Multiplication Stimulating Activity (MSA) Can Substitute for Insulin to Stimulate the Secretion of Testicular Transferrin by Cultured Sertoli Cells

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ABSTRACT

Both MSA and insulin were found to stimulate transferrin production in cultured Sertoli cells to the same extent in the absence or presence of follitropin, testosterone, or retinol. Sertoli cells were responsive to 30-fold lower concentrations of MSA than of insulin. MSA and insulin together stimulated transferrin secretion to the same extent as either hormone alone. These results, and what is presently understood about the relationship of MSA and insulin, suggest that insulin can substitute for the action of an MSA-like peptide in the stimulation of testicular transferrin secretion by Sertoli cells.

INTRODUCTION

Sertoli cells are testicular cells which are found in the seminiferous tubules. Within these tubules is a specialized serum-free environment created primarily by the Sertoli cells (Waites and Neeves, 1977). It has previously been demonstrated that Sertoli cells can be isolated and maintained in serum-free cell culture and these cultured Sertoli cells are under hormonal control (Dorrington and Fritz, 1975; Griswold and Skinner, 1982). We have shown that Sertoli cells synthesize and secrete a transferrin-like protein (Skinner and Griswold, 1980). Recently, with a radioimmunoassay for transferrin we have determined that a variety of hormones and a vitamin regulate testicular transferrin secretion by cultured Sertoli cells (Skinner and Griswold, 1982). It was found that follitropin, insulin, testosterone, and the retinoids all stimulate transferrin production. However, the presence of insulin was required for either the retinoids or testosterone to significantly stimulate transferrin production. In the presence of insulin the responsiveness of Sertoli cells to follitropin and testosterone increased 2-fold and to the retinoids 3-fold.

Multiplication stimulating activity (MSA) is a series of structurally related peptides of approximately 10,000 daltons present in serum. MSA has similar properties to the non-suppressible insulin-like activity present in serum and to somatomedins present in serum (Dulak and Temin, 1973). A component of MSA is structurally homologous to insulin-like growth factor II (IGF II) (Marquardt et al., 1973). In addition, MSA has been shown

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to have insulin-like activity with several different tissue types (Rechler et al., 1977).

This study presents a comparison of the regulatory action of insulin and MSA on transferrin production by cultured rat Sertoli cells.

METHODS

Chemicals. Multiplication stimulating activity (MSA), isolated from buffalo rat liver cell conditioned medium, was obtained from Collaborative Research Inc. Medium for cell culture (Ham's F-12) was made from a powdered formulation supplied by Gibco. Purified rat transferrin and rabbit anti-rat transferrin were obtained from Cappel Laboratories. ^{125}I was obtained from New England Nuclear Corp. FSH (NIAMDD ovine FSH-S13) was obtained from the National Pituitary Agency, NIH. All other chemicals were obtained from Sigma.

Cell Culture. Sertoli cells from 20 day old rats were prepared and cultured as previously described (Dorrington and Fritz, 1975; Skinner and Griswold, 1980). Cells were cultured in F-12 medium in 24 well dishes (Linbro Scientific, Inc.). Approximately 5×10^5 cells were plated per well and were maintained in the absence of serum. The medium (1 ml) from each well was collected and replenished every 2 days for the duration of the culture. The samples that were assayed were collected between days 4 and 6 of cell culture. The number of cells in each well were determined by a Coulter ZF cell counter after the cells were removed with 0.5 ml of 1.7% trypsin solution in isotonic saline. Hormones and vitamin A were added to the designated cultures for the duration of the cell culture in the following final concentrations: FSH (25 ng/ml), testosterone (0.7 μM), and retinol (0.35 μM).

Radioimmunoassay. Rat serum transferrin was iodinated with ^{125}I using a chloramine-T procedure previously described (Skinner and Griswold, 1982). A quantitative radioimmunoasay for testicular transferrin using rabbit anti-rat serum transferrin was performed as previously described (Skinner and Griswold, 1982). This assay was shown not to cross react with any of the medium components, hormones, or any of the Sertoli cell secreted proteins except testicular transferrin (Skinner and Griswold, 1980; 1982).

RESULTS

The hormonal stimulation of testicular transferrin secretion was previously found to reach a maximum during the 48 hr collection on day 6 of Sertoli cell culture (Skinner and Griswold, 1982). Therefore, transferrin concentrations were determined on medium samples collected at this time. It was found that MSA did stimulate transferrin production approximately the same amount that insulin did. Dose response curves were determined for both MSA and insulin and are shown in Figure 1. The MSA concentration required for a half maximal stimulation was approximately 5 nM and for a

maximum response was 10 nM. For insulin the concentration required for a half maximal stimulation was 150 nM and for a maximum response was 833 nM.

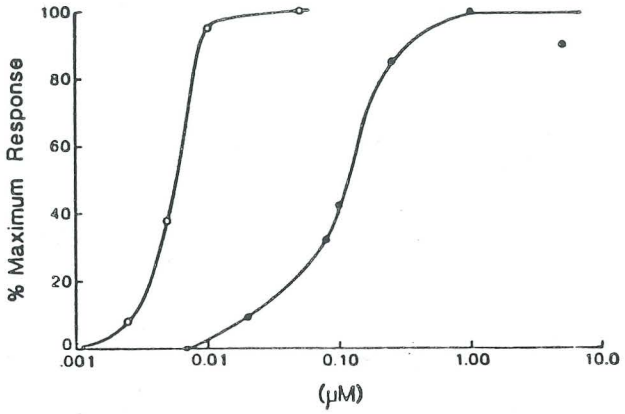

Fig. 1. Insulin and MSA dose response curves. The amount of transferrin was determined with a transferrin radioimmunoassay from a 48 hr medium collection on day 6 of a Sertoli cell culture. Varying concentrations of MSA (o) and insulin (•) were added to the cultures and the resultant amount of transferrin expressed as a % maximum response. Values represent the mean of three different determinations which had a variance between 10 and 15%.

The magnitude of the maximum stimulation obtained with MSA and insulin is shown in Table 1. Both MSA and insulin produced approximately a 2-fold stimulation of transferrin secretion over control

TABLE I. TESTICULAR TRANSFERRIN PRODUCTION IN THE PRESENCE OF MSA AND INSULIN. The secretion of transferrin (ng Tf/10^5 cells) was determined with a radioimmunoassay on day 6 of culture from Sertoli cells cultured from 20 day old rats. Values represent a mean and one standard deviation from 9 different determinations. Values were analyzed statistically with Duncan's multiple range test for variable values. The values with different superscript letters are significantly different from each other at $P \leq 0.05$ (Duncan, 1955).

Treatment	Concentration	ng Tf/10^5 Cells
Control	---	25 ± 5^a
Insulin	833 nM (5 μg/ml)	48 ± 8^b
MSA	10 nM (.1 μg/ml)	46 ± 7^b
Insulin + MSA	same	42 ± 6^b

levels. Also shown in Table 1 is the stimulation obtained in the presence of both MSA and insulin in the same culture well. The response was not additive and resembled that of either hormone alone.

The response of Sertoli cells to insulin and MSA in the presence of follitropin, testosterone, and vitamin A are shown in Table 2. The same magnitude of stimulation was obtained with either insulin or MSA. These results suggest that insulin and MSA stimulate transferrin production by Sertoli cells the same amount in the absence or presence of other hormones and vitamin A.

It was also found that in the presence of MSA the Sertoli cell numbers at the end of the culture period were 2-fold increased over the number of cells in control cultures. This increase was not found with insulin, follitropin, testosterone, or the retinoids. This MSA-induced increase in cell number was attributed to an increase in cell plating efficiency and not to cell division (data not shown).

TABLE II. EFFECT OF OTHER HORMONES AND VITAMIN A ON THE STIMULATION OF TESTICULAR TRANSFERRIN PRODUCTION BY MSA AND INSULIN
Sertoli cells from 20 day old rats were placed in cell culture as described in the Methods. The amount of transferrin is expressed as ng Tf/10^5 cells and was determined with a radioimmunoassay on day 6 of culture. Each column represents an experiment where insulin or MSA or neither insulin nor MSA (control) were added to the cultures. Each line represents the different hormones or vitamin added to the cultures in those experiments (F = follicle stimulating hormone, T = testosterone, R = retinol, C = no F, T or R). Values represent the mean and one standard deviation. The number in brackets indicates the number of different determinations. Values for each treatment were analyzed statistically with Duncan's multiple range test for variable values. The values with different superscript letters for a given treatment differ from each other at $P \leq 0.05$ (Duncan, 1955).

	Control	+ Insulin	+ MSA
F	$73 \pm 10\ (3)^a$	$132 \pm 16\ (3)^b$	$130 \pm 12\ (3)^b$
T	$37 \pm 4\ (3)^a$	$70 \pm 8\ (3)^b$	$75 \pm 11\ (3)^b$
R	$41 \pm 4\ (3)^a$	$102 \pm 10\ (3)^b$	$109 \pm 9\ (3)^b$
C	$25 \pm 5\ (9)^a$	$48 \pm 8\ (9)^b$	$46 \pm 7\ (9)^b$

DISCUSSION

An analysis of the dose response curves of insulin and MSA revealed that MSA was more than 30-fold more potent than insulin in promoting transferrin secretion. An MSA concentration of 10 nM stimulated transferrin production. This concentration is similar to the physiological concentrations of MSA in rat serum (10 to 50 nM) previously reported by Moses et al. (1980). In contrast, an 833 nM concentration of insulin was required for a maximum stimulation while the physiological concentration of insulin has been reported to be less than 1 nM in serum (Rosenzweig et al., 1980). The magnitude of the stimulation of transferrin secretion by cultured rat Sertoli cells for both insulin and MSA was the same. Also both MSA and insulin increase the responsiveness of the Sertoli cells to the other hormones and vitamins in a similar way. The stimulation of transferrin production in the presence of both insulin and MSA together was the same as with either hormone alone and was not additive. MSA has weak insulin-like activity as determined by the measurement of glucose oxidation in rat adipose tissue (Rechler et al., 1977). In addition, MSA will compete for insulin receptors and insulin can bind to MSA receptors (Rechler et al., 1977).

Based on these results, and what is already understood about the relationship between MSA and insulin, we feel that in cell culture insulin can substitute, at high concentration, for the physiological requirement of Sertoli cells for MSA. This finding does not exclude the possibility that insulin may have a physiological regulatory action on some other cellular parameter of Sertoli cell functions.

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