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Ornithine Decarboxylase Activity in Porcine Reproductive Tissues

Pak, S.C. and J.R. Diehl

Department of Obstetrics and Gynecology,
University of Illinois at Chicago, Chicago, IL 60612
Animal, Dairy and Veterinary Sciences Department,
Clemson University, Clemson, SC 29634*

Gilt에 있어서 Tissue내에 함유되어 있는 Ornithine Decarboxylase의 활성

박 석 천 · J.R. Diehl

Department of Obstetrics and Gynecology,
University of Illinois at Chicago, Chicago, IL 60612
Animal, Dairy and Veterinary Sciences Department,
Clemson University, Clemson, SC 29634*

적 요

성숙한 gilt를 이용하여 estrous cycle과 pregnancy기간 동안 ODC효소의 tissue내 함량을 측정하는 것이 본 연구의 주 목적이었다. 하루 한 마리의 gilt를 estrous cycle day 3, 10, 17, 18, 19, 20 그리고 pregnancy day 11, 12, 13, 14, 18, 19, 20, 48, 50, 52에 도살하였다. 시상하부, 뇌하수체, 자궁, 난소 및 골격근에서 조직을 제거하였으며, 이들 조직들을 완충액에 넣어 균질화 시켰으며 그 상청액은 단백질량과 ODC의 활력도를 측정하였는데 사용되었다. Radiolabled ornithine에서 발생하는 $^{14}CO_2$양은 0.124~4 mg범위내에서 단백질첨가량과 배양시간에 비례하였다. OCD는 특히 estrous cycle day 19일에 뇌하수체, 난소 그리고 자궁에서 활발한 활동을 보여주었다. 한편, 시상하부와 골격근에서 ODC는 그 어떤 활동도 보이지 않았다. 자궁조직은 다른 조직들에 비해 보다 활발한 ODC활동을 보여 주었다 ($P < 0.05$).

I. INTRODUCTION

As well as being the first, it is well established that ornithine decarboxylase(ODC) is the rate-limiting enzyme in polyamine biosynthesis. The specific function of the polyamines is still obscure, however, their biosynthesis is shown to be essential for normal cellular growth and differentiation(Tabor & Tabor, 1984).

Relating the activity of ODC of various tissues to different stages of mammalian reproduction will increase our understanding of endocrinology and fetal and maternal interactions associated with pregnancy.

Involvement of ODC in some aspects of mammalian reproduction has been reproted. In the pituitary gland of the rat, ODC activity peaks on afternoon of proestrus(Persson, et al., 1985). Furter, both the role of ODC in expression of the LH surge and the involvement of an ODC inhibitor, α-difluoromethylornithine(DFMO), in reporductive hormonal regulation have been reproted(Aslam, et al., 1987 ; Nicholson, et al., 1988 ; Nicholson & Wynne-Jones, 1989).

Our preliminary data showed a suppressive action of DFMO on LH secretion during the follicular phase. Therfore, the question of activity of ODC in selected tissues during various reproductive stages was of considerable interest. This study was desinged to investigate the tissue levels of ODC activity during the estrous cycle and pregnancy in the pig.

II. MATERIALS AND METHODS

1. Animals and preparation of tissue extracts

Cycling female crossbred pigs weighing approximately 110 kg were used. One animal was sacrificed at the university abattoir on estrous cycle days 3, 10, 17, 18, 19, 20 and during pregnancy on day 11, 12, 13, 14, 18, 19, 20, 48, 50 and 52. Tissues from the hypothalamus, pituitary, uterus, ovary and skeletal muscle were removed within 30 min of exsanguination, frozen in liquid nitrogen and kept at −80℃ until analyzed for ODC.

2. Measurement of ODC activity

For the measurement of ODC activity, frozen tissues were thawed, cut into small pieces, weighed and homogenized at 0~4℃ in a buffer (3 ml/g tissue) containing 0.02 M of Na_2HPO_4, 1.25 mM of DTT and 10 mM EDTA. The homogenates were centrifuged for 30 min at 20,000 × g at 4℃ and supernatants were analyzed for protein concentration using the method of Bradford(1976). Enzyme activity was determined by the relese of $^{14}CO_2$ from DL-[1-^{14}C] ornithine monochloride(American Radiolabeled Chemicals, Inc., St. Louis, MO; 55 mCi/mM) according to the method of Pegg, Lockwood and Williams-Ashman(1970) with modifications. The standard reaction mixture contained 50 μmol tris-HCl buffer, pH 7.2, 0.05 μmol pyridoxal-5'-phosphate(cofactor, freshly prepared), 2.5 μmol 2-mercaptoethanol, 5 μmol DTT, 0.2 μCi DL-[1-^{14}C] ornithine monochloride and sample extract in a parallel increase in the amount of $^{14}CO_2$ recovered with an increase in total volume of 1.0 ml. The use of DTT in the reaction mixture allowed the detection of lower levels of ODC. Since low(0.2 μCi) and high(0.5 μCi) amounts of ^{14}C-ornithine resulted in a parallel increase in the amount of $^{14}CO_2$ recovered with an increase in a protein concentration and incubation time(Fig. 1 and 2), it was decided to use 0.2 μCi of ^{14}C-ornithine in this study. A stoppered vial was used as a reaction vessel to trap the evolved CO_2. The well was filled with 3 × 2 cm pieces of Whatman 3 MM chromatography paper soaked with 0.1 M hyamine hydroxide in methanol. Based on results presented in Fig. 1, the enzyme reaction was started by adding 2 mg protein. Both ovarian and muscle samples showed a linear increase in $^{14}CO_2$ produced with increased sample protein up to at least 4 mg. Each sample was assayed in duplicate along with blank and total count tubes. Enzyme activity of one tissue type was determined in a single assay. The intraassay coefficients of variation of hypothalamus, pituitary, uterus, ovary and skeletal muscle were 9.8%, 9.5%, 7.5%, 4.5% and 9.6%, respectively. After 1 hr of incubation at 37℃ in a water bath, 1 ml of 10% TCA was injected through the rubber stopper to stop the reaction. The incubation time of 1 hr was derived from the conclusion that $^{14}CO_2$ produced was independent of incubation time(Fig.2). The incubation was continued for an additional 30 min at 37℃ to capture all remaining CO_2 released. At the end of incubation, the filters were removed from the reaction vessel and

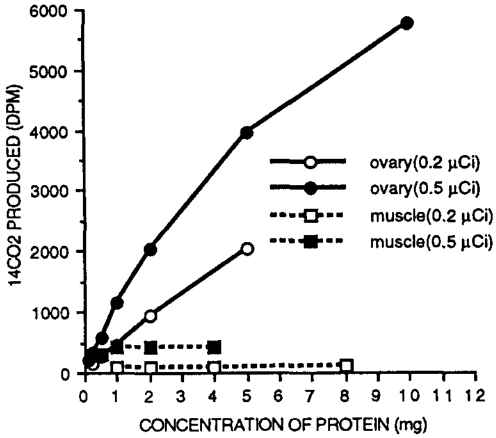

Fig. 1. Effect of amount of radiolabeled ornithine on $^{14}CO_2$ production. Both ovarian and muscle homogenates from an estrous day 3 gilt showed that production of $^{14}CO_2$ was independent of the ^{14}C-ornithine concentration and protein concentration.

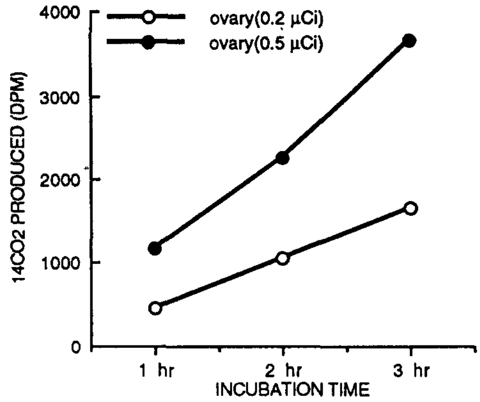

Fig. 2. Effect of incubation time of reaction mixture on $^{14}CO_2$ production expressed as dpm. An ovarian sample from an estrous day 3 gilt showed that production of $^{14}CO_2$ was independent of incubation time.

placed in scintillation vials containing 4 ml of Liquid Scintillation Cocktail and counted. The enzymatic activity was expressed as pmol $^{14}CO_2$ released / mg protein / h. The minimum detectable ODC activity was 1.1 pmol / mg protein / h.

3. Statistical analysis

All results are presented as means ± SD. Differences between tissues and status of animal(pregnancy or not) were analyzed by a split-plot analysis of variance(Steel & Torrie, 1980). Differences were considered significant if Pvalues from the test were less than 5%.

Ⅲ. RESULTS

From a statistical standpoint, the only comparison possible was among tissues since only one animal was sampled each day. As a result of this analysis, only the uterus possessed a significant amount of enzyme activity.

Hypothalamic tissue(Fig. 3) had no detectable ODC activity throuhgout the study. Animals in the estrous cycle had 0.73±0.16 units / mg protein of ODC activity while animals during pregnancy had 0.82±0.19 units / mg protein when averaged across days. The hypothalamus had the lowest rate of enzyme activity of any tissue tested($p > 0.05$).

The pituitary(Fig. 4) had more ODC activity on day 19 than any other days of the estrous cycle. Tissue collected during pregnancy had more enzyme activity than during the estrous cycle and was highest on day 14. The average enzyme activity across days of estrus and pregnancy was 1.9±0.46 and 2.83±1.01 units / mg protein, respectively.

Uterine ODC activity was high on days 10 and 19 of the estrous cycle(Fig. 5). In comparison to the other tissues, the uterus had the highest ODC activity regardless of animal status($p < 0.05$). Pregnancy days 11, 12 and 50 also showed vigorous activity. The average enzyme activity

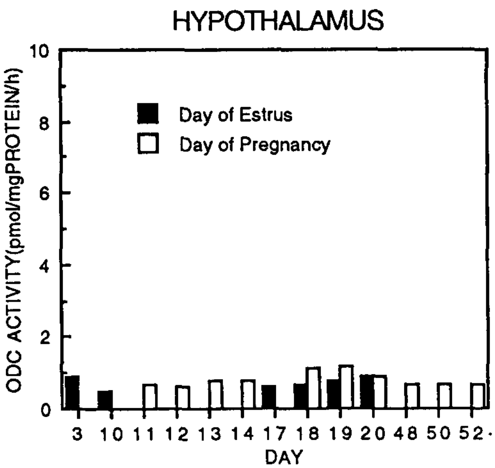

Fig. 3. Ornithine decarboxylase activity in hypothalamnus during the pig estrous cycle and pregnancy.

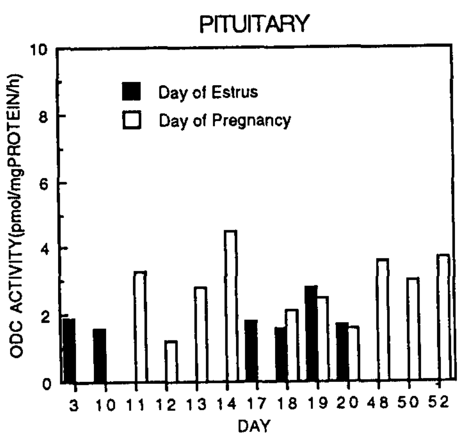

Fig. 4. Ornithine decarboxylase activity in pituitary during the pig estrous cycle and pregnancy.

across days of estrus and pregnancy was 55.2±117.6 and 8.1±14.8 units/mg protein, respectively.

Although it had lower peaks of enzyme activity than uterus, the day 10 and 19 ovary(Fig. 6) possessed higher ODC activity than other days during the estrous cycle while more activity was evident during pregnancy on days 18, 20 and 48. The average enzyme activity across days of estrus and pregnancy was 3.8±1.8 and 5.6±2.2 units/mg protein, respectively.

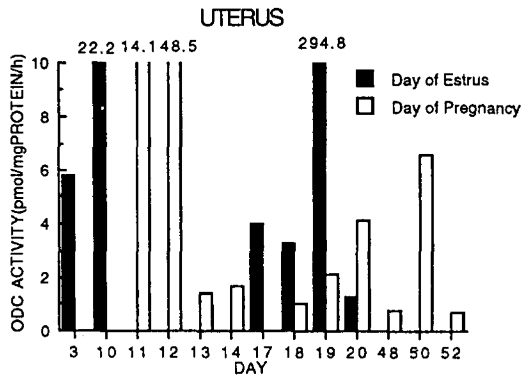

Fig. 5. Ornithine decarboxylase activity in uterus during the pig estrous cycle and pregnancy.

Skeletal muscle ODC activity was low among all days and reproductive states(Fig. 7). The activity was barely above the minimum detectable amount. The average enzyme activity across days of estrus and pregnancy was 1.4±0.5 and 1.4±0.6 units/mg protein.

Ⅳ. DISCUSSION

Studies of ODC activity in specific tissues during a given interval of time are rare; therefore, the enzyme activity was measured in different tissues of pigs at different stages of the estrous cycle and pregnancy in this study.

While not possible to assess statistically due to the number of animals sampled(1/day), it appears that ODC has biologically important activites in pituitary, ovary and uterus. The highest ODC activity appeared in the pituitary during proestrus of the gilt which suggests its

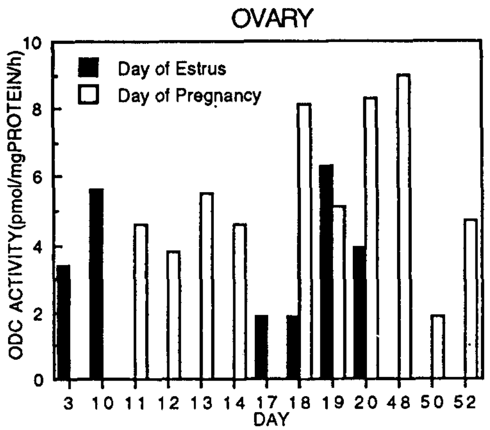

Fig. 6. Ornithine decarboxylase activity in ovary during the pig estrous cycle and pregnancy.

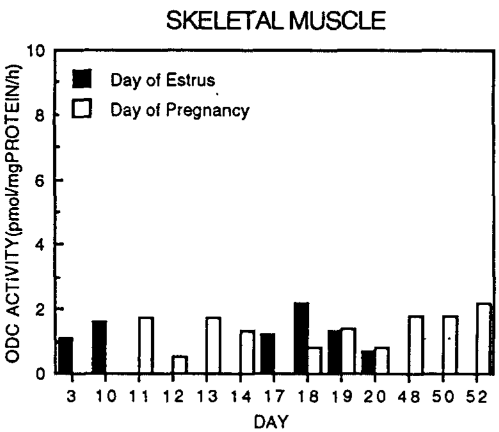

Fig. 7. Ornithine decarboxylase activity in skeletal muscle during the pig estrous cycle and pregnancy.

involvement in LH secretion. Further, involvement of ODC in the LH surge(Aslam et al., 1987; Nicholson et al., 1988) seems likely because it showed higher activity on day 19 than days 17 and 18 of the estrous cycle. This suggests that estrogen may have an enhancing effect on ODC.

During the estrous cycle, uterine tissue showed its greatest ODC activity on days 10 and 19. The day 19 activity level of 294.8 units /mg protein was a dramatic increase. Pregnancy day 11 values of 14.1 units /mg protein and day 12 values of 48.5 units /mg protein suggest a relationship between ODC activity and the dramatic morphological changes of porcine embryo that also occur during this period of time. This interval also corresponds with the times that significant quantities of estrogen secretion from conceptus occurs(Perry et al., 1973; Geisert et al., 1982). The results reported herein strongly imply that estrogens can induce an increase in ODC activity in selected tissues. Further support for this hypothesis is found in the data for ODC activity in the ovary and pituitary. In both tissues, the high enzyme activity was found at a time when estrogen levels are high.

The fact that skeletal muscle had little ODC activity was expected since there is little proliferative activity on the tissue level when these samples were taken. It was chosen specifically for that reason. Since ODC activity has been shown to be an indicator of proliferation, it was not surprising that this tissue had low enzyme activity. Alternatively, ODC was high in uterus during days 11, 12, 20 and 50 of pregnancy, a series of days when uterine proliferation might be expected.

All together, we found that ODC had some relationship with known biological functions of pituitary, ovary and uterus while it showed no such activities in hypothalamus and skeletal muscle of sexually matured pigs.

V. SUMMARY

The tissue levels of ornithine decarboxylase (ODC) during the estrous cycle and pregnancy were investigated in the pig. Sexually mature female cycling pigs were used. One animal was

sacrificed on estrous cycle days 3, 10, 17, 18, 19, 20 and during pregnancy on day 11, 12, 13, 14, 18, 19, 20, 48, 50 and 52. Tissues from the hypothalamus, pituitary, uterus, ovary and skeletal muscle were removed. They were homogenized in buffer, and supernatants were used for measurement of protein concentration and ODC activity. The release of $^{14}CO_2$ from radiolabeled ornithine was proportional to the amout of protein added over the range of 0.125~4 mg and to the incubation time. ODC appered to have some relationship with the biological functions of the pituitary, ovary and uterus during the reproductive period, especially on day 19 of the estrous cycle, while it showed no such activities in hypothalamus and skeletal muscle of mature pigs. Uterine tissues had significantly more ODC activity than other tissues tested($p < 0.05$).

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