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SYNTHESIS OF THE RABBIT UTERINE PROTEIN, BLASTOKININ: ONSET AND AGE DEPENDENT CHANGES

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Abstract

Changes in the capacity to synthesize blastokinin in response to ovarian steroids at different stages of maturity were determined using rabbits of 1, 3, 6, 16 and 24+ weeks of age. The 3-week-old neonate is the youngest age in which we could achieve induction of blastokinin. At that age, treatment either with estradiol-17β and progesterone together or with progesterone alone, but *not* with estradiol-17β alone, resulted in the appearance of blastokinin in uterine fluids.

Introduction

The synthesis of blastokinin (Krishnan and Daniel, 1967) in the rabbit uterus is regulated primarily by progesterone (Urzua et al., 1970; Beier, 1970; Arthur and Daniel, 1972; Garcea et al., 1974; Beato and Baier, 1975). Rahman et al. (1975) found that mature does produced 8 to 10 times as much blastokinin in response to various steroid treatments as did immature animals. Here, we have studied age-dependent changes in blastokinin synthesis in response to steroids and have identified the onset of the response by identifying the earliest age at which synthesis is elicited.

Materials and Methods

Age-response analysis: New Zealand white rabbits of the following ages were used: 1-week, 3-week, 6-week, 4-month, and mature ($\geq$ 6 months). Hormonal injections were made using a propylene glycol carrier via a subcutaneous route according to the following schedules:

Day	Steroid	Amt/kg body wt.
1	Estradiol-17β	200 µg
2	Progesterone	3.0 mg
3	Progesterone	3.0 mg
4	Progesterone	3.0 mg
5	Progesterone	3.0 mg

This schedule has been found to give optimum blastokinin synthesis in the mature castrate doe (Arthur and Daniel, 1972; Murray and Daniel, 1973). For comparison, three groups of 4 animals each at 3 and at 6 weeks of age were given 5 injections of the following hormones in propylene glycol carrier at daily intervals: estradiol-17β, 200 µg/kg body wt; progesterone, 3.0 mg/kg body wt; or the carrier alone. One-half hour after the final injection in each case the animals were killed by cervical dislocation and the uteri excised. The uteri were flushed with 1-5 ml sterile 0.9% NaCl. The flushings were centrifuged at 7,000 RPM on an RC2-B for 15 minutes to remove cell debris and were then frozen at —20°C until analyzed. The flushed uteri were cut open longitudinally and the endometrium was scraped off, suspended in 1-5 ml sterile saline solution (depending on the quantity of endometrium), and homogenized using 20 strokes of a glass on glass homogenizer. The homogenate was centrifuged at 8,000 RPM in an RC2-B for 20 minutes, and the supernatant was decanted and stored at —20°C until analyzed.

Uterine flushings and endometrial homogenate supernatants were assayed by electrophoresis for the presence of blastokinin using the polyacrylamide-gel slab system of ORTEC. Gradient gels of 8, 6, and 4.0% polyacrylamide in 0.375M tri-sulfate buffer and a 0.065M tri-borate tank buffer, pH 8.6 were used. A pulsed power supply (ORTEC) was used, applying 325 volts and a graded step up of pulse for maximum resolution. Each sample was standardized for protein concentration using the method of Lowry et al. (1951) and a 200 µg sample was loaded into each well. One well contained purified rabbit blastokinin as a reference standard. Gels were stained with 0.1% amido black in 10% acetic acid for 1 hour and destained in 10% acetic acid. Stained gels were analyzed for relative protein concentration using an ORTEC model 4310 densitometer with integrator. Blastokinin/total protein was expressed as the percentage of area under the blastokinin peak on the densitometric tracing as compared to area under all protein peaks.

Double immunodiffusion was performed using 1% agar noble on microslides with samples and a purified blastokinin (BKN) standard tested against Bullock and Connell's (1973) guinea pig anti-serum to rabbit blastokinin.

Results and Discussion

Figure 1 shows the results of the experiment for determining the earliest age at which a doe can synthesize blastokinin in response to exogenous steroids administered by the protocol described. Relative blastokinin concentration in uterine flushings was plotted against the age of the animal at the beginning of the 5-day steroid treatment. It is readily apparent that the response increased with the age of the doe up to 6 months, the time of sexual maturity. Electrophoresis (see Fig. 2) and double immunodiffusion plates testing uterine flushing against monospecific guinea pig anti-blastokinin (see Fig. 3) demonstrate that the earliest age at which a neonate is capable of any detectable response is 3 weeks, but considerable variability exists in young animals. Progesterone alone will stimulate blastokinin synthesis in both the 3-week and 6-week old animals while estradiol-17β and the propylene glycol carrier are unable to do so.

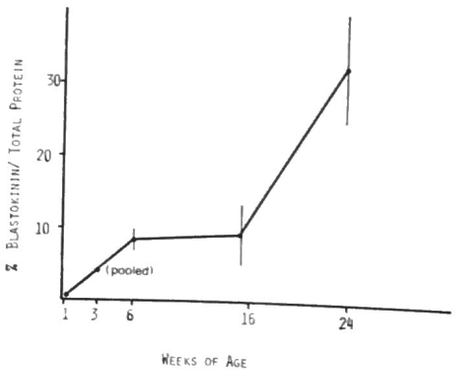

FIG. 1: *Age dependent response of rabbit uterus to estradiol-17β and progesterone treatment in inducing blastokinin synthesis.*

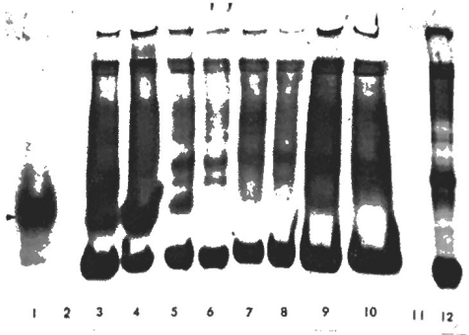

FIG. 2: *Electrophoresis of uterine flushings (UF) from rabbits after treatment with estradiol-17β (E) and/or progesterone (P).*

Well no.

1	purified rabbit blastokinin
2	blank
3	6-week UF, E and P treated
4	6-week UF, P treated, animal A
5	6-week UF, P treated, animal B
6	6-week UF, P treated, animal C
7	6-week UF, E treated
8	6-week UF, propylene glycol treated
9	3-week UF, E and P treated
10	1-week UF, E and P treated
11	blank
12	rabbit serum

Note individual differences in relative blastokinin amounts in P-treated animals A, B, and C.

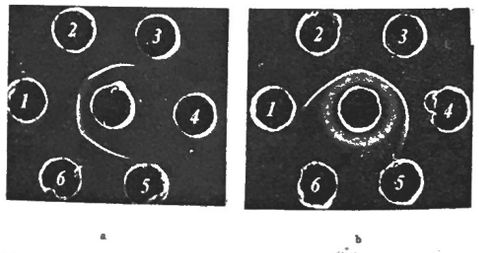

FIG. 3: *a. Double immunodiffusion of uterine flushings of 6-week-old rabbits under different steroid treatments. well 1: progesterone (P)-treated (animal A); well 2: P-treated (B); well 3: P-treated (C); well 4: estradiol-17β treated; well 5: propylene glycol treated; well 6: BKN; center: guinea pig anti-BKN. b. Double immunodiffusion of uterine flushings from young rabbits treated with estradiol-17β and progesterone as described in the text. well 1: 1-week old; well 2: 3-week; well 3: 6-week; well 4: BKN; wells 5 and 6: saline; center: guinea pig anti-BKN.*

We were unable to demonstrate blastokinin in the 3- and 6-week uterine endometrial homogenates from steroid treated animals. Mayol and Longenecker (1974) found that blastokinin could be demonstrated in immature uterine homogenates only after 2-3 days of estrogen priming followed by 5 days of progesterone treatment. It is possible that our protocol, which called for only 1 day of estrogen treatment followed by 4 days of progesterone treatment, was not sufficient to induce blastokinin in the uterine *endometrium;* but these data are confusing in that from this same protocol we found blastokinin in the uterine *secretion.* One might suspect that in these young animals blastokinin may be produced at some extrauterine site, then transported to and accumulated in the uterine secretions; but such a conclusion is contrary to our current knowledge of this substance. For the 4-month and mature ($\geq$ 6 months) animals, as was the case with the uterine flushings, the relative proportion of blastokinin to total protein in the endometrial homogenates of animals treated with our protocol was less than in the normal rabbit uterus of early pregnancy.

Urzua et al. (1970) obtained more blastokinin from oophorectomized rabbits treated for 4 days with 1-2 mg progesterone plus 0.5-1 µg estradiol-17β/kg body weight than with progesterone alone. Estradiol alone, up to 8µg/kg, did not induce blastokinin production. Conversely, Rahman et al. (1975) noted that blastokinin synthesis could be induced in castrated does by administration of estradiol and/or progesterone, but the relative amount of blastokinin produced in immature animals was only 10-15% of that produced by mature does under the same treatment. Both mature and immature animals produced more blastokinin after treatment with 2 mg progesterone plus 2 µg estradiol/kg wt than with either steroid alone at the same concentration, or together or alone at 10 µg estradiol/kg and a variety of lower concentrations of progesterone. Bullock and Willen (1974), working with adult castrated does, found that the proportion of uterine fluid protein represented by blastokinin "was similar . . . after treatment with either 3 mg/kg or 1 mg/kg of progesterone alone," but the addition of 5 µg estradiol/kg with either dose of progesterone "significantly reduced the proportion of blastokinin." They suggest, as Beier and colleagues had earlier concluded (see Beier (1974) for review) that estrogen might suppress progesterone-induced synthesis of blastokinin. Arthur and Daniel (1972) obtained maximal BKN production after administration to castrated does of 1-6 mg progesterone/kg body wt for 4 days but significantly less at concentrations below the 1 mg level. Addition of 100 µg estradiol-17β daily, or on days 1 and 4 of the schedule for progesterone, did not influence significantly the concentration of blastokinin produced. Obviously, the protocols noted above differ in many respects; but all are consistent in supporting the hypothesis of progesterone regulation of BKN synthesis. They do not answer the question of need for estrogen priming, because the uteri of normal mature does will have already been exposed to estrogen prior to castration. We show here that immature rabbits at the earliest ages *capable* of synthesizing blastokinin do so in response to progesterone whether estradiol is also administered or not—results which do not permit a clear conclusion to the question of priming but point again to the difficulty of voiding an animal completely of estrogen.

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