INVOLVEMENT OF POLYAMINES IN THE PROGESTIN-INDUCED STIMULATION OF ENDOMETRIAL GLYCOGEN SYNTHESIS DURING ORGAN CULTURE

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SUMMARY

Addition of progesterone to an organ culture of endometrial fragments increased glycogen levels 2-3 fold. Concomitant with this increase in glycogen was an increase in S-adenosyl-L-methionine decarboxylase activity and the conversion of putrescine into spermidine. When an inhibitor of S-adenosyl-L-methionine decarboxylase, methylglyoxyl bis(guanylhydrazone), was added to the culture with progesterone, glycogen synthesis was blocked. Inhibition of glycogen synthesis was reversed by the addition of spermidine, suggesting that spermidine synthesis may be necessary for the progestin-induced accumulation of endometrial glycogen.

Progesterone action on the uterus is essential for the development and maintenance of secretory endometrium and pregnancy. Numerous biochemical events are associated with these phenomena, one of which is the accumulation of endometrial glycogen. Recent studies demonstrated that uterine glycogen metabolism can be studied in an organ culture system which employs endometrial fragments (1,2). Experiments with this system indicated that progesterone-induced accumulation of glycogen was dependent upon prior estrogen priming, concomitant insulin action, and continued RNA and protein synthesis (2). These observations suggest that organ culture can be used to elucidate the mechanism of progestin action on the uterus.

Polyamine levels and the activities of their synthesizing enzymes are increased in rapidly growing tissues (3,4). A rapid rise in polyamine levels in uterus and prostate has been demonstrated following treatment with estradiol and testosterone, respectively (5-7). In parallel experiments, the sex steroid increased ornithine decarboxylase activity as well as S-adenosyl-L-methionine decarboxylase, the enzyme which catalyzes the conver-

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TABLE I

Effect of Progesterone on Endometrial Glycogen, Polyamines, and AdoMet Decarboxylase Activity During Organ Culture

Addition	Glycogen[1] (μg glycogen/ 100 mg wet tissue	Polyamines[1] Putrescine	Spermidine	Spermine	AdoMet Decarboxylase ($dpm\ ^{14}CO_2$/mg protein) x 10^{-3}
		(dpm/100 mg wet tissue) x 10^{-2}			
Control	58 ± 14	305 ± 33	67 ± 21	23 ± 18	5.4
Progesterone	152 ± 59	326 ± 55	148 ± 18	22 ± 6	17.9
Progesterone + MGBG	62 ± 23	278 ± 47	2 ± 1	1 ± 1	0.01*

[1] Results are the mean ± SD of four experiments. Endometrial fragments were cultured 24 h with vehicle (control), 6 μM progesterone, or 6 μM progesterone + 4 μM MGBG which was added 2 h prior to the steroid. After harvesting, glycogen, polyamines, and AdoMet decarboxylase were assayed as described in Methods.

* Enzyme activity measured in the presence of MGBG.

sion of putrescine to spermidine, the rate-limiting step in polyamine synthesis (5,7). In the present study, we have measured the effect of progesterone on polyamine levels and AdoMet decarboxylase activity in endometrium in organ culture. In addition, a potent inhibitor of AdoMet decarboxylase, methylglyoxyl bis(guanylhydrazone) (8) was used to determine the role of polyamine synthesis in the progesterone-induced stimulation of glycogen. The results demonstrate that progesterone increases polyamine and endometrial glycogen levels. In addition, progesterone induction of glycogen accumulation is blocked when spermidine synthesis is inhibited with MGBG.

METHODS

Endometrium was excised from estrogen-primed guinea pig uteri and placed in culture as previously described (2). The only modifications of published procedures were a culture period of 24 h and the omission of a fetal calf serum supplement to the Trowell's T8 medium which contains 22 mM glucose. Each culture contained [^{14}C]putrescine (1.0 μCi/ml, specific activity 51.8 Ci/mole) and progesterone (6 μM) or steroid-free media (control). In those experiments which employ MGBG (4 μM), the inhibitor was added 2 h prior to the steroid and [^{14}C]putrescine. [^{14}C]MGBG (0.4 Ci/mole) was synthesized by the procedure of Oliverio and Denham (9) and used to determine the effect of spermidine on tissue uptake of MGBG. Spermidine (1 mM) was added to the media as indicated.

Abbreviations: AdoMet, S-adenosyl-L-methionine; MGBG, methylglyoxyl bis (guanylhydrazone)

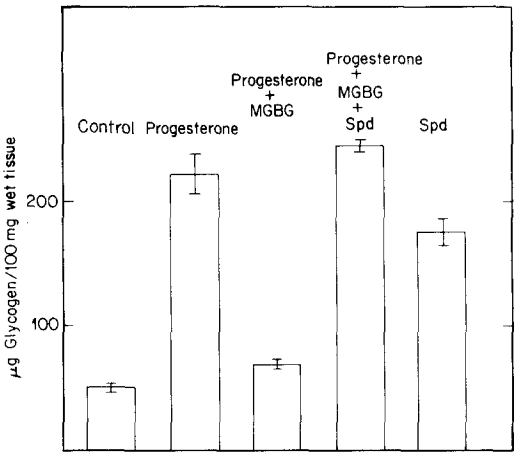

Figure 1 Effect of MGBG and spermidine on glycogen synthesis. Endometrial fragments were cultured 24 h with vehicle (control), 6 μM progesterone, 6 μM progesterone + 4 μM MGBG, 6 μM progesterone + 4 μM MGBG + 1 mM spermidine (Spd), or 1 mM spermidine. MGBG was added 2 h prior to other additives. Glycogen was determined as described in Methods. Bars and brackets indicate the average and range of duplicate determinations.

Four cultures were used for each experimental point. At the end of the incubation period, the tissue was frozen on dry ice and weighed. Two cultures were used to measure glycogen colorimetrically as previously reported (2). The third culture was homogenized in 1 ml of 5% (w/v) trichloroacetic acid and used for assay of radioactivity present in putrescine and polyamines. Synthesis of polyamines was measured by counting the incorporation of label from [^{14}C]putrescine into the polyamines which were separated by high-voltage paper electrophoresis and located by ninhydrin spray (7). The fourth culture was homogenized in 1 ml 20 mM Tris-HCl, 5 mM dithiothreitol, pH 7.3, centrifuged at 15,000 x g for 30 min and AdoMet decarboxylase activity in the supernatant fraction quantified by measuring $^{14}CO_2$ released from AdoMet ^{14}COOH (10). Each assay contained in 0.25 ml: 0.1 ml undialyzed tissue extract, 0.2 mM AdoMet-^{14}COOH (10 Ci/mole), 2.5 mM putrescine, 50 mM Na phosphate, pH 7.0, and 2.5 mM dithiothreitol.

RESULTS

The effect of progesterone on endometrial glycogen and polyamine synthesis are summarized in Table I. [^{14}C]putrescine was added to the media to enable measurement of conversion into polyamines. The uptake of [^{14}C] putrescine by the tissue was unaffected by hormone or MGBG (Table I). Progesterone addition to the culture media increased glycogen levels 2-3

TABLE II

Effect of Spermidine on Tissue Level of [^{14}C]MGBG

Addition	Tissue Level of [^{14}C]MGBG (dpm/mg wet tissue)
Control	7.2
Spermidine	7.9

Endometrial fragments were incubated 24 h with [^{14}C]MGBG (4 μM) or [^{14}C]MGBG (4 μM) + spermidine (1 mM). The [^{14}C]MGBG was added 2 h prior to the spermidine. The tissue was washed in media, blotted and dissolved in warm 30% KOH before counting.

fold over untreated controls. There was a concomitant increase in the activity of AdoMet decarboxylase as well as an increase in the conversion of putrescine into spermidine. Although putrescine was converted to polyamines, the radioactivity present in putrescine did not decrease in the presence of progesterone. Presumably, this was due to the fact that equilibration of endogenous putrescine with exogeneous [^{14}C]putrescine was rapid compared to polyamine synthesis.

When sufficient MGBG was added to the culture media to inhibit polyamine synthesis, glycogen synthesis was also blocked (Table I). The effect of MGBG on glycogen synthesis could be reversed by the addition of spermidine to the media (Fig. 1). In this latter experiment, polyamine synthesis could not be measured due to the exogenous spermidine. The addition of spermidine alone also increased glycogen levels in endometrium cultured in the absence of progesterone.

Spermidine could have reversed the effect of MGBG by preventing the uptake of the drug. This possibility was tested by addition of [^{14}C]MGBG in the presence or absence of spermidine. Since MGBG is not metabolized by mammalian cells (11), the radioactivity present in the tissue correlates directly with the inhibitor. There was no effect of spermidine on the tissue level of [^{14}C]MGBG (Table II).

DISCUSSION

Polyamine synthesis involves the conversion of arginine to spermidine via ornithine and putrescine intermediates. Several investigators have reported increased polyamine and/or enzyme levels in target tissues following the administration of sex steroids (5-7,12,13). In the uterus, these increases are among the earliest reported responses to estrogen stimulation (5,12,13). Additional studies have shown that polyamine synthesis fluctuates with the reproductive cycle, being elevated in concert with rising estrogen and progestin levels (14,15). In the present study, addition of progesterone to the organ culture media resulted in an increase in endometrial glycogen and an increase in the conversion of putrescine to spermidine. This increase in spermidine concentration correlated well with the increase in AdoMet decarboxylase activity.

In order to assess the role of polyamine synthesis in the progesterone-induced accumulation of endometrial glycogen, we made use of a competitive inhibitor of AdoMet decarboxylase, MGBG (8). In the organ culture system, addition of MGBG followed by progesterone resulted in an inhibition of the accumulation of glycogen. The effect of the inhibitor was overcome by addition of spermidine. Thus, spermidine synthesis may be a necessary step for the progestin-induced accumulation of endometrial glycogen.

The present results are strikingly similar to those from the organ culture of mouse mammary epithelium which requires insulin, prolactin, and hydrocortisone for polyamine synthesis and an increase in milk protein synthesis (16-19). In the culture of mouse mammary epithelium, spermidine can partially substitute for the glucocorticoid (16-18). Similarly, spermidine may replace progesterone or insulin in the endometrial cultures depending upon incubation conditions. We previously demonstrated that insulin is an obligatory component of the media and that continued RNA and protein synthesis are necessary for the progesterone-induced expression of glycogen synthesis (2). The latter studies, when viewed in context with those on

mouse mammary epithelium (16-19), suggest the effect of spermidine may be to increase a specific enzyme necessary for glycogen synthesis in uterus. Further experiments are required to determine the primary site of control and the mechanism by which progesterone and spermidine increase glycogen synthesis.

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REFERENCES

1. Csermely, T., Demers, L.M. and Hughes, E.C. (1969) Ob. Gyn. 34, 252-259.
2. Demers, L.M., Feil, P.D., and Bardin, C.W. (1977) N.Y. Acad. Sci., in press.
3. Tabor, C.W., and Tabor, H. (1976) Ann. Rev. Biochem. 45, 285-306.
4. Raina, A., and Jänne, J. (1975) Med. Biol. 53, 121-147
5. Russell, D.H., and Snyder, S.H. (1971) Endocrinology 88, 1397-1403.
6. Moulton, B.C., and Leonard, S.L. (1969) Endocrinology 84, 1461-1465.
7. Pegg, A.E., Lockwood, D.H., and Williams-Ashman, H.G. (1970) Biochem. J. 117, 17-31.
8. Williams-Ashman, H.G., and Schenone, A. (1972) Biochem. Biophys. Res. Comm. 46, 288-295.
9. Oliverio, V.T., and Denham, C. (1963) J. Pharm. Sci. 52, 202-203.
10. Pegg, A.E., and Conover, C. (1976) Biochem. Biophys. Res. Comm. 69, 766-774.
11. Mihich, E. (1975) In: Handbook of Experimental Pharmacology, A.C. Sartorelli and D.G. Johns, eds., Vol. 38, pp. 766-788.
12. Cohen, S., O'Malley, B.W., and Stastny, M. (1970) Science 170, 336-338.
13. Kaye, A.M., Icekson, I., and Lindner, H.R. (1971) Biochem. Biophys. Acta 252, 150-159.
14. Hernandez, O., Ballesteros, L.M., Mendez, D., and Rosado, A. (1973) Endocrinology 92, 1107-1112.
15. Lundgren, D.W., Farrell, P.M., Cohen, L.F., and Hankins, J. (1976) Proc. Soc. Exp. Biol. Med. 152, 81-85.
16. Oka, T., and Perry, J.W. (1974) J. Biol. Chem. 249, 3586-3591.
17. Oka, T., and Perry, J.W. (1974) J. Biol. Chem. 249, 7647-7652.
18. Oka, T. (1974) Science 184, 78-80.
19. Rillema, J. (1976) Biochem. Biophys. Res. Comm. 70, 45-49.