $ACTH_{4-10}$ and ovine β-lipotropin (gifts of Dr. A.J. Thody), human β-lipotropin,

γ_1-MSH and γ-MSH precursor (gifts of Dr. P. Lowry), and corticotrophin-like intermediate lobe peptide (CLIP) (gift of Professor H. Yajima).*

Steroid estimation.

Steroids were extracted from the incubation media and estimated using the comprehensive glc methods described earlier (19). Fractionation of ovine β-lipotropin was performed using the HPLC system described elsewhere (20).

RESULTS

Results of stimulation of glomerulosa and fasciculata/reticularis cells by different concentrations of the various peptides are shown in Fig. 1. These results were necessarily obtained in a fairly large number of experiments over a period of time. It is well known that while quantitative reproducibility of results is good within a single experiment based on a uniform cell crop, there may be considerable variation between experiments in, for example, the basal (unstimulated) or the maximal steroid output (e.g. ref. 21). In our hands, for example, maximal corticosterone output by 5×10^5 fasciculata/reticularis cells under ACTH stimulation varies between 500 ng and 1500 ng. Similarly, maximal corticosterone output under α-MSH stimulation may vary between 150-250 ng per 10^5 glomerulosa cells. For this reason, to facilitate comparison between experiments, the results are plotted not as amounts of steroid produced, but are normalised as percentages of the maximal steroid output. For valid comparison of glomerulosa and inner zone results, values in Fig. 1 are for corticosterone alone; other steroids were stimulated in relation to corticosterone as previously described (14,15,18).

In these experiments $ACTH_{1-24}$ stimulated corticosterone output by fasciculata/reticularis cells with an ED-50 of about 5×10^{-11} moles l^{-1}, which

---

* γ_1-MSH = Tyr-Val-Met-Gly-His-Phe-Arg-Trp-Asp-Arg-Phe-NH_2
γ-MSH precursor = pro-opiocortin$_{-105 \text{ to } -29}$ (Nakanishi et al. numbering, ref. 26)
CLIP = $ACTH_{18-39}$

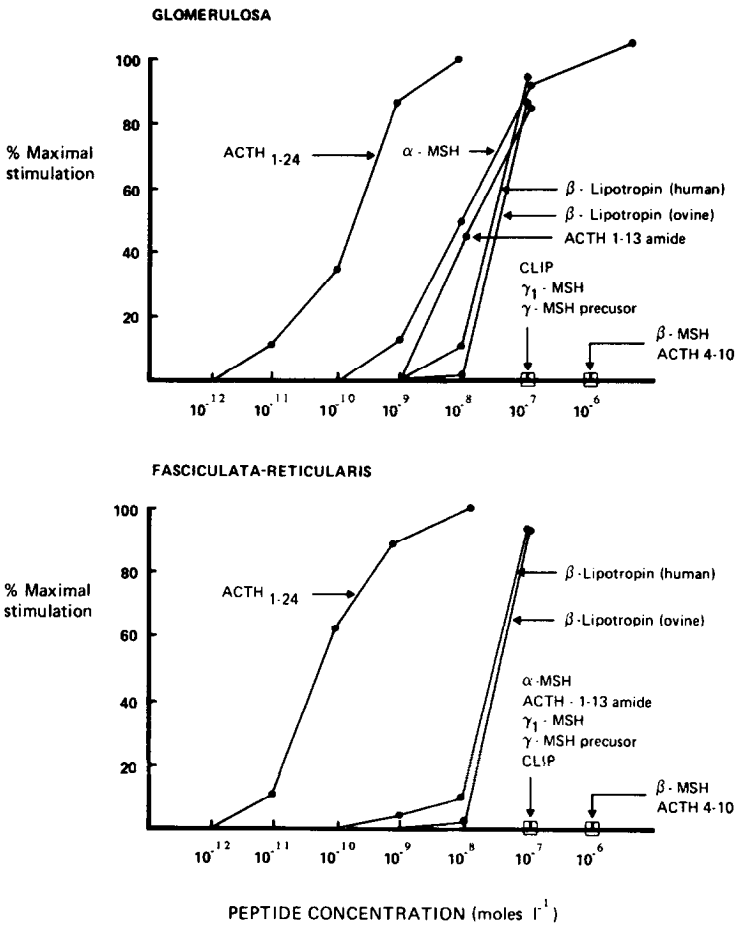

Fig. 1: Effect of pituitary peptides on stimulating steroidogenesis in suspensions of glomerulosa (upper Fig.) and fasciculata/reticularis cells (lower Fig.). Each point is the mean of two observations.

was slightly lower than for glomerulosa. β-Lipotropin of both ovine and human origin also stimulated both cell types with ED-50 two to three orders of magnitude greater than for $ACTH_{1-24}$. There were no essential differences between the effects of any of these three stimulants on the two cell types. In contrast α-MSH and $ACTH_{1-13}$-amide stimulated glomerulosa cells alone, and were without effect on the fasciculata/reticularis preparations at the concentrations used. The ED-50 for the effects of these peptides on the glomerulosa was about 10^{-8} moles l^{-1} in both cases. The other peptides assayed were without any effect on either cell

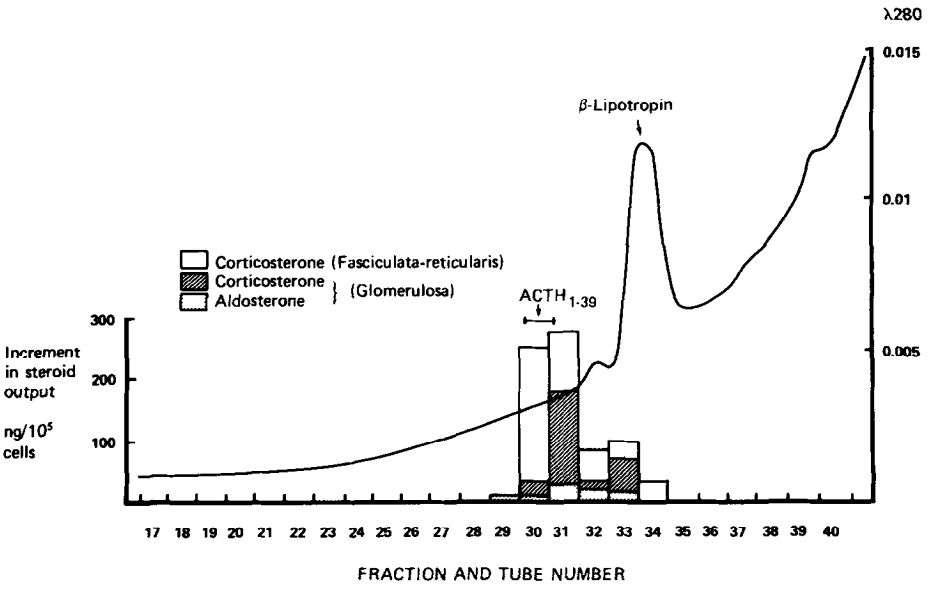

Fig. 2: Elution profile for ovine β-lipotropin subjected to HPLC fractionation, with bioassay data for the eluted fractions.

type under the conditions used. When fractions obtained by HPLC chromatography of the ovine β-lipotropin were assayed for their capacity to stimulate steroidogenesis, (Fig. 2), biologically active material (as judged by the above assay) eluted as two peaks before the elution position of β-lipotropin. The major peak of the biological activity was coincident with the normal position at which human $ACTH_{1-39}$ elutes.

DISCUSSION

From experimental studies in the rat, sheep and dog and from clinical studies in man, it has become clear in recent years that one or more pituitary components may exert specific effects on the zona glomerulosa of the adrenal cortex, and that the secretion of these components may account for enhanced aldosterone secretion in some circumstances (7,13). Clear identification of these factors has however remained elusive. In recent months three groups

have suggested different candidates for this role. Thus Matsuoka et al (16) proposed that β-lipotropin may have a special effect on glomerulosa function, and Bravo et al (17) have isolated a new glycoprotein from human urine which also stimulates glomerulosa aldosterone output. We in turn have examined the biological activity in posterior pituitary extracts, and find that a substantial part is associated with α-MSH (14).

In attempting to evaluate the likelihood that any of these might have a physiological role, it is first of all important to draw attention to the fact that under physiological conditions glomerulosa and inner adrenocortical function is often completely dissociated (3). This is particularly clear with regard to the response of the tissue to sodium depletion, in which aldosterone secretion is enhanced without any effect on cortisol secretion, for example, in the sheep or in isolated inner zone tissue in the rat (3,22,23). Consequently ACTH is ruled out as a significant mediator of glomerulosa function in these conditions. It is therefore clear that the pituitary glomerulosa stimulant we now seek is likely to be without effect on the fasciculata/reticularis. In this respect it is most important to emphasise that the two samples of β-lipotropin we assayed each stimulated both tissue types equally, and closely resemble ACTH in this respect. In the case of the ovine sample, biological activity was clearly separated from β-lipotropin by HPLC and appeared to be associated with one or more ACTH-like components (Fig. 2). From the data in Fig. 1, it is furthermore clear that a contamination of the unfractionated β-lipotropin sample by only 0.1% ACTH would account for its observed activity. Matsuoka et al. (16) also observed that inner zone function was very markedly stimulated by β-lipotropin. In this connection it should be noted too that Bravo et al (17) also find that their glycoprotein is a good stimulator of cortisol secretion.

Of the peptides we tested only α-MSH and $ACTH_{1-13}$ amide possessed the required specificity of action on the glomerulosa cells of the rat adrenal. It is interesting that the peptide structure/function relationships are different in glomerulosa stimulating and melanocyte expanding activity, since neither β-MSH, γ_1-MSH nor the γ-MSH precursors, nor $ACTH_{4-10}$ stimulated the glomerulosa cells (cf. e.g. ref. 24). They were of course only tested singly, and this does not conflict with the suggested possibility of a potentiating role for γ-MSH in ACTH action, presumably also likely to be effective in both glomerulosa and inner zones (25).

Whether α-MSH is indeed the pituitary glomerulosa stimulant under physiological conditions is a matter for further study. We nevertheless conclude that its actions are such that the physiologically important glomerulosa stimulating pituitary peptide will quite probably be found to be similar to α-MSH.

ACKNOWLEDGEMENTS

We are grateful to the MRC for project grant support to GPV and the MRC and SRC for grant support to HRM. We are also most grateful to Dr. D. M. Burley and Dr. P. Desaulles (Ciba-Geigy), Dr. P. Lowry (St. Bartholomew's Medical College), Dr. A. J. Thody (University of Newcastle-upon-Tyne) and Professor H. Yajima (University of Kyoto) for the generous gift of peptides used in this study.

REFERENCES

1. Haning, R., Tait S. A. S. and Tait J. F. (1970) Endocrinology 87, 1147-1167.
2. Williams G. H. and Dluhy R. G. (1972) Am. J. Med., 53, 585-605.
3. Müller J. (1971) Regulation of Aldosterone Biosynthesis, Springer-Verlag, Berlin.
4. Tait J. F. and Tait S. A. S. (1979) J. Endocrinol. 83, 3P-24P.
5. Peart W. S. (1970), Am. J. Clin. Pathol. 54, 324-330.
6. Spät A. and Jozan S. (1975), J. Endocrinol. 65, 55-63.

7. Palmore W.P., Anderson R. and Mulrow P.J. (1970), Endocrinology, 86, 728-734.

8. Coghlan J.P., Blair-West J.R., Denton D.A., Fei D.T., Robinson P.M., Scoggins B.A. and Wright R.D. (1979), J.Endocrinol. 81, 55P-67P.

9. Palmore W.P. and Mulrow P.J. (1967) Science 158, 1482-1484.

10. Lee T.C., Van der Wal B. and de Wied D. (1964), J.Endocrinol. 42, 465-475.

11. Ganong W.F., Pemberton P.L. and van Brunt E.E. (1967), Endocrinology, 81, 1147-1150.

12. McCaa R.E., Young D.B., Bower J.D. and McCaa C.S. (1974), Circulation Res.34 & 35 (Suppl.1), 1-15 & 1-25.

13. Williams G.H., Rose L.I., Dluhy R.G., Dingman J.F. and Lauler D. (1971) J.Clin.Endocr. 32, 27-35.

14. Vinson G.P., Whitehouse B.J., Dell A., Etienne T. and Morris H.R. (1980) Nature (Lond.) 284, 464-467.

15. Vinson G.P., Whitehouse B.J. and Thody A.J. (1981) Peptides, submitted.

16. Matsuoka H., Mulrow P.J. and Li C.H. (1980) Science 209, 307-308.

17. Bravo, E.L., Saito I., Zanella T., Sen S. and Bumpus F.M. (1980) J.Clin,Endocr. 51, 176-178.

18. Vinson G.P., Whitehouse B.J., Goddard C. and Sibley C.P. (1979) J.Steroid Biochem.11, 175-183.

19. Vinson G.P., Goddard C. and Whitehouse B.J. (1978) J.Steroid Biochem. 9, 657-665.

20. Morris H.R., Etienne A.T., Dell A. and Albuquerque R. (1980), J. Neurochem. 34, 574-582.

21. Bing R.F. and Schulster D. (1977), J.Endocrin. 74, 261-272.

22. Blair-West J.R., Coghlan J.P., Denton D.A., Munrow J.A., Wintour M., and Wright R.D. (1964) J.Clin.Invest., 43, 1576.

23. Baniukiewicz S., Brodie A., Flood C., Motta M., Okamoto M., Tait, J.F. Tait S.A.S., Blair-West J.R., Coghlan J.P., Denton D.A., Goding J.R., Scoggins B.A., Wintour M. and Wright R.D. (1968), Functions of the Adrenal Cortex (ed.K.W. McKerns) (Vol. I pp 153-232), North Holland Publishing Co., Amsterdam.

24. Baker B. (1979), in "Hormones and Evolution" (ed. E.J.W. Barrington) Vol.II pp 643-722, Academic Press, New York and London.

25. Pederson R.C. and Brownie A.C. (1980) Proc.Nat.Acad.Sci., USA, 77 2239-2243.

26. Nakanishi S., Inoue A., Kita T., Nakamura M., Chang A.C.Y., Cohen S.N. and Numa S., Nature, Lond., 278, 423-427.