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ADRENOCORTICAL RESPONSE TO CORTICOTROPIN IS INHIBITED BY γ_3-MSH ANTISERA IN NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS

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In order to further investigate the coordinate action of pro-corticotropin/endorphin-derived peptides on adrenal steroidogenesis, we have evaluated the effects of highly specific antisera to synthetic rat γ_3-MSH (1-27) peptide (γ_3-MSH Ab) on corticosterone, 18-hydroxycorticosterone and aldosterone responses to ACTH (1-24) in chronically cannulated spontaneously hypertensive rats (SHR) and their normotensive controls (WKY). Antisera eliminated the ACTH induced rise of all three corticosteroids. It had no effect on basal corticosteroid levels. Our results offer further evidence that the potentiating action of γ_3-MSH may play an important role in modulating ACTH induced steroidogenesis.

Corticotropin (ACTH) is synthesized in the rat pituitary as a pro-ACTH/endorphin precursor called pro-opiomelanocortin (1-3). The precursor molecule contains the amino acid sequences for β-lipotropin (β-LPH) and a third region at the amino-terminal end of the molecule which has been designated the 16K fragment (apparent molecular weight, 16,000) (4). This N-terminal region contains a melanotropin (MSH)-like sequence (γ-MSH) which is partially homologous with the α-and β-MSH segments of the precursor (5,6).

Following trypsin exposure of the intact N-terminal glycoprotein, it potentiates ACTH-induced corticosterone biosynthesis in vitro and stimulates cholesterol ester hydrolase activity (7). Furthermore, the synthetic γ_3-MSH peptide stimulates the activity of adrenocortical cholesterol

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ester hydrolase and is capable of synergistically potentiating ACTH-stimulated corticosterone and aldosterone biosynthesis in hypophysectomized rats (8). Herein we have examined the effect of highly specific antisera to synthetic γ_3-MSH on corticosterone, 18-hydroxycorticosterone (18-OHB) and aldosterone responses to ACTH (1-24) in intact chronically cannulated spontaneously hypertensive rats (SHR) and their normotensive controls (WKY).

MATERIALS AND METHODS

Antisera (γ_3-MSH Ab) was raised in male New Zealand rabbits against a synthetic peptide consisting of rat γ-MSH with a carboxyl terminal extension of 15 additional residues (rat γ_3-MSH; 1-27). This synthetic peptide was generously supplied to us by Dr. Nicholas Ling of the Salk Institute. After bleeding the rabbits the antisera and normal rabbit serum were precipitated with saturated ammonium sulfate, extensively dialyzed and reconstituted with saline. This antisera has been demonstrated in our laboratory (Sharp et al. submitted) to have negligible cross reactivity with purified β-LPH (NIH), synthetic human β-endorphin (61-91), and synthetic α-MSH (Peninsula laboratories). There was less than 1% cross-reactivity with purified rat ACTH (NIH) and none with ACTH (1-24) or synthetic ACTH (1-39) (NIH). G-50 Sephadex chromatography of homogenates of rat anterior pituitary demonstrated two peaks of immunoreactivity corresponding to the 11K and 6K fragments recently reported (7,8). The total molar amount of γ_3-MSH immunoreactivity detected in this homogenate approximates the β-endorphin immunoreactivity. Serial dilution of pituitary homogenates was parallel to the standard curve for rat γ_3-MSH.

Twelve month old SHR and WKY rats had polyethylene catheters (PE-50) inserted into the left common carotid artery and a PE-10 catheter inserted into the right internal jugular vein under nembutal anesthesia (9). Catheters were exteriorized and kept patent by flushing with heparinized saline. Ninety-six hours after surgical placement (0800 to 0900 h), the catheters were passed outside the cages, and the rats were studied in a conscious unrestrained state. Blood pressures were determined via the indwelling arterial catheter using physiographic recording. Twenty-four SHR and 24 WKY rats then had basal blood samples (0.5 ml) obtained through the arterial cannula and an equal volume of normal saline replaced. Twelve of the SHR and WKY rats received 250 µl of γ_3-MSH antibody and another 12 of the SHR and WKY received 250 µl of normal rabbit serum (NRS) through the venous cannula. Thirty minutes later another blood sample (0.5 ml) was obtained through the arterial cannula and all rats were given 5 ng ACTH (1-24) in normal saline (total volume 0.5 ml) through the venous cannula. Fifteen minutes later blood samples were obtained from all rats. Blood samples were pooled so that there was 6 samples for each of four treatment group at each sampling point.

Plasma for aldosterone was extracted with methylene chloride and separated from other steroids using a Sephadex LH-20 column. The extracted aldosterone was measured by RIA as previously described (9). Assay sensitivity is 0.4 ng/dl and the intra-assay CV is 7%. Plasma 18-OHB was measured by RIA using antisera raised against 18-OHB lactone (10). Plasma corticosterone was measured by RIA as previously described (9).

Comparison of plasma, corticosterone, 18-OHB and aldosterone responses to various treatments in the SHR and WKY rats was performed using multivariant analysis (10). All results are expressed as mean $\pm$ SEM.

RESULTS

Basal systolic blood pressures were greater ($p<0.001$) for the SHR (172 ± 7 mm Hg) than for the WKY (124 ± 7 mm Hg). Basal levels of corticosterone, 18-OHB and aldosterone were similar in the SHR and the WKY groups. Neither NRS or γ_3-MSH antibody significantly affected plasma levels.

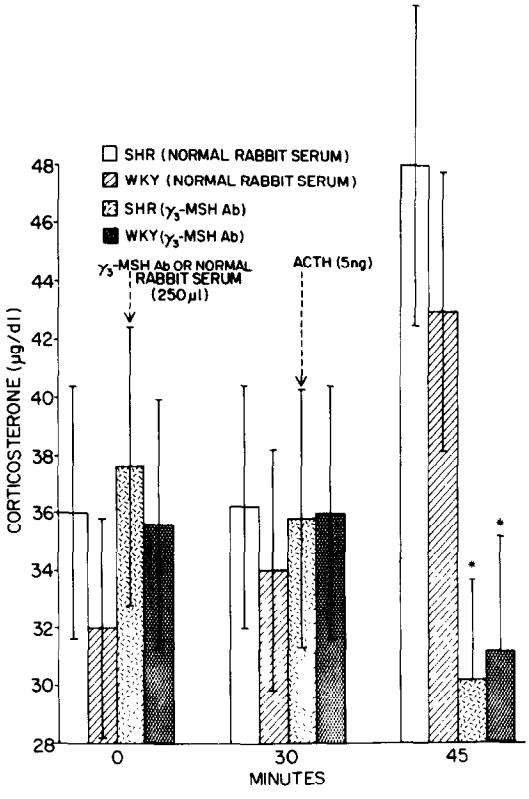

Figure 1. Effect of γ_3-MSH Ab on the corticosterone response to ACTH in SHR and WKY rats (n=6).

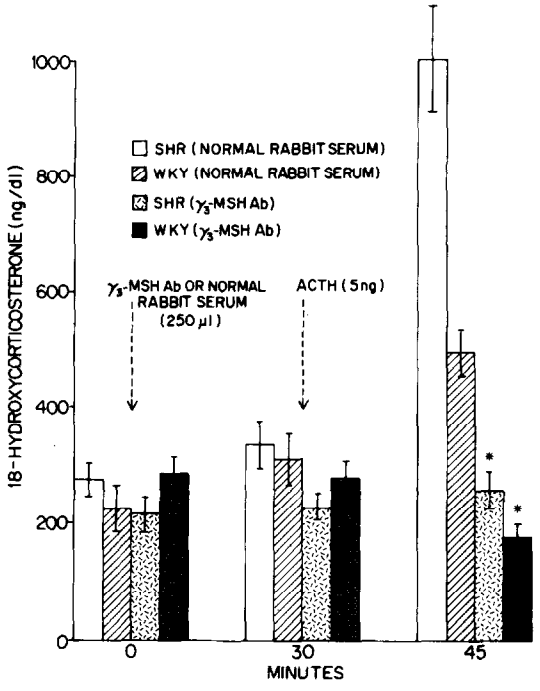

Figure 2. Effect of γ3-MSH Ab on 18-hydroxycorticosterone response to ACTH in SHR and WKY rats (n=6).

Administration of 5 ng ACTH following NRS resulted in a rise ($p<0.05$) in plasma corticosterone from 36±4.2 to 48±5.5 ng/dl in the SHR and from 34±4.2 to 43±4.8 ng/dl in the WKY (Fig 1). However, γ3-MSH antibody inhibited this response in both groups.

ACTH following NRS caused a rise ($p<0.01$) in 18-OHB from 335.1±40 to 998.4±91 ng/dl in the SHR (Fig 2) and from 306.1±44 to 492±40 ng/dl (p 0.05) in the WKY. However, the administration of γ3-MSH antibody eliminated the plasma 18-OHB response to ACTH in both the SHR and WKY.

Plasma aldosterone rose ($p<0.05$) from 9.6±1.4 to 13.6 ±2.6 ng/dl in the SHR and from 7.6±0.8 to 13.5±2.5 ng/dl in the WKY (Fig 3). The aldosterone response to ACTH was eliminated by γ3-MSH Ab in both groups.

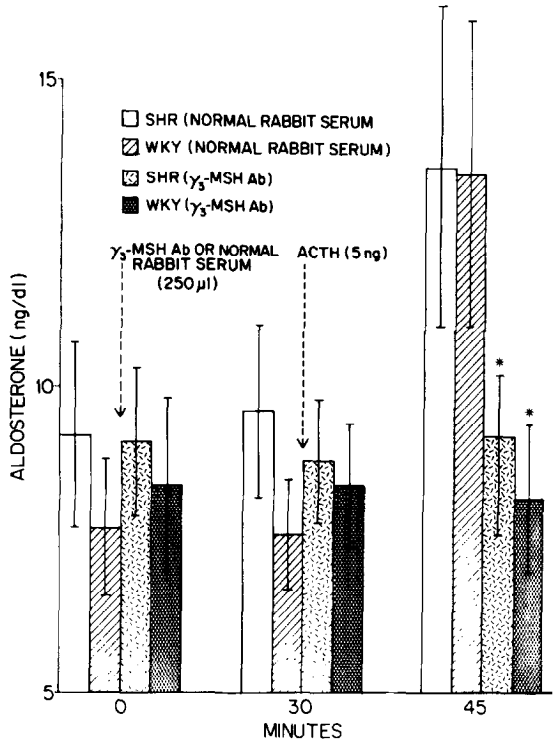

Figure 3. Effect of γ_3-MSH Ab on aldosterone response to ACTH in SHR and WKY rats (N=6).

DISCUSSION

Although the exact biological significance of γ_3-MSH like fragments from the N-terminal peptide of pro-opiomelanocrotin remains to be elucidated, Pedersen et al (7,8) have recently reported that γ_3-MSH potentiated the effect of ACTH on serum corticosterone and aldosterone secretion in hypophysectomized rats. The intact N-terminal peptide had no activity unless it was subjected to trypsinization, presumably releasing the active peptide inside its structure containing γ_3-MSH (7). Several laboratories (11,12) have confirmed the presence of γ-MSH in the pituitary and circulation of several species, including human beings. Thus, there is considerable support for γ_3-MSH playing a potentially important role in adrenocortical control.

In the present study we have demonstrated that the administration of a highly specific antibody to γ_3-MSH eliminated corticosterone, 18-OHB and aldosterone responses to ACTH. However, γ_3-MSH Ab did not affect basal levels of these corticosteroids. This suggests that endogenous γ_3-MSH like peptides may play an important role in potentiating ACTH induced steroidogenesis. The observation that corticosterone and 18-OHB as well as aldosterone responses to ACTH were affected by γ_3-MSH antibody is consistent with previous observations (8) that γ_3-MSH potentiates steroid output from the inner zones of the adrenal cortex. It has also been shown that both γ_3-MSH and human pro-γ-MSH are potent ACTH-independent stimulators of aldosterone secretion by a primary culture of adrenocortical adenoma cells from a patient with primary hyperaldosteronism (13). However, in our study γ_3-MSH antibody did not significantly affect corticosterone, 18-OHB and aldosterone levels in the absence of ACTH; it remains uncertain whether γ_3-MSH has a direct steroidogenic action on normal tissue.

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