J. Endocr. (1975), **64**, 187–188
With 1 plate
Printed in Great Britain

INDUCTION OF BLASTOKININ SYNTHESIS BY R 2323 IN OVARIECTOMIZED RABBITS

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(*Received* 4 *July* 1974)

The synthetic steroid R 2323 (Sakiz & Azadian-Boulanger, 1971) is being tested clinically as a contraceptive. From studies of its inhibitory action on endometrial proliferation, progesterone-maintained pregnancy in castrated animals and implantation, this trienic steroid has been shown to have strong anti-progesterone activity and weak progestomimetic activity (Azadian-Boulanger, Secchi & Sakiz, 1973; Sakiz, Azadian-Boulanger & Raynaud, 1973). Raynaud, Philibert & Azadian-Boulanger (1974) maintain that it exerts its action by competition for the corresponding hormonal receptor rather than directly impeding hormonal activity. We were interested in determining whether the mode of action for R 2323 might be found in its possible influence on the induction of uterine protein synthesis, specifically that of blastokinin (Krishnan & Daniel, 1967) in the rabbit.

Blastokinin synthesis can be induced in castrated does by administration of progesterone (Urzua, Stambaugh, Flickinger & Mastroianni, 1970; Arthur & Daniel, 1972). Arthur & Chang (1974) found that some oral contraceptive steroids (specifically those containing the C-19 methyl group) also induce blastokinin synthesis. The same procedures as described by Arthur & Daniel (1972) were used for testing whether R 2323 would induce blastokinin synthesis in the rabbit uterus.

Three mature, virgin, New Zealand White rabbits were ovariectomized and retained untreated for 4 weeks thereafter. They were then injected s.c. daily for 4 days with propylene glycol containing 3 mg R 2323. Within 2 h after the last injection the animals were killed by cervical dislocation, the uteri excised, and each horn carefully flushed with 2·5 ml sterile physiological saline. The flushings were centrifuged to remove cellular debris, then the protein content of an aliquot of the supernatant was determined by the procedure of Lowry, Rosebrough, Farr & Randall (1951). The remaining supernatant was exposed to vacuum dialysis to concentrate the macromolecular components. These concentrated fluids were then used as samples for polyacrylamide gel electrophoresis with the ORTEC pulse-power system using gradient gels that ranged from 4·5 to 8%. The gels were fixed with 10% acetic acid and stained with Amido Black. Three additional castrated animals were injected with propylene glycol carrier alone to serve as controls.

The same concentrated uterine flushing samples were used to test the specificity of any blastokinin they might contain by double diffusion using the monospecific anti-

serum to rabbit blastokinin described by Johnson, Cowan & Daniel (1972). R 2323 was kindly supplied by Roussel-Uclaf, France.

Electrophoresis patterns obtained from the uterine flushings of all rabbits receiving R 2323 contained a distinct band which migrated identically with a blastokinin sample isolated from an untreated uterus on day 5 *post coitum* (Plate). This band is absent in serum from the same animal, and from the uterine fluids of all the control animals. On the Ouchterlony plates resulting from the test of the uterine flushings against an anti-blastokinin serum, a line of identity was detectable between the precipitation arc formed from the antiserum and R 2323, and that formed from the test blastokinin, in all cases.

The mean total uterine fluid protein content of three animals receiving exogenous R 2323 was 220 ± 20 (S.E.M.) μg/uterus. For animals that received progesterone by the same system it was $2{\cdot}5 \pm 0{\cdot}6$ mg protein/uterus, and $5{\cdot}6 \pm 0{\cdot}4$ mg protein/uterus were obtained from normal animals on day 5 *post coitum*. Obviously, the protein content of uteri from animals treated with R 2323 samples is low, being only 10% of that from progesterone-treated animals and approximately 4% of that from normal pregnant animals.

We conclude that R 2323 is capable of inducing, in the rabbit uterus, the synthesis (or secretion) of a specific protein which is identical both electrophoretically and immunologically with the blastokinin of a normal animal, but that the total amount of protein produced under the influence of this synthetic steroid is significantly less than that found under the influence of progesterone or in the untreated pregnant animal. These results would seem to provide peripheral support for the contention made in the introduction of this paper, namely that this compound is weakly progestomimetic.

REFERENCES

Arthur, A. T. & Chang, M. C. (1974). *Fertility & Sterility* **25**, 217–221.

Arthur, A. T. & Daniel, J. C. (1972). *Fertility & Sterility* **23**, 115–122.

Azadian-Boulanger, G., Secchi, J. & Sakiz, E. (1973). *Proc. 7th World Congr. on Fertility and Sterility, Tokyo 1971, I.C.S.* no. 278, pp. 129–133. Ed. T. Hasegawa. Amsterdam: Excerpta Medica Foundation.

Johnson, M. H., Cowan, B. D. & Daniel, J. C. (1972). *Fertility & Sterility* **23**, 93–100.

Krishnan, R. S. & Daniel, J. C. (1967). *Science* (*N.Y.*) **158**, 490–492.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *Journal of Biological Chemistry* **193**, 265–275.

Raynaud, J. P., Philibert, D. & Azadian-Boulanger, B. (1974). In *The physiology and genetics of reproduction*, vol. 1, pp. 143–160. Eds E. M. Coutinho & F. Fuchs. New York: Plenum Press.

Sakiz, E. & Azadian-Boulanger, G. (1971). In *Proc. 3rd Int. Congr. Hormonal Steroids, Hamburg 1970, I.C.S.* no. 219, pp. 865–871. Eds V. H. T. James & L. Martini. Amsterdam: Excerpta Medica Foundation.

Sakiz, E., Azadian-Boulanger, G. & Raynaud, J. P. (1973). In *Proc. 4th Int. Congr. Endocr., Washington, D.C., 1972, I.C.S.* no. 273, pp. 988–994. Eds R. O. Scow, F. J. G. Ebling & I. W. Henderson. Amsterdam: Excerpta Medica Foundation.

Urzua, M. A., Stambaugh, R., Flickinger, G. & Mastroianni, L. (1970). *Fertility & Sterility* **21**, 860–865.

DESCRIPTION OF PLATE

Protein band patterns obtained from polyacrylamide gel electrophoresis of:

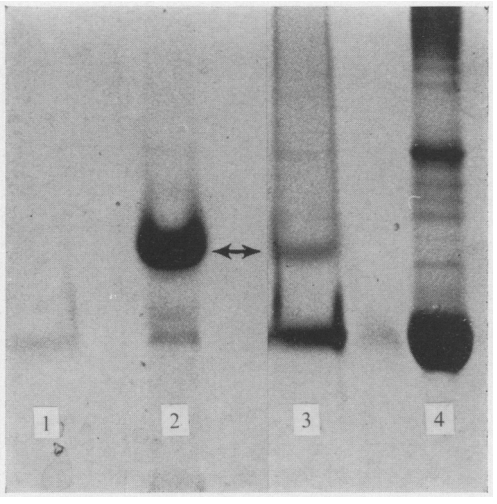
(1) Uterine fluids of a castrated doe after treatment with propylene glycol carrier.

(2) Blastokinin isolated from the uterine fluids of a 5-day pregnant rabbit.

(3) Uterine fluids of a castrated doe after treatment with R 2323 in propylene glycol carrier.

(4) Serum from 5-day pregnant rabbit.

Arrow indicates blastokinin band.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