INHIBITION OF ORNITHINE DECARBOXYLASE *IN VIVO* IN RAT OVARY

S. K. Guha and J. Jänne

Department of Biochemistry, University of Helsinki, SF-00170 Helsinki 17, Finland

Received January 20,1977

SUMMARY: Various diamines injected intraperitoneally to the rat powerfully inhibited ovarian ornithine decarboxylase (EC 4.1.1.17) stimulated by human chorionic gonadotrophin (HCG). 1,3-Diaminopropane and putrescine (1,4-diaminobutane) partially prevented the rise in the enzyme activity by HCG in the prepubertal rat ovaries whereas cadaverine (1,5-diaminopentane) and most notably 1,6-diaminohexane virtually completely abolished the stimulation by the latter hormone. The inhibition of ornithine decarboxylase by the diamines *in vivo* apparently occurred through alterations of the enzyme levels since none of the amines did inhibit ornithine decarboxylase activity *in vitro* under the assay conditions used.
A close correlation existed between progesterone content and ornithine decarboxylase activity in the ovary of prepubertal rats under a variety of experimental conditions used.
The inhibition of ornithine decarboxylase activity by injections of 1,6-diaminohexane was accompanied by a partial prevention of the enhancement in ovarian progesterone accumulation by HCG.

A stimulation of rat ovary by gonadotrophic hormones, endogenous or exogenous, resulted in a marked enhancement in the biosynthesis of polyamines (putrescine and spermidine), as reflected by dramatically elevated activities of L-ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase (EC 4.1.1.50) (1-6). The timing of the stimulation of ovarian ornithine decarboxylase in the rat immediately after the surge of luteinizing hormone (LH), *i.e.* just a few hours prior to ovulation, has given rise to justified suggestions of the possible involvement of putrescine in the ovulatory process itself (1,3). It appears that the activity of ornithine decarboxylase in rat ovary is mainly, if not necessarily exclusively, regulated by LH since the administration of various steroid hormones or follicle stimulating hormone (FSH) has been reported to be without any effect on ovarian ornithine decarboxylase (1). Moreover, the activity of ornithine decarboxylase as well as putrescine content remained at low levels in rat ovary during the first half of pregnancy but were abruptly elevated when placenta was formed (5,7). The latter finding likewise supports the assumption that progesterone that is actively synthesized by the ovary during the early pregnancy do not activate ovarian putrescine synthesis. The close

Copyright © 1977 by Academic Press, Inc.
All rights of reproduction in any form reserved.

ISSN 0006-291X

association of the stimulation of ornithine decarboxylase with the ovulatory process (1,6) and the fluctuation of the enzyme activity in the ovary during pregnancy suggest that an enhanced synthesis of putrescine (and spermidine) may play a regulatory role in the cascade of gonadotrophic hormone action on mammalian ovary.

Based on our earlier experience of the repression type inhibition of liver ornithine decarboxylase *in vivo* by a variety of "unphysiological" diamines (8-10), we have extended these studies in order to develop a selective inhibitor of ovarian ornithine decarboxylase. Unlike regenerating rat liver (8), where 1,3-diaminopropane serves as an excellent inhibitor of ornithine decarboxylase (8), it appears that diamines of longer carbon chains are more potent inhibitors of putrescine synthesis in rat ovary.

MATERIALS AND METHODS

Immature female rats (weighing 40-60 g) of either Wistar or Sprague-Dawley strains were used in all experiments.

The animals were killed by decapitation, the ovaries removed, freed from any extraneous tissue and homogenized with 25 volumes of Tris-HCl buffer (pH 7.1) containing 5 mM dithiothreitol and 0.1 mM EDTA. The homogenates were centrifuged for 30 min at 100 000 x g and the resultant supernatant fractions were used for the assay of ornithine decarboxylase activity and for the measurement of progesterone content (see below).

Human chorionic gonadotrophin (HCG, Pregnyl^{R}, Organon) was dissolved in physiological saline and administered intraperitoneally.

DL-[1-^{14}C]ornithine (sp. radioactivity mCi/mmole) was purchased from the Radiochemical Centre (Amersham,Bucks.,England).

1,3-Diaminopropane (Fluka AG,Buchs SG,Switzerland), cadaverine (Calbiochem,San Diego,Calif.,U.S.A.), putrescine (Calbiochem) and 1,6-diaminohexane (BDH Chemicals Ltd.Poole,England) were dissolved in water and neutralized before injections.

Ornithine decarboxylase activity was assayed in the presence of saturating concentration of L-ornithine (2 mM) and 5 mM dithiothreitol as described earlier (2). Ovarian progesterone content was measured using radioimmunoassay with anti-progesterone antibody raised in rabbits (11).

Protein was measured by the method of Lowry *et al.* (12) using bovine serum albumin as the standard.

RESULTS

As shown in Table 1, single injection of HCG resulted in a more than 25-fold stimulation of ovarian ornithine decarboxylase activity at 4 h after the administration of the hormone. Noteworthy is also the fact that the specific activity

Table 1. Effect of different diamines *in vivo* on ovarian ornithine decarboxylase activity of prepubertal rats after treatment with human chorionic gonadotrophin (HCG). The animals received 25 I.U. of HCG (or saline) without or with the diamines (125 μmoles/100 g body wt.) 4 h before death. The amine injection was repeated again 2 h before death. The number of rats in each group is given in parentheses.

Treatment		Ornithine decarboxylase activity (nmoles/mg protein per 30 min ±S.D.)	
Saline	(4)	0.89 ± 0.29***	(4%)
HCG	(4)	23.45 ± 5.24	(100%)
HCG + diaminopropane $[NH_2(CH_2)_3NH_2]$	(4)	9.10 ± 0.79***	(35%)
HCG + putrescine $[NH_2(CH_2)_4NH_2]$	(4)	13.90 ±10.00	(59%)
HCG + cadaverine $[NH_2(CH_2)_5NH_2]$	(3)	6.80 ± 1.40**	(29%)
HCG + diaminohexane $[NH_2(CH_2)_6NH_2]$	(4)	1.20 ± 1.10***	(5%)

The significance of the differences (as compared with the group receiving HCG alone): ** $p < 0.01$, *** $p < 0.001$

of ornithine decarboxylase in stimulated rat ovary represents by far the highest activity of this enzyme ever recorded in rat tissues, being 10 to 20 times higher (per mg protein) than that found in regenerating rat liver, for example (13). Injections of various diamines (125 μmoles/100 g body wt.) given with HCG (4 h before death) and 2 h later (2 h before death) markedly inhibited ovarian ornithine decarboxylase activity stimulated by HCG (Table 1). While 1,3-diaminopropane and putrescine (1,4-diaminobutane) only partially (40-65%) prevented the stimulation of the enzyme activity elicited by HCG, cadaverine (1,5-diaminopentane) and most notably 1,6-diaminohexane virtually completely abolished the enhancement of ornithine decarboxylase activity (Table 1).

Table 2. Effect of different diamines *in vitro* on ovarian ornithine decarboxylase activity stimulated by an adminstration of human chorionic gonadotrophin (HCG). The animals were injected with 25 I.U. of HCG 4 h before death and ornithine decarboxylase activity was assayed using undialyzed cytosol fractions of the ovary as the source of the enzyme.

Additions	Concentration (mM)	Ornithine decarboxylase activity (nmoles/mg protein per 30 min)	
None	-	20.45	(100%)
Diaminopropane	2	20.05	(98%)
Putrescine	2	17.93	(88%)
Cadaverine	2	19.58	(96%)
Diaminohexane	2	19.36	(95%)

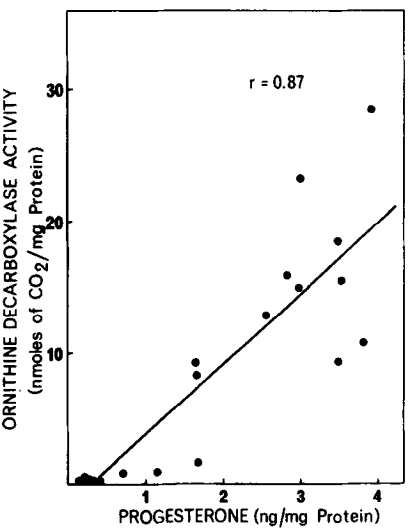

Fig. 1. The relationship between ovarian ornithine decarboxylase activity and cytosolic progesterone concentration in prepubertal rats . The values are obtained from animals without any treatment and from animals receiving HCG without or with 1,6-diaminohexane. Experimental details as in Table 3.

Table 3. Effect of human chorionic gonadotrophin (HCG) without or with 1,6-diaminohexane on ovarian ornithine decarboxylase activity and progesterone concentration of prepubertal rats. The animals received 25 I.U. of HCG (or saline) without or with diaminohexane (125 µmoles/100 g body wt.) 4 h before death. The injection of the amine was repeated again 2 h before death. The number of animals in each group is given in parentheses.

Treatment		Ornithine decarboxylase activity (nmoles/mg protein per 30 min ±S.D.)	Progesterone concentration (ng/mg protein ± S.D.)
Saline	(6)	0.22 ± 0.14***	0.24 ± 0.08***
HCG	(6)	18.60 ± 6.30	3.44 ± 0.39
HCG + diaminohexane	(8)	7.40 ± 5.70**	1.95 ± 0.93**

The significance of the differences (as compared with the group receiving HCG alone): ** $p < 0.01$, *** $p < 0.001$

The varying degree of inhibition exerted by the amines is most likely attributable to differences of their uptake by ovarian tissue.

The fact that the inhibitory effect exerted by the diamines on ovarian ornithine decarboxylase was not due to any direct feedback inhibition of the enzyme activity is demonstrated in Table 2. Equimolar concentration of the diamines, with respect to L-ornithine, did not depress ornithine decarboxylase activity *in vitro* to any appreciable extent (Table 2).

There appeared to exist a remarkably close relationship between ovarian ornithine decarboxylase activity and the progesterone content in the ovarian cytosol fraction, as shown in Fig. 1. The individual values have been obtained from ovaries of immature rats not treated with HCG, of HCG-treated animals and of rats in which the stimulation of ornithine decarboxylase activity was partially or totally inhibited by injections of 1,6-diaminohexane. The correlation of these two variables, ovarian ornithine decarboxylase activity and progesterone concentration, was highly significant ($p < 0.001$) with a regression coefficient as high as 0.87 (Fig. 1).

The intimate association of ornithine decarboxylase activity and progesterone concentration in the ovary of immature rats is also seen in Table 3. As shown,

an injection of HCG resulted in about 85-fold enhancement in ovarian ornithine decarboxylase activity with a concomitant 14-fold increase in cytosolic progesterone concentration. The inhibition (60%) of ornithine decarboxylase activity by 1,6-diaminohexane was accompanied by a significant decline (43%) of ovarian progesterone concentration. It remains to be established whether these two changes are casually related.

DISCUSSION

Large body of evidence indicates that a major way, if not necessarily the sole one, for the control of mammalian polyamine synthesis is the regulation of ornithine decarboxylase, the rate-controlling enzyme of the polyamine pathway, by various amines through a repression type mechanism (14-16). The fact that not only natural polyamines (putrescine, spermidine and spermine) but also some structurally related diamines, which do not normally occur in mammalian tissues (8-10), exert similar inhibitory action on ornithine decarboxylase *in vivo*, has opened new avenues for the search of a specific inhibitor of the synthesis of natural polyamines. The use of exogenous amines for the inhibition of ornithine decarboxylase, however, is rational only if these amines do not take over the physiological functions of the natural polyamines. Although the latter eventuality has to be investigated experimentally in every case, there exists already some evidence in support of the view that the requirement for putrescine and spermidine can not be met by unphysiological amines. The inhibition of putrescine and spermidine accumulation after partial hepatectomy by 1,3-diaminopropane thus resulted in a marked decrease in the synthesis of liver DNA (9). Similarly, the inhibition of ornithine decarboxylase activity by α-methyl ornithine was associated with a marked reduction of DNA synthesis in rat hepatoma cells that could only be reversed by natural polyamines but not by 1,3-diaminopropane or cadaverine (17).

The present experiments likewise suggest that 1,6-diaminohexane might be unable to replace putrescine in rat ovary, assuming that putrescine and/or spermidine are needed for full stimulation of steroidogenesis following treatment with HCG. Although it is highly improbable that polyamines would have any direct influence on the synthesis of ovarian steroids, they conceivably may play a permissive role possibly optimizing the intracellular conditions for the ovulatory process (1). The moderate inhibition of progesterone accumulation might thus be reflection of a general disturbance of ovarian metabolism resulted for the inhibition of putrescine synthesis. In this respect, the system may be resemble

that in organ cultures of mouse mammary gland where the secretion of milk proteins is stimulated by insulin, prolactin and cortisol. The enhancement in milk protein synthesis was preceded by an increase in the concentration of intracellular spermidine, the inhibition of which by methylglyoxal bis (guanyl-hydrazone) resulted in an abolition of the hormone effects. The inhibition by methylglyoxal bis (guanyl hydrazone) was overcome by spermidine, which in fact, appeared to fulfill the requirements for cortisol but not for insulin or prolactin (18). More data are obviously needed to elucidate the role and importance of the enhanced preovulatory synthesis of polyamines for the metabolism and functions of mammalian ovary. However, the repression of ornithine decarboxylase by suitable amines may offer some new approaches and tools for a selective prevention of the synthesis and accumulation of the natural polyamines during hormone action.

ACKNOWLEDGEMENTS

The help of Dr. Pekka Lähteenmäki, Mrs. Marjatta Tevilin and Mrs. Tarja Ahonen in progesterone assays and Miss Merja Kärkkäinen in enzyme assays is gratefully acknowledged. This investigation was supported by a grant from the Ford Foundation. Sujit K. Guha is recipient of research fellowship from the Sigrid Jusélius Foundation (Helsinki, Finland).

REFERENCES

1. Kobayashi, Y., Kupelian, J. and Maudsley, D. V. (1971) Science 172, 379-380.
2. Jänne, J. and Williams-Ashman, H. G. (1971) J. Biol. Chem. 246, 1725-1732.
3. Williams-Ashman, H. G., Jänne, J., Coppoc, G. L., Geroch, M. E. and Schenone, A. (1972) Adv. Enz. Regul. 10, 225-245.
4. Kaye, A. M., Icekson, I., Lamprecht, S. A., Gruss, R. and Tsafriri, A. (1973) Biochemistry 12, 3072-3076.
5. Guha, S. K. and Jänne, J. (1976) Acta Endocrinol. 81, 793-800.
6. Maudsley, D. V. and Kobayashi, Y. (1974) Biochem. Pharmacol. 23, 2697-2703.
7. Guha, S. K. and Jänne, J. (1976) Biochim. Biophys. Acta 437, 244-252.
8. Pösö, H. and Jänne, J. (1976a) Biochem. Biophys. Res. Commun. 69, 885-892.
9. Pösö, H. and Jänne, J. (1976b) Biochem. J. 158, 485-488.
10. Pösö, H. (1977) Acta Chem. Scand., in the press.
11. Thorneycroft, I. H. and Stone, S. C. (1972) Contraception 5, 129-146
12. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275.
13. Raina, A., Jänne, J., Hannonen, P. and Hölttä, E. (1970) Ann. N. Y. Acad. Sci. 171, 697-708.
14. Kay, J. E. and Lindsay, V. J. (1973) Biochem. J. 132, 791-796.
15. Clark, J. L. (1974) Biochemistry 13, 4668-4674.
16. Jänne, J. and Hölttä, E. (1974) Biochem. Biophys. Res. Commun. 61, 449-456.
17. Mamont, P. S., Böhlem, P., McCann, P. P., Bey, P., Schuber, F. and Tardif, C. (1976) Proc. Natl. Acad. Sci. USA 73, 1626-1630.
18. Oka, T. and Perry, J. W. (1974) J. Biol. Chem. 249, 7647-7652.