PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **179**, 318-323 (1985)

Sites of Action of β-Melanocyte Stimulating Hormone in Aldosterone Biosynthesis in the Rat (42103)

MINORU YAMAKADO, ROBERTO FRANCO-SAENZ, AND PATRICK J. MULROW

Department of Medicine, Medical College of Ohio, Toledo, Ohio 43699

Abstract. The sites of action of β-melanocyte stimulating hormone (β-MSH) on aldosterone biosynthesis were studied using collagenase-dispersed adrenal glomerulosa cells from rats maintained on either normal or sodium-deficient diets for 2 weeks. Isolated cells were treated with a cyanoketone derivative (WIN 19,578) to isolate the early and late steps in aldosterone biosynthesis. WIN 19,578 (1 μM) completely blocked aldosterone production stimulated by sodium depletion, AII, ACTH, and β-MSH. β-MSH (1 μM) significantly stimulated pregnenolone production (early step) and the conversion of corticosterone to aldosterone (late step) in aldosterone biosynthesis. The effect of β-MSH was similar to AII and ACTH. Sodium depletion enhanced the effect of β-MSH only on the late step in aldosterone biosynthesis. In conclusion, β-MSH stimulates both the early and late steps of aldosterone biosynthesis. These results suggest that β-MSH or peptides containing β-MSH may play a role in the regulation of aldosterone production.

The regulation of aldosterone secretion cannot be completely explained by the known stimulators. Experimental and clinical studies (1–3) suggest that a non-ACTH pituitary factor(s) may play a role in the regulation of aldosterone secretion during sodium deficiency.

Recently, we reported that similar to the parent compound β-lipotropin (4, 5), β-melanocyte stimulating hormone (β-MSH) stimulates aldosterone production in isolated rat adrenal cell preparations. Sodium depletion enhances the sensitivity of the adrenal glomerulosa cells to β-MSH causing significant stimulation of aldosterone production by doses of β-MSH that are within the physiological range (5, 6). These studies suggested that β-MSH or peptides containing β-MSH may be one of the non-ACTH pituitary factor(s) that regulate aldosterone secretion during sodium depletion in the rat. It has been shown that angiotensin II and ACTH stimulate both the early (7–10) and late steps (10, 11) of aldosterone biosynthesis. Sodium deficiency has also been shown to stimulate both the early and late steps in aldosterone biosynthesis (9, 11–17).

In these experiments, we studied the sites of action of β-MSH on the aldosterone biosynthetic pathway under normal sodium and sodium-deficient diets in isolated rat adrenal glomerulosa cell preparations.

Materials and Methods. *Materials.* Synthetic human β-MSH was purchased from Peninsula Laboratories (San Carlos, Calif.). Synthetic ACTH$^{1\text{-}24}$ (ACTH) was provided from Ciba-Geigy Corporation (Summit, N.J.). Synthetic (Asp^1, Ile^5) angiotensin II (AII), corticosterone, and bovine serum albumin (BSA) were obtained from Sigma Chemical Company (St. Louis, Mo.), collagenase was from Worthington Biochemical Corporation (Freehold, N.J.), Medium 199 was from Gibco Laboratories (Grand Island Biological Company, Grand Island, N.Y.), and a cyanoketone derivative WIN 19,578 was kindly provided by the Sterling Winthrop Research Institute (Rensselaer, N.Y.).

Twenty female Sprague–Dawley rats weighing 200 g were used in each experiment. Rats were maintained on a normal sodium diet containing 1% NaCl (Wayne Lab-Blox; Allied Mills, Chicago, Ill.) for at least 5 days and then on either a sodium-deficient diet or normal sodium diet for 2 weeks. The sodium-deficient diet containing a negligible amount of sodium was prepared by ICN Nutritional Biochemicals (Cleveland, Ohio). Rats were killed by decapitation between 0830 and 0930, the adrenal glands were immediately

0037-9727/85 $1.50

removed and dissected free of fat and the capsular portions (glomerulosa cells) were separated from decapsular portions (fasciculata-reticularis cells) by manual squeezing. Collagenase-dispersed rat adrenal glomerulosa cells were prepared according to the method previously described in this laboratory (18). Cell counts were performed microscopically in a hemocytometer and cell viability was tested by trypan blue stain (19). In this cell suspension, the degree of contamination with zona fasciculata cells was 7.0 ± 1.4% (mean ± SE) and most cells (~95%) were viable.

The glomerulosa cell suspensions were incubated in duplicate in 1 ml of Medium 199 containing 2 mg/ml BSA with synthetic β-MSH (1 μ*M*), AII, (10 n*M*), and ACTH (10 n*M*) for 2 hr at 37°C under 95% O_2, 5% CO_2 such doses induce maximal aldosterone response in glomerulosa cells (5). To isolate the early and late portions of the aldosterone biosynthetic pathway, the glomerulosa cells were incubated with a cyanoketone derivative WIN 19,578 (1 μ*M*: an inhibitor of 3β-hydroxysteroid dehydrogenase; 4α-5-epoxy, 17β-hydroxy-3-oxo-androstane-2α-carbononitrile) to isolate the early and late portions of the aldosterone biosynthetic pathway. This cyanoketone derivative inhibits the conversion of pregnenolone to progesterone but does not affect other steps in aldosterone biosynthesis (10). Then corticosterone at concentrations of 100 ng/ml to 5 μg/ml was added as an exogenous precursor steroid of aldosterone. In this system, it is possible to study the effects of stimulators on both the early and late steps in aldosterone biosynthesis by measuring endogenous pregnenolone production as an index of activity of the early step of aldosterone production and the conversion of exogenous corticosterone to aldosterone, as an index of the late step in aldosterone biosynthesis.

Aldosterone and corticosterone in the incubation medium were measured by a specific radioimmunoassay previously described (18, 20, 21) and pregnenolone in the incubation medium was measured by direct radioimmunoassay using a highly specific antiserum provided by Dr. D. L. DiPietro (22).

All data are expressed as the means of a group ± standard error and significance was calculated by two-way analysis of variance. A *P* value of less than 0.05 was considered significant.

Results. Table I shows the effect of WIN 19,578 on aldosterone production by unstimulated cells, ACTH-stimulated, and Angiotensin II-stimulated glomerulosa. WIN 19,578 caused a 77% inhibition of basal or unstimulated aldosterone production and completely blocked aldosterone production stimulated by ACTH and Angiotensin II.

Figure 1 shows the effects of AII, ACTH, and β-MSH on pregnenolone production from endogenous cholesterol. Under normal sodium diet, AII, ACTH, and β-MSH significantly stimulated pregnenolone production from the control of 87 ± 17 ng/10^5 cells to 439 ± 76, 1102 ± 165, and 447 ± 58, respectively. ACTH was the most potent stimulus of pregnenolone production, while the effects

TABLE I. EFFECT OF WIN 19,578 ON UNSTIMULATED, ACTH AND AII STIMULATED ALDOSTERONE PRODUCTION BY DISPERSED GLOMERULOSA CELLS

		Aldosterone ($ng/10^5$ cells)	
Unstimulated cells	$n = 11$	24.26 ± 1.53	$p < 0.001$
Unstimulated cells + WIN 19,578 (1 μ*M*)	$n = 11$	5.60 ± 0.51	
ACTH (10 n*M*)	$n = 4$	94.71 ± 7.96	$p < 0.001$
ACTH (10 n*M*) + WIN 19,578 (1 μ*M*)	$n = 5$	4.34 ± 0.37	
Angiotensin II (10 n*M*)	$n = 6$	58.07 ± 6.53	$p < 0.001$
Angiotensin II (10 n*M*) + WIN 19,578 (1 μ*M*)	$n = 7$	5.66 ± 0.58	

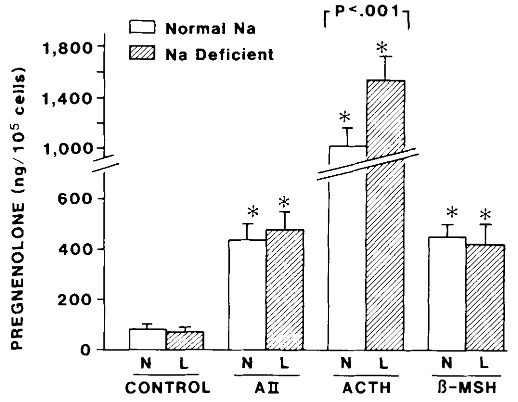

FIG. 1. Effect of AII, ACTH, and β-MSH on pregnenolone production from endogenous cholesterol in isolated glomerulosa cells from rats maintained on normal sodium (open bars) and sodium-deficient diets (hatched bars) for 2 weeks. The bars represent the means ± SE of six different experiments. *Significantly different from the each control value ($P < 0.01$).

of AII and β-MSH were similar. Sodium depletion did not enhance pregnenolone accumulation from endogenous cholesterol by itself nor pregnenolone production by AII and β-MSH, whereas the effect of ACTH was significantly enhanced by sodium depletion. Table II shows the effect of stimulators on aldosterone production from exogenous corticosterone (500 ng/ml). AII, ACTH, and B-MSH significantly stimulated aldosterone production from exogenous corticosterone under normal and sodium-deficient diets. Sodium depletion by itself significantly stimulated aldosterone production and potentiated the effects of ACTH, AII, and β-MSH. Although the absolute levels of aldosterone were highest with ACTH during both normal and sodium-deficient diets, sodium depletion did not enhance the effect of ACTH as reflected by the increment from control (13.7 ± 2.0 vs 16.0 ± 2.9, P: not significant).

Under normal sodium diet AII significantly stimulated the conversion of corticosterone to aldosterone from the control of 7.9% ± 0.7 to 9.0 ± 0.7. Sodium depletion by itself stimulated the conversion from 7.9% ± 0.7 to 12.8 ± 0.8, while AII significantly potentiated the conversion from 12.8% ± 0.8 to 15.3 ± 0.6. The increment of the percentage change was significant from 1.2% ± 0.3 to 2.5 ± 0.5 after sodium depletion. Similarly, ACTH significantly stimulated the conversion of corticosterone to aldosterone under normal and sodium-deficient diets from the control of 7.9% ± 0.7 to 10.7 ± 0.8 and 12.8% ± 0.8 to 16.3 ± 0.6, respectively. However, the increment of the percentage change was not significant from 2.8% ± 0.5 to 3.2 ± 0.6. Therefore the effect of ACTH on the late step in aldosterone biosynthesis was not enhanced by sodium depletion. β-MSH also significantly stimulated the conversion under normal and sodium-deficient diets from the control of 7.9% ± 0.7 to 9.2 ± 0.8 and 12.8% ± 0.8 to 15.5 ± 0.9, respectively. The increment of the percentage change was significant from 1.3% ± 0.4 to 2.7 ± 0.7 after sodium depletion.

Discussion. Our previous studies showed that h-β-LPH stimulates aldosterone produc-

TABLE II. EFFECTS OF AII, ACTH, AND β-MSH ON THE CONVERSION OF EXOGENOUS CORTICOSTERONE (500 ng) TO ALDOSTERONE

		Normal Na		Na deficient	
	No. of experiments[a]	Aldosterone (ng/10^5 cells)	Increment from control	Aldosterone (ng/10^5 cells)	Increment from control
Control	10	36.9 ± 3.3		65.0 ± 4.0	
AII (10 nM)	10	43.5 ± 3.3*	6.5 ± 1.4	76.4 ± 5.0*	12.6 ± 2.2***
ACTH (10 nM)	10	51.8 ± 4.2**	13.7 ± 2.0	79.9 ± 3.1**	16.0 ± 2.9
β-MSH (1 μM)	10	44.5 ± 3.7**	7.6 ± 2.1	77.2 ± 3.6**	13.4 ± 3.4***

Note. Mean ± SE; *$P < 0.01$, **$P < 0.001$ vs Control, ***$P < 0.05$ comparison of increment of normal Na vs Na deficient.

[a] Individual experiments performed in duplicate.

tion in isolated rat adrenal cell preparations and that β-MSH which corresponds to residues 35 to 56 of h-β-LPH contains the active core necessary for aldosterone stimulation. Also we have shown that sodium depletion enhances the sensitivity of the adrenal glomerulosa cells to β-MSH (5, 6). In the present study, β-MSH stimulated pregnenolone production and the conversion of corticosterone to aldosterone, both the early and late steps in aldosterone biosynthesis. It has been demonstrated that sodium depletion stimulates aldosterone biosynthesis at multiple sites (9, 11–16). In our study, sodium depletion by itself did not increase basal pregnenolone accumulation from endogenous cholesterol in isolated rat glomerulosa cells. This result is in contrast with a previous report by Aguilera *et al.* (17) which demonstrated that short-term sodium restriction increased the mitochondrial accumulation of cholesterol and its conversion to pregnenolone, as well as the conversion of corticosterone to aldosterone. Also Kramer *et al.* (23) showed that 10 days of sodium depletion increased the rate of cholesterol side-chain cleavage in glomerulosa cell mitochondria. A possible explanation for this discrepancy is that we used adrenal glomerulosa cells whereas Aguilera *et al.* and Kramer *et al.* used adrenal mitochondria. It is possible that by using intact cells we may have not been able to detect small changes in pregnenolone production occurring in the mitochondria of the sodium depleted rats.

Sodium depletion did not enhance pregnenolone production in control cells nor the effect of AII and β-MSH, but markedly enhanced the effect of ACTH on the early step in aldosterone biosynthesis. It has been reported that ACTH stimulates steroidogenesis by regulating the mitochondrial precursor pool of cholesterol (24) and cholesterol side-chain cleavage activity (25). An increase in the mitochondrial pool of cholesterol has been found in capsular cells from sodium restricted rats (17). Shima *et al.* (26) reported that cholesterol esterification and hydrolysis were significantly higher in decapsular than in capsular cells. They suggested that decapsular cells may be more active in cholesterol mobilization which involves intracellular transfer and availability of cholesterol. The higher levels of pregnenolone after ACTH may be partly explained by contamination of fasciculata cells, which are known to be sensitive to ACTH. However, the further increase in the response after sodium depletion is not readily explainable. The actions of AII and ACTH on the early step in aldosterone biosynthesis are consistent with previous reports (10). β-MSH stimulated the early step similar to AII and ACTH.

Sodium depletion by itself significantly stimulated the late step of the aldosterone biosynthetic pathway causing a near doubling of the percentage conversion of corticosterone to aldosterone. This result is consistent with previously reported work. β-MSH significantly stimulated the conversion of corticosterone to aldosterone under a normal and sodium-deficient diet. During sodium depletion, β-MSH significantly potentiated the conversion of corticosterone to aldosterone. The increment of the percentage conversion of corticosterone to aldosterone by β-MSH was about threefold after sodium depletion. It has been reported that AII and ACTH have a direct stimulatory effect on the late step of aldosterone biosynthetic pathway (10, 11). Similar to AII and ACTH, β-MSH stimulates the late step in aldosterone biosynthesis. In this study, plasma potassium concentration was slightly but significantly increased after 2 weeks of dietary sodium depletion (5). Potassium ion is an important regulator of adrenal function in the rat, playing a significant role in the mediation of the zona glomerulosa response to sodium depletion (27–29); also, potassium ion is known to stimulate both the early and late steps in aldosterone biosynthesis (8, 10, 11, 30, 33). It is possible that the increased plasma potassium concentration during sodium depletion may have played a role in the effect of AII, ACTH, and β-MSH on aldosterone biosynthesis.

In conclusion, β-MSH stimulates pregnenolone production and the conversion of corticosterone to aldosterone, both the early and late steps in aldosterone biosynthesis. Sodium depletion enhanced the effect of β-MSH only on the late step in aldosterone biosynthesis.

We thank Dr. D. L. DiPietro, Department of Obstetrics and Gynecology, Vanderbilt University, Nashville, Tennessee, for kindly providing the pregnenolone antibody and the Sterling Winthrop Research Institute for providing the cyanoketone derivative, WIN 19,578.

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Received July 26, 1984. P.S.E.B.M. 1985, Vol. 179.
Accepted March 14, 1985.