Vol. 144, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 14, 1987 Pages 166-171

THE IHIBITION OF 3βHSD ACTIVITY IN PORCINE GRANULOSA CELLS BY 4-MA, A POTENT 5α-REDUCTASE INHIBITOR

W.K. Chan, C.Y. Fong, H.H. Tiong, and C.H. Tan

Department of Zoology, National University of Singapore, Singapore

Received February 27, 1987

Addition of 17β-N,N,-diethylcarbamoyl-4-methyl-4-aza-5α-androstan-3-one, a potent 5α-reductase inhibitor, to granulosa cell cultures inhibited the FSH-stimulated progesterone synthesis during both the initial 48h induction period and the subsequent 6h test period in a dose-dependent fashion. Besides being a more potent inhibitor of FSH-stimulated progesterone synthesis than testosterone, 4-MA also synergised with the androgen to inhibit progesterone synthesis. These results indicate that 4-MA has a direct inhibitory action on 3β-HSD.

4-MA is a member of a series of 4-aza-3-oxosteroids that were found to be highly effective reversible inhibitors of 5α-reductase (EC 1.3.1.22) (1,2). Since 5αDHT is implicated in the pathogensis of androgen-dependent diseases like acne vulgaris, hirsutism and benign prostatic hypertrophy (3-5), 4-MA might be useful in the treatment of these conditions. However, little is known about the action of 4-MA on steroid biosynthesis in steroidogenic tissues, although it has no steroidal effects in the rat (6). But, being a 5α-reduced steroid (1), it might inhibit 3β-HSD activites since 5α-reduced androgens are known to inhibit the enzyme activities in porcine granulosa cells (7).

Although 4-MA has proved useful in our studies on the modulation of FSH-stimulated aromatase induction by 5-reduced

Abbreviations used: FSH, follicle-stimulating hormone; 4-MA, 17β-N,N-diethylcarbamoyl-4-methyl-4-aza-5α-androstan-3-one; 3β HSD, 3β-hydroxysteroid dehydrogenase; T, testosterone; Eagle's MEM, Eagle's Minimum Essential Medium.

0006-291X/87 $1.50

androgens, the following experiments were designed to ascertain if FSH-stimulated progesterone synthesis in porcine granulosa cells is also inhibited by 4-MA. Our results demonstrate that 4-MA is indeed a potent inhibitor of 3βHSD.

MATERIALS AND METHODS

All incubations were carried out in Eagle's MEM, modified as previously described (8). Steroids were obtained from Sigma Chemical CO., St. Louis, MO, while 4-MA was a generous gift from Dr. G.H. Rasmusson (Merck, Sharp and Dohme Research Laboratories, Rahway, N.J.). Ovine follicle-stimulating hormone (NIADDK-oFSH-S13) was used in all incubations at the final concentration of 1 µg/ml.

Ovaries were collected from immature prepubertal gilts (4-6 months old) at a local abattoir. Granulosa cells were isolated from non-atretic medium sized follicles (4-6mm) as previously described (9,10) and equally distributed into NUNC plastic multiwell plates in such a way that each well contained one follicle-equivalent of granulosa cells in 1 ml of MEM (approximately 80 µg cell protein/ well were used to establish the cultures).

The cells were incubated at 37o C in a humidified atmosphere of 5% CO2/ 95% air for an initial induction period of 48h. During this induction period, granulosa cells were cultured with or FSH in the presence or absence of increasing doses of 4-MA (0.001 - 10 µM). In other experiments T (1 µM) together with FSH and increasing doses of 4-MA were added during the induction period. After the induction period, the medium was removed and the cell monolayers were washed before been cultured for a further 6 h test period in the presence of fresh medium containing only pregnenolone (5 µM) as substrate. The production of progesterone during the test period is taken as an index of 3β-HSD activity. At the end of the induction and test periods, the medium was removed and stored at -20oC until assayed.

The progesterone content of the unextracted culture medium was measured by a specific radioimmunoassay validated previously (11). The total cellular protein content of each well was determined by the method of Lowry et. al. (12), using bovine serum albumin as standard. The viability of the cells was checked using the trypan blue exclusion test (13). Before culture, the viability of the cells was 95% and was slightly reduced (85%) after culture.

All experimental data are presented as the mean + SEM of quadruplicate cultures. The experimental data were evaluated statistically by analysis of variance followed by Duncan's New Multiple Range Test (14).

RESULTS

Figure 1 shows that the presence of 4-MA (0.001 - 10 µM) during the induction period significantly ($P>0.05$) inhibited the FSH-stimulated progesterone synthesis during both the induction

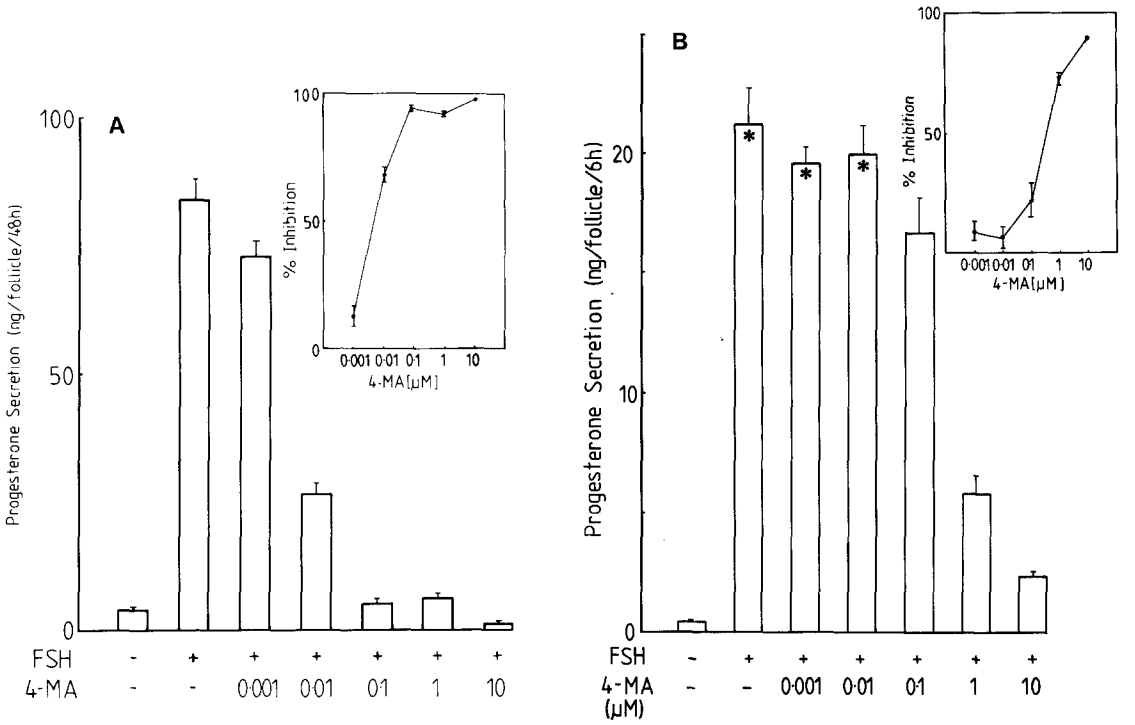

Fig. 1. Effect of 4-MA on FSH-stimulated progesterone synthesis. Granulosa cells were cultured with or without FSH, in the presence or absence of 4-MA (0.001 - 10 μM) during the initial 48 h induction period (A), followed by a 6 h test period (B) when only pregnenolone was added. Each point represents the mean ± SEM of four incubations. The inserts show the percentage inhibition (relative to FSH-stimulated culture) of progesterone synthesis due to the presence of 4-MA.

and test periods in a dose-dependent manner. At a concentration of 0.1 μM, 4-MA inhibited the FSH-stimulated progesterone synthesis by 94% during the induction, although the inhibition was less pronounced during the test period (22%).

The addition of T (1μM) during the induction period also significantly ($P>0.05$) inhibited the progesterone synthesis during both the induction and test periods by 59% and 36% respectively (Fig. 2). However, T was 5 times less potent than 4-MA in inhibiting progesterone synthesis (Fig. 2). More importantly, 4-MA appears to act synergistically with T to further decrease progesterone synthesis during both the induction and test periods (Fig. 3).

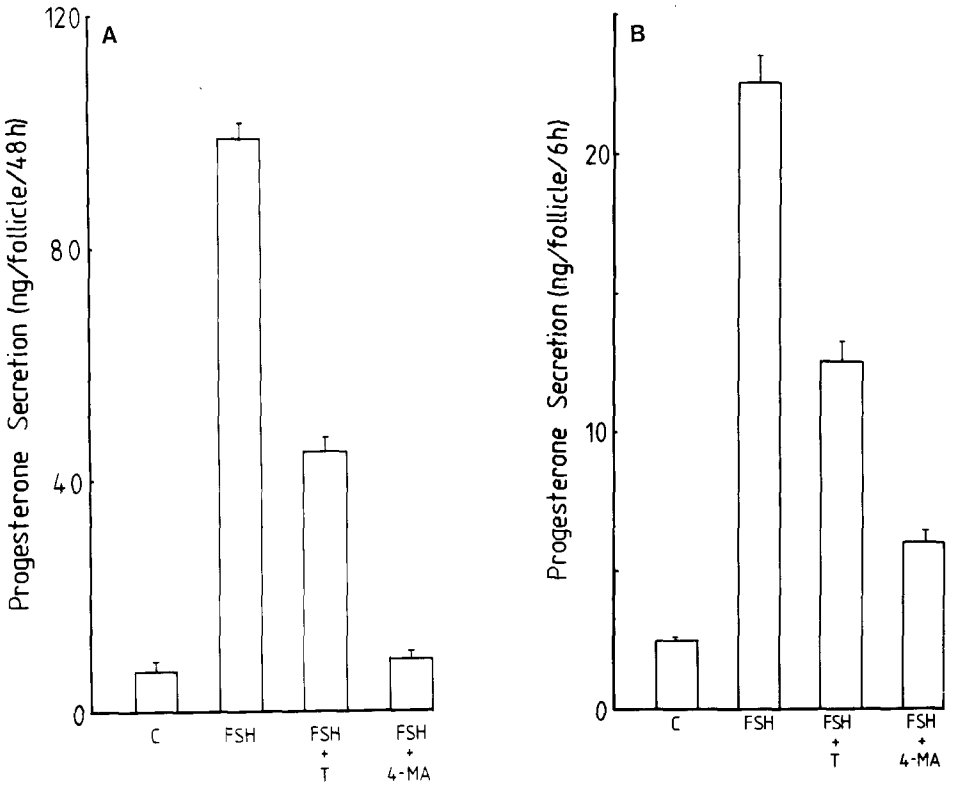

Fig. 2. Effect of 4-MA and T on FSH-stimulated progesterone synthesis. Granulosa cells were cultured with no hormones (C), FSH, FSH + T (1 μM) or FSH + 4-MA (1 μM) during the 48 h induction period (A), followed by a 6 h test period (B). Each point represents the mean ± SEM of four incubations.

DISCUSSION

The present results indicate for the first time that the addition of 4-MA to porcine granulosa cells stimulated with FSH inhibited the progesterone synthesis in a dose-related fashion. This decrease might be attributable mainly to a direct inhibitory action of 4-MA on the induction of 3 HSD activity, since the inhibitory action persisted during the test period when 4-MA was removed and fresh medium containing pregnenolone as substrate was added. The present experiments also indicate that 4-MA is capable of synergizing with T to inhibit FSH-stimulated progesterone synthesis. Since 4-MA has been shown to have a moderate affinity for the androgen receptors (1, 15), it is

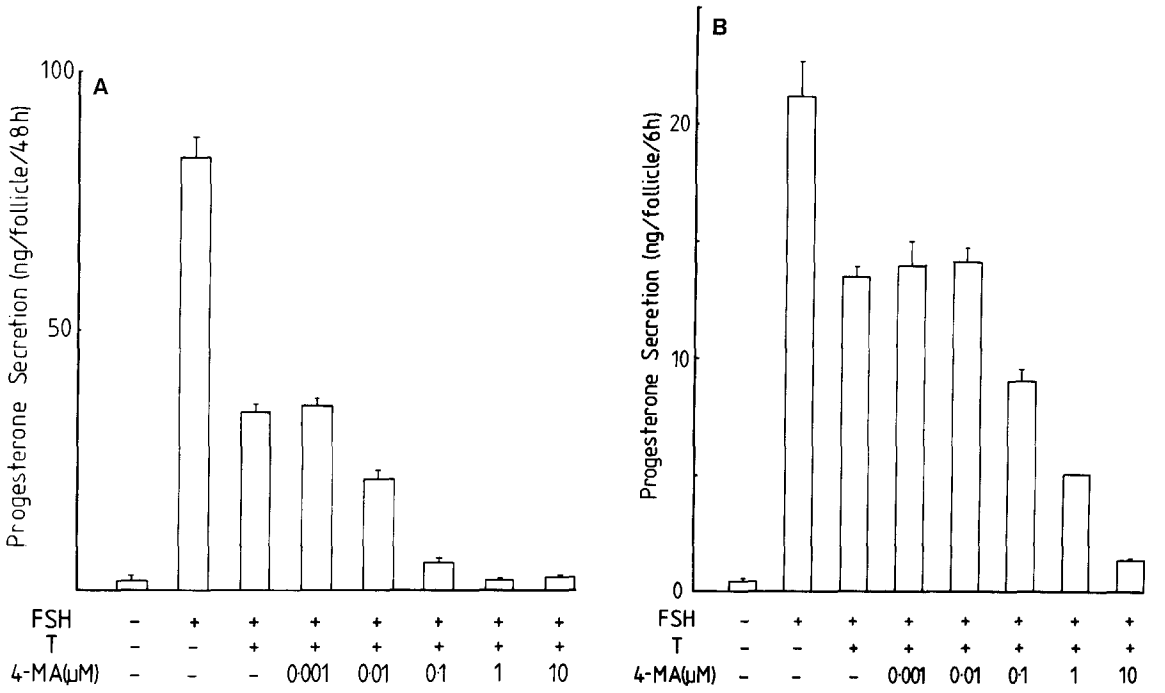

Fig. 3. Effect of 4-MA on FSH+T-stimulated progesterone synthesis. Granulosa cells were cultured with no hormones, with FSH or with FSH + T (1 μM) in the absence or presence of 4-MA (0.001 - 10 μM) during the initial 48 h induction period (A), followed by a 6 h test period (B) when only pregnenolone (5 μM) was added. Each point represents the mean ± SEM of four incubations.

likely that the inhibitory action of 4-MA on the induction of progesterone synthesis is mediated by androgen receptors as in the case of androgenic modulation of aromatase activity (16, 17).

In an earlier study, 4-MA was shown to be devoid of estrogenic, progestational or anti-progestational activity, and also has no significant gonadotropin inhibiting potency (6). Therefore, it has been suggested that 4-MA might be useful as a theraupetic agent in the treatment of androgen-dependent diseases (2) since it was thought to inhibit 5α-reductase only (1). Although the inhibitory action of 4-MA on FSH-stimulated progesterone synthesis will probably not affect its theraupetic use in males for treating androgen-dependent diseases, it might be detrimental to females as possible side-effects could occur

since progesterone-dependent events would be affected by the inhibition of 3βHSD. Thus, any potential therapy involving the use of 5α-reduced androgens like 4-MA to treat androgen-dependent diseases in females must take these findings into consideration.

ACKNOWLEDGEMENT. We are gratefully to the National Pituitary Agency NIAMDD, for the ovine FSH; Dr. P.R. Edwards (Middlesex Hospital Medical School, England) for providing the RIA curve-fitting program; G. Barbe and Professor D.T. Armstrong (MRC Group in Reproductive Biology, London, Ontario) for the specific progesterone antiserum; Dr. G.H. Rasmusson (Merck, Sharp and Dohme Research Laboratories, Rahway, N.J.) for 4-MA.

REFERENCES

1. Liang, T.M., Rasmusson, G.H., and Brooks, J.R. (1983) J. Steroid Biochem. 19, 385-390.
2. Rasmusson, G.H., Liang, T.M., and Brooks, J.R. (1983) in A.K. Roy and J.H. Clark (editors), Gene Regulation by Steroid Hormones II. p 311-334, Springer-Verlag, N.Y.
3. Zoppi, S., Cocconi, M., Natali, A. Costantini, A., Serio, M., Martini, L., and Motta, M. (1986) J. Clin. Endrocinol. Metab. 63, 269-271.
4. Sansone, G., and Reisner, R.M. (1971) J. Invest. Dermatol. 56, 366-371.
5. Kutten, F., Mowszowicz, I., Schaison, G., and Mauvais-Jarvis, P. (1977) J. Endocrinol. 75, 83-91.
6. Brooks, J.R. Berman, C., Hichens, M., Primka, R.L., Reynolds, G.F. and Rasmusson, G.H. (1982) Proc. Soc. exp. Biol. Med. 169, 67-73.
7. Tan, C.H., and Armstrong, D.T. (1984) Proc. 3rd Joint Mtg of British Endocrine Soc., Edinburgh, Mar. 1984, p 105. Abstract No. 129.
8. Dorrington, J.H., and Armstrong, D.T. (1975) Proc. Natl. Acad. Sci. USA 72, 2677-2681.
9. Chan, W.K., and Tan, C.H. (1986) J. Endocrinol. 108, 335-341.
10. Chan, W.K., and Tan, C.H. (1986) Endocrinology 119, 2353-2359.
11. Leung, P.C.K. and Armstrong, D.T. (1979) Endocrinology 104, 1411-1417.
12. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
13. Tennant, J.R. (1964) Transplantation 2, 685-694.
14. Steel, R.G.D., and Torrie, J.H. (1960) Principles and Procedures of Statistics, McGraw-Hill, New York.
15. Liang, T., and Heiss, C.E. (1981) J. Biol. Chem. 256, 7998-8005.
16. Hillier, S.G., and De Zwart, F.A. (1981) Endocrinology 109, 1303-1305.
17. Daniel, S.A.J., and Armstrong, D.T. (1984) Endocrinology 114, 1975-1982.