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SYNTHESIS OF THE RABBIT UTERINE PROTEIN, BLASTOKININ: CHANGES IN RATE PRIOR TO IMPLANTATION

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ABSTRACT

Activity of the rabbit uterus in synthesizing blastokinin during the period between estrus and day 8 *post coitum* has been studied. The specific radioactivity of blastokinin, obtained after incorporation of ^{3}H-labelled amino acids introduced into the uterine lumen and used as a relative measure of synthesis, is high at estrus and progressively declines thereafter. During this same period the concentration of blastokinin in the uterine fluids begins to increase on day 3, reaches its maximum on day 6, and declines thereafter. The rate of synthesis of blastokinin calculated from these data is low up to the second day of pregnancy, accelerates on day 3 to reach its highest level on days 4 to 5 and reduces rapidly by day 6. Several interpretations of these data are offered, but all point to a critical change on day 6, one day preceding implantation.

INTRODUCTION

Blastokinin (BKN), the uterine-specific glycoprotein of the rabbit, is known to be produced during early pregnancy or pseudopregnancy (Krishnan and Daniel, 1967; Beier, 1968; Johnson, 1972; Bullock and Connell, 1973; Barfield et al., 1976). In the studies reported here we have been interested in determining the daily changes in rate of synthesis of BKN by mature animals during the time between estrus and implantation.

METHODS AND MATERIALS

Synthetic pattern of blastokinin during early pregnancy: For this study two or three New Zealand white female rabbits were used on each of days 0 (estrus) 2, 3, 4, 5, 6, 7, and 8 *post coitum*. The pattern of blastokinin synthesis was assayed using radio-labelled amino acid incorporation according to the method of Murray and Daniel (1973). Amino acids used for labelling (New England Nuclear, Boston, Mass.) were 4,5-^{3}H-L-leucine (specific activity 44.2 C./mole), 4,5-^{3}H-L-lysine (specific activity 33.6 C./mmole), and 3-^{3}H-L-glutamic acid (specific activity 16.2 C./mmole). The incubation solution consisted of a mixture of equal amounts of radioactivity contributed by each of the three amino acids in sterile 0.9% NaCl so that the final solution contained 50 μC/ml. One milliliter of this solution was injected into the lumen of each uterine horn of the test animals using standard surgical conditions; to assure retention of the solution, each horn was ligated at both the tubal and the cervical end. After 6 hours, the time period found to yield maximum incorporation of amino acids, the animals were killed by cervical dislocation and the uteri removed. Uterine fluid was collected by flushing each horn with 5 ml 0.9% sterile NaCl injected with a 10 ml syringe through an 18-gauge needle which had been secured in the utero-tubal end of the horn with a haemostat and by flushing through an 18-gauge needle which had been secured by clamping at the cervical end of the horn. The collected flushings were assayed for total protein by the method of Lowry et al. (1951) and concentrated in an Amicon ultrafiltration unit using a UM-10 membrane. Flushings from each rabbit were subjected to gel filtration at 4°C through Sephadex G-200 as previously described by Murray et al. (1972). The eluent was 0.02 M citrate buffer, pH 7.4, and fractions of 0.5 ml were collected. One tenth milliliter aliquots from even-numbered fractions were placed with 0.4 ml H_2O in 10 ml of scintillation solution [0.5% PPO (2,5-diphenyloxazole), 0.01% POPOP (1,4-bis (2-(5-phenyloxazolyl)) benzene), 22% Triton-X (Sigma, St. Louis, Missouri) brought to a volume of 1 liter with toluene.] Counts were made in a Beckman LS-230 liquid scintillation counter. The odd-numbered aliquots were analyzed for protein concentration. The protein profile and distribution of radioactivity of each animal were graphed together (Fig. 1), and the specific activity of blastokinin was determined using the highest protein value and the highest counts/minute of that peak.

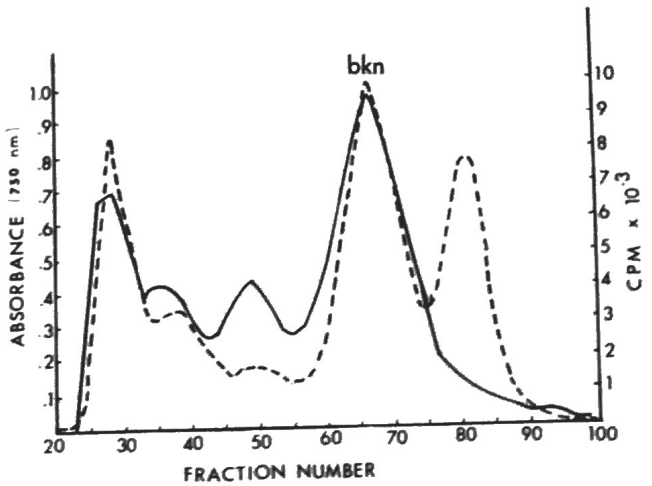

FIG. 1. *Example of scintillation and protein profiles from G-200 chromatography of rabbit uterine flushing; from a 6-day pregnant rabbit. (-----) = counts per minute; (———) = absorbance at 750 nm for Lowry protein determination. Blastokinin peak is designated as bkn.*

RESULTS AND DISCUSSION

Other publications from this laboratory have shown how the concentration of blastokinin, expressed as percentage of total protein, changes in the uterine fluids on progressive days of early pregnancy (Krishnan and Daniel, 1967; Daniel, 1969, 1971, 1976). Samples, carefully collected on day 5-6, may contain up to 75-80% blastokinin. Data collected from hundreds of animals over an eight-year period show that the amount of total protein/uterus and the relative proportion that is blastokinin are predictable when the flushing procedures are consistent (Daniel, 1976). Similarly, the amount of blastokinin from our surgically treated animals was consistent for successive days of pregnancy, although the relative percentages of blastokinin were lower in these animals than in intact ones, possibly because of tissue trauma resulting from the injection procedure. It is probable that our quantitative data are minimal and may not reflect the total amount of blastokinin present because, as noted by Mayol and Longenecker (1974), blastokinin can still be detected after multiple flushing (up to 10 times) of a uterine horn.

The specific activities of blastokinin isolated from rabbit uteri 6 hours after infusion of tritium labelled percursors into the lumen are listed in Table 1 for each day between estrus and implantation. This activity is high at estrus and through the second day *post coitum*. This would seem to indicate that most, if not all, of the minute amount of blastokinin present at that time is the product of continual and immediate synthesis. Thereafter, the specific activity drops and continues to decline through day 8. By day 7 it has reached a level analogous to that of albumin from the same samples, which had remained relatively constant throughout this preimplantation period (ranging from 15-50 CPM x 10^3/mg protein) and which probably represents either the normal synthetic activity of albumin or nonspecific binding of the labelled precursors. Obviously some (progressively more) of the blastokinin present in the uterus between days 3 and 7 was not synthesized during the 6-hour period of exposure to available labelled precursors and thus must represent accumulation from prior synthetic activity. Using the specific activities of the proportion of blastokinin being synthesized, knowing the amount of blastokinin present at any one time (Table 1), and assuming that the specific activity for estrous animals represents maximal potential incorporation (i.e., 100%), it is possible to calculate the approximate rate of blastokinin synthesis. Table 1 lists the results of these calculations, which indicate that the rate of synthesis starts to rise on day 3, is accelerating rapidly by day 4, reaches a peak on day 5, drops thereafter, and is virtually stopped by day 7.

Obviously, blastokinin accumulates through day 6, and synthesis is still progressing even though the rate has started to decline at that time. By day 7, a major drop occurs in total blastokinin and new synthesis has almost stopped. Thereafter, sometime between days 6 and 7, a major utilization of the substance must have been initiated or it must be being degraded, absorbed, or transported elsewhere. Thus the search for a function for this molecule might best be focused at this time immediately prior to implantation. An alternate interpretation of the blastokinin-accumulation pattern might involve a constant rate of utilization as long as that rate remains less than the rate of synthesis up to day 6. Such an interpretation would implicate a role for blastokinin that would be functional mainly during the period between days 3 to 6 *post coitum*.

Changes in the rates of degradation, absorption, etc. *could* also account for all or part of the observed changes, but it seems inefficient for a specific molecule to be synthesized in large amounts within a very restricted time period and be rapidly eliminated by non-utilitarian processes. If that were the case, however, it would still point to a change coincidental with the 24-hour period preceding implantation. In the work reported here, we have not measured these parameters and therefore cannot accurately discuss "turnover" as the balance of the interactive phenomena.

TABLE I: *The specific activities of blastokinin isolated from rabbit uteri 6 hours after infusion of tritium labeled precursors into the lumen.*

Sample (n)	Specific Activity of H^3AA Labelled BKN. CPM/mg Prot.	Total mg BKN/Uterus	Calculated Rate mg BKN Synthesized/hr
	(Range)	(Range)	
Estrus (2)	661,490 ± 228,967	.51 ± .12	.085
D-2 (2)	351,500	.098 *	.008
D-3 (2)	291,791 ± 25,708	.80 ± .26	.058
D-4 (3)	226,450 ± 64,846	4.14 ± .95	.236
D-5 (2)	214,028 ± 50,584	5.00 ± .85	.270
D-6 (2)	88,975 ± 22,935	6.56 ± .02	.147
D-7 (2)	41,149 ± 4,211	3.84 ± 1.13	.040
D-8 (2)	21,445 ± 7,412	1.83 ± .07	.009

* Second value for mg BKN was undetectable with our methods