A quantitative relationship between conceptus number and ovarian steroid dehydrogenase activity in pregnant rats

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RECEIVED 23 September 1983

ABSTRACT

The effects of a conceptus-derived substance on the activity of 3β-hydroxysteroid dehydrogenase (3β-HSD) and 20α-HSD in the ovary were studied in the rat. On day 7 of pregnancy (day 1 = insemination), rats were laparotomized and the desired number of conceptuses was aspirated from the uterus; thus, rats carrying one, two, three, four, five to seven or eight to ten conceptuses were prepared. They were autopsied on day 15 and 3β-HSD and 20α-HSD activity in the corpus luteum (CL) or non-luteal ovarian tissue (NLO) was determined. Conceptus number was directly related to 3β-HSD and inversely related to 20α-HSD activity in the CL. The serum progesterone level and CL weight were also directly related to conceptus number. Neither 3β-HSD nor 20α-HSD activity in the NLO was affected by conceptus number. These results indicated that 3β-HSD and 20α-HSD in the CL are probably regulated by placental hormone secreted in proportion to the number of conceptuses; in the NLO these enzymes may be controlled by a different mechanism.


INTRODUCTION

It has been demonstrated that luteal function in the pregnant rat is mainly regulated by placental luteotrophins during the second half of pregnancy (Greenwald & Rothchild, 1968). A quantitative relationship between the number of conceptuses (i.e. the amount of placental hormones) and the production of progesterone or the weight of the corpora lutea (CL) has been demonstrated in pregnant rats (Kato, Morishige & Rothchild, 1979; Ochiai & Rothchild, 1981). However, little is known at present about how the placental hormones influence luteal function. It is well known that at least two enzymes, 3β-hydroxysteroid dehydrogenase (3β-HSD) and 20α-HSD, play essential roles in the regulation of progesterone secretion. Rodway & Kuhn (1975) showed that ovarian 20α-HSD activity was higher in rats bearing one or two conceptus(es) than in those bearing a full complement of conceptuses. Ovarian 3β-HSD activity is also increased during the second half of pregnancy (Marcal, Chew, Salomoń & Sherman, 1975), when the placenta secretes large amounts of rat placental lactogen (rPL) (Shiu, Kelly & Friesen, 1973), and probably rat chorioic gonadotrophin (Haour, Tell & Sanchez, 1976; Wide & Hobson, 1978; Blank, Dufau & Friesen, 1979; Robertson & Friesen, 1981). It is therefore likely that the luteotrophic effects of the placenta may be mediated through these ovarian enzymes. The present study was undertaken to determine the quantitative relationship between conceptus number and the activity of 3β-HSD or 20α-HSD in the CL of the pregnant rat.

MATERIALS AND METHODS

Sprague–Dawley rats, 220–280 g, were housed with free access to food and water. Day 1 of pregnancy was the day on which sperm were found in the vaginal smear. Pregnancy was confirmed at laparotomy on day 7 by the presence of embryonic swellings in the uterus, and those rats bearing at least eight conceptuses were used in this experiment.

The number of conceptuses was altered on day 7 of pregnancy according to the method of Kato et al. (1979). In brief, a small incision was made on the antimesometrial surface of an embryonic swelling and its contents were aspirated with a glass pipette attached


Printed in Great Britain
to a suction line. In this way, rats carrying one, two, three, four, five to seven or eight to ten conceptuses were prepared. In order to avoid the non-specific effect of uterine surgery, those rats bearing large numbers of conceptuses received several incisions between the embryonic swellings, but without disturbing the conceptuses.

The rats were autopsied on day 15 of pregnancy. The CL and the non-luteal ovarian tissue (NLO) were dissected from each pair of ovaries, weighed quickly, and then suspended in ice-cold phosphate-buffered saline (PBS; 0·05 M-sodium phosphate buffer, pH 7-4, containing 0·14 M-NaCl) at a concentration of 10 mg wet weight of tissue/ml PBS. All further preparatory steps were carried out at 4°C. The tissues were homogenized in a glass homogenizer and centrifuged at 800 g for 20 min. The supernatant fraction was collected and glycerol was added as a cryoprotective agent at a final concentration of 20%. The supernatant fractions were snap-frozen in a dry ice–methanol bath and stored at -70°C.

Blood samples were collected at the time of autopsy and allowed to clot at 4°C; the serum samples were stored at -70°C until their progesterone levels were determined.

The activity of 3β-HSD was determined according to the method of Marcal et al. (1975). The supernatant fraction (10 or 20 μl) was added to 0·02 M-Tris buffer, pH 7·5, containing NAD (2·5 mmol/l), bovine serum albumin (200 μg) and 5 μg pregnenolone (Sigma Chemical Co., St Louis, Missouri, U.S.A.) (in 10 μl N,N'-dimethylformamide) in a total volume of 0·52 ml. The tubes for the zero-time control were immediately placed in boiling water for 10 min before incubation. Other tubes were incubated at 37°C for 10 min. The reaction was terminated by placing the tubes in boiling water for 10 min. All tubes were centrifuged at 1800 g for 10 min at 4°C, and the supernatant fractions were stored at -70°C until assayed for progesterone. The zero-time control values were subtracted from the experimental values to correct for the endogenous progesterone and for cross-reaction of pregnenolone with anti-progesterone antibodies in the progesterone radioimmunoassay. The activity of 3β-HSD was expressed as nmol progesterone/min per mg protein.

The activity of 20α-HSD was determined by measuring the conversion rate of 20α-hydroxyprogren-4-en-3-one to progesterone. The assay was a modification of the method described by Eckstein & Nimrod (1979). However, since the progesterone concentration was determined by a radioimmunoassay in our study, the optimum conditions of each parameter (e.g. pH, incubation time, concentrations of substrate, tissue homogenate or NADPH) were retested by varying one parameter at a time. Aliquots (50–100 μl) of the supernatant fraction were added to 0·1 M-Tris buffer, pH 8·0, containing 1·12 mM-EDTA, 1·13 mM-cysteine, 11·3 mM-nicotinamide, 0·547 mM-NADP and 10 ng 20α-hydroxyprogren-4-en-3-one (Sigma Chemical Co.) in a total volume of 0·6 ml. The subsequent assay procedure and the calculation of 20α-HSD activity were essentially the same as those described for 3β-HSD activity, except that an incubation time of 20 min was used for the determination of 20α-HSD activity. Under these conditions there was a linear relationship between the amounts of the homogenate added (with the range of 25–150 μl) and the conversion rate of 20α-hydroxyprogren-4-en-3-one to progesterone. The coefficients of variation for the intra- and interassay variability were 10·1 and 18·2%, respectively. The activity of 20α-HSD was expressed as pmol progesterone/min per mg protein.

The progesterone concentration was determined by a radioimmunoassay as described previously (Kato, Ueda, Tsutsui et al. 1982). The cross-reactions of pregnenolone and 20α-dihydroprogesterone in the assay were 0·33 and 2·5%, respectively. Intra- and interassay coefficients of variation were 7·4% and 9·6%, respectively. Protein concentration was determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

The statistical significance of the difference between groups was analysed by an analysis of variance and Duncan's multiple range test; P < 0·05 was considered to be significant.

RESULTS

In agreement with previous findings (Kato et al. 1979) it has been found that on day 15 of pregnancy rats bearing more conceptuses had higher levels of circulating progesterone and a higher total CL weight. Also, in confirmation of Marcal et al. (1975) and Bast & Melampy (1972), it has been shown that in the whole ovaries of rats bearing a full complement of conceptuses the activity of 3β-HSD reached a maximum on days 15–18 of pregnancy, whereas the activity of 20α-HSD reached its nadir on day 18.

Figure 1 shows the relationship between the number of conceptuses and 3β-HSD or 20α-HSD activity in the CL and NLO. The activity of 3β-HSD was significantly (P < 0·05) lower in rats bearing one or two conceptuses than in those with three or more and in rats bearing three conceptuses than in those with eight to ten. In contrast, 20α-HSD activity in the CL was significantly (P < 0·05) higher in rats bearing one or two conceptuses than in those bearing four or more conceptuses. There was, however, no relationship between conceptus number and the activity of either enzyme in the NLO.

It is not clear how the placental hormone regulates these enzymes. However, it is worth noting that oestrogen maintains ovarian 3ß-HSD activity in pregnant rats (Rodway & Rothchild, 1980), whereas 20α-HSD activity is suppressed by oestrogen (Rodway & Rothchild, 1980) or by prolactin (Armstrong, 1968; Wiest, Kidwell & Balogh, 1968). The placenta secretes a large amount of rPL, as well as androgen (Rembiesa, Marchut & Warchol, 1972; Chan & Leathem, 1975; Sridaran, Basuray & Gibori, 1981), which is readily converted to oestrogen in the CL (Elbaum & Keyes, 1976; Gibori & Keyes, 1978). Furthermore, there is a direct relationship between conceptus number and the circulating levels of rPL (Robertson & Friesen, 1981; Ochiai, Kato, Kelly & Rothchild, 1983) or oestrogen (Csapo & Wiest, 1973). Since rPL has a prolactin-like activity, it is reasonable to suppose that 3ß-HSD and 20α-HSD in the CL are regulated by rPL and androgen from the placenta.

On the other hand, Ochiai et al. (1983) noted that hypophysectomy on day 12 in rats bearing a single conceptus induced a rise in progesterone secretion on day 15, without increasing the circulating rPL levels. These data indicate that the pituitary gland exerts luteolytic effects after day 12 of pregnancy. The luteolytic effect of the pituitary gland on day 15, however, would be blocked by the placental hormone, because hypophysectomy in rats with a full complement of conceptuses induces little change in serum progesterone levels (Pepe & Rothchild, 1972; Ochiai et al. 1983). It is difficult to explain how the pituitary gland acts to exert luteolytic effects after day 12 of pregnancy and how the placental hormone influences the luteolytic effects of a pituitary factor. We have reported that serum lutphinizing hormone levels are significantly higher in rats bearing a single conceptus on day 15 than in rats bearing a full complement of conceptuses (Kato et al. 1982). Furthermore, in a pilot study, hypophysectomy on day 12 induced a significant increase in ovarian 3ß-HSD activity in rats bearing a single conceptus (H. Kato, F. Miyauchi, K. Ueda & H. Tamura, unpublished findings). Luteininizing hormone (or human chorionic gonadotrophin) or follicle-stimulating hormone has been reported to increase ovarian 20α-HSD activity in pregnant rats (Wiest et al. 1968; Bast & Melampy, 1972; Eckstein & Nimrod, 1979). These findings strongly suggest the possibility that the placental hormone acts through the pituitary gland or in cooperation with a pituitary factor to regulate 3ß-HSD and 20α-HSD in the CL.

The present study also showed that neither 3ß-HSD nor 20α-HSD activities in the NLO were affected by conceptus number, which indicates that these two enzymes in the NLO are not controlled directly by the conceptus-derived substances. Recently, Taya &

**DISCUSSION**

It is well documented that serum progesterone in intact pregnant rats reaches peak levels by about day 15 (Pepe & Rothchild, 1974; Gibori, Antczak & Rothchild, 1977). The present study has confirmed previous findings (Marcel et al. 1975) that the activity of 3ß-HSD in the ovary also reaches a maximum by day 15 of pregnancy. The luteal secretion of progesterone in intact pregnant rats would, therefore, be at the maximum by about day 15. It has also been confirmed that the weight of CL and serum progesterone levels on day 15 of pregnancy are directly related to conceptus number (Kato et al. 1979). The present study further showed that 3ß-HSD activity in the CL was directly related to conceptus number, whereas there was an inverse relationship between conceptus number and 20α-HSD activity in the CL (Fig. 1). These results indicate that the effect of the placental hormones on progesterone secretion is mediated through these enzymes.
Greenwald (1981) suggested that the steroidogenesis in the NLO is regulated in a different manner from that in the CL.

ACKNOWLEDGEMENTS

We are indebted to Dr I. Rothchild, Case Western Reserve University School of Medicine, Cleveland, Ohio, U.S.A., for helpful suggestions.

REFERENCES


