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EVIDENCE THAT ARGININE VASOTOCIN INHIBITS HUMAN CHORIONIC GONADOTROPIN AND CYCLIC ADENOSINE 3',5' MONOPHOSPHATE STIMULATED OVARIAN STEROIDOGENESIS*

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Summary: A study of the ovary as a site of action of arginine vasotocin, a nonapeptide synthesized in the pineal gland of vertebrates, was conducted in the rat. Progesterone production stimulated by either human Chorionic Gonadotropin or 8Br-cyclic Adenosine 3',5' Monophosphate was significantly reduced in rats pretreated with arginine vasotocin. The affinity and receptor number for human chorionic gonadotropin were uneffected by pretreatment with arginine vasotocin. These results suggest that the antigonadotropic action attributed to arginine vasotocin may be due to an inhibition of ovarian steroidogenesis at a point distal to cyclic Adenosine 3',5' Monophosphate formation.

INTRODUCTION

Arginine vasotocin (AVT) is a nonapeptide synthesized and stored in the pineal gland of Vertebrates (1-3). Previous studies have established that AVT possesses antigonadotropic actions such as acting as an abortifacient (4), blocking ovulation (5), and causing a reduction in sex organ weight (6). The site and the mechanism of inhibition of the hypothalamo-hypophyseal-gonadal axis by AVT have not been defined, however (7). The present study investigated the effect of AVT on the ovary as a possible site of its antigonadotropic actions.

MATERIALS AND METHODS

Female 24 to 26 day old Sprague-Dawley rats were used in the present studies. The rats were made pseudopregnant by subcutaneous injection with

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Abbreviations: AVT, Arginine Vasotocin; hCG, human Chorionic Gonadotropin; cAMP, cyclic Adenosine 3',5' Monophosphate; K_d, Equilibrium dissociation constant.

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50 IU pregnant mare's serum gonadotropin followed 56 h later by 25 IU hCG as described by Parlow (8). Six days after the hCG injections the rats received three subcutaneous injections of either 0.9% NaCl or AVT (4 μg dissolved in 100 ml saline). Twenty-four hours following the injections, the rats were then killed and a collagenase dispersed luteal cell suspension was prepared as described earlier (9). Approximately 1×10^6 luteal cells (0.1 ml) were incubated in tubes containing 0.3 ml Medium 199 in the presence or absence of hCG or 8Br-cAMP. The incubation was carried out for three hours in a metabolic shaking incubator at 37°C in the presence of 95% oxygen and 5% carbon dioxide. At the end of the incubation, the reaction was stopped by placing the tubes in a boiling water bath for three minutes. To the samples. 0.6 ml of water and 10 ul of [^{3}H]-progesterone (approx. 10,000 cpm) were added to monitor recovery. The tubes were stored at 4°C overnight and the samples were extracted with petroleum ether and assayed for progesterone by radioimmunoassay (10).

A second group of similarly injected rats were killed and the specific binding of [^{125}I]-hCG to partially purified luteal cell plasma membranes was determined by the method described earlier from this laboratory (11). Aliquots of purified membrane were incubated for 90 minutes at 37°C in 0.3 ml 0.25 M sucrose containing 10 mM Tris HCl (pH 7.5) in the presence of varying concentrations of [^{125}]-hCG (45,000 cpm/ng). At each concentration of [^{125}I]-hCG the cells were incubated with and without unlabeled hCG (10 μg) to determine specific binding. The binding reaction was stopped by adding 1 ml of 0.25 M sucrose Tris HCl followed by centrifugation at 10,000 rpm for 10 min in a Sorvall refrigerated centrifuge fitted with a SS34 rotor. The pellet was washed once with 1 ml of fresh 0.25 M sucrose. The sedimented membrane was counted for bound radioactivity in a gamma counter (Searle Analytic Mark I). The specific binding was determined by subtracting the nonspecific binding from the total binding. DNA was estimated by the colorimetric method of Burton (12).

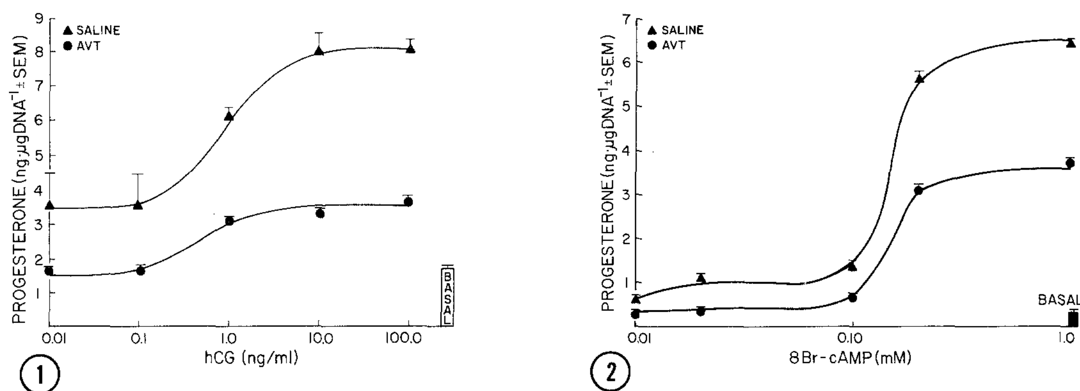

Fig. 1. Effect of increasing concentrations of hCG on steroidogenesis by luteal cells in the presence or absence of pretreatment with AVT. Rats were pretreated with saline or AVT. Luteal cells were incubated with the indicated concentrations of hCG for 3 hours in an atmosphere of 95% oxygen and 5% carbon dioxide. The samples were processed for progesterone assay as described in Materials and Methods. Results are expressed as the mean of triplicate samples on duplicate determinations.

Fig. 2. Effect of increasing concentrations of 8Br-cAMP on steroidogenesis by luteal cells in the presence or absence of preinjection of AVT. Incubation conditions were similar to Figure 1 except the indicated concentrations of 8Br-cAMP were added.

RESULTS AND DISCUSSION

Effect of AVT on hCG-stimulated steroidogenesis by rat luteal cells. Figure 1 shows that increasing concentrations of hCG stimulated progesterone production in a dose related manner resulting in a 4-fold stimulation. At all concentrations of hCG, pretreatment with AVT produced a 50% reduction of gonadotropin-induced steroidogenesis. The reduction was significant (Student's T-test, $p < 0.01$) at concentrations of hCG greater than 0.1 ng/ml.

Effect of AVT on 8Bromo-cyclic AMP-stimulated steroidogenesis by rat luteal cells. Figure 2 demostrates that increasing concentrations of 8Br-cAMP stimulated progesterone production in a dose related manner from 0.01 to 1.0 mM concentrations. Pretreatment with AVT inhibited steroidogenesis at all concentrations of 8Br-cAMP, and was significant ($p < 0.01$) at concentrations of 8Br-cAMP greater than 0.1 mM.

Effect of AVT on [^{125}I]-hCG binding to purified membrane preparations. In order to determine whether the inhibitory effect of AVT on gonadotropin-

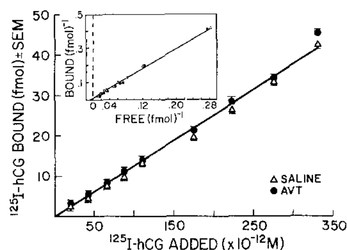

Fig. 3. Effect of increasing concentrations of [^{125}I]-hCG on hormone binding to partially purified luteal cell plasma membrane preparations in the presence or absence of preinjection of AVT. Varying concentrations of [^{125}I]-hCG were incubated for 90 minutes with the membrane preparations. The results indicate specific binding. All assays were carried out in triplicate.

Inset. Data from Figure 3 plotted according to Klotz (13).

induced ovarian steroidogenesis resulted from changes in the binding properties of the gonadotropin receptors, the binding kinetics in partially purified membrane preparations of luteal cells were determined with and without pretreatment with AVT. Figure 3 shows the effect of increasing concentrations of [^{125}I]-hCG on the binding of hormone to luteal cell membranes prepared from both groups. There was no difference in the binding kinetics between membranes prepared from the control and AVT treated animals. Equilibrium data plotted according to Klotz (13) demonstrate a single class of gonadotropin binding sites with an equilibrium dissociation constant (K_d) of 2.66×10^{-10}M (see inset in Fig. 3). The value for the K_d is consistent with previously reported figures (9). AVT treatment did not alter either the K_d or the number of gonadotropin binding sites.

The data presented in this study demonstrate that AVT has significant inhibitory effects directly on ovarian steroidogenesis. Progesterone production is diminished following AVT pretreatment when stimulated with either hCG or the cAMP analogue. In addition, AVT pretreatment produced no effect in the ability of the membrane preparation to bind ^{125}I-hCG. This suggests that the site of inhibition of ovarian steroidogenesis by AVT is distal to gonadotropin receptor interaction and cyclic AMP formation, but rather at a site after cyclic AMP formation. Whereas the site and mechanism of the antigonadotropic

actions of AVT had not been identified, these new findings suggest that one of the sites of the antigonadotropic action of AVT is at the ovarian level by inhibiting gonadotropin stimulated steroidogenesis.

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