

## Cyclic AMP and cyclic GMP in rabbit blastocysts

S. K. Dey, F. Kimura\*, A. Mukherjee† and Z. Dickmann

*Department of Gynecology & Obstetrics and Ralph L. Smith Human Development Research Center, University of Kansas Medical Center, Kansas City, Kansas 66103, U.S.A.;*

*\*Department of Physiology, Yokohama City University, School of Medicine, Yokohama, Japan and*

*†Department of Internal Medicine, Ischemic Heart Center, University of Texas Health Science Center, Dallas, Texas 75235, U.S.A.*

**Summary.** Concentrations of both nucleotides were significantly higher in Day-6 than in Day-5 blastocysts but the ratio of cAMP to cGMP changed from 0.5 to 1.5.

### Introduction

Recent findings suggest that the regulation of cellular events such as hormone secretion, cell growth and differentiation appears to involve an inverse relationship between the intracellular concentrations of cyclic AMP (cAMP) and cyclic GMP (cGMP) (Sharma, Ahmed, Sutliff & Brush, 1974; Ratner, 1976; Moriyama, Hasegawa & Murayama, 1976). Furthermore, an agonist–antagonist hypothesis has been proposed for the interaction between these two nucleotides in modulating certain tissue responses (Goldberg, O’Dea & Haddox, 1974). Nevertheless, except for a single report on levels of cAMP in mouse preimplantation embryos (Fisher & Gunaga, 1975), no information is available about the contents of cAMP and cGMP in the preimplantation embryo of any mammal. We therefore studied the levels of cAMP and cGMP in rabbit blastocysts.

### Materials and Methods

Adult virgin female domestic rabbits were induced to superovulate (Kennelly & Foote, 1965) and were then mated by two fertile bucks in close succession. At 120 and 144 h *post coitum* (Days 5 and 6 of pregnancy, respectively), the females were killed and the uteri were flushed with chilled saline (0.9% NaCl) to recover the blastocysts. The blastocysts were washed once in chilled saline, their diameters were recorded and the normality of their morphology was checked rapidly under a dissecting microscope. The blastocysts were then frozen in a solid CO<sub>2</sub>–acetone mixture and stored at –80°C until the assays were performed. Groups of 25–40 Day-5 blastocysts and 10–20 Day-6 blastocysts were used in each assay. The blastocysts were transferred into ice-cold glass homogenizers containing 0.6 ml trichloroacetic acid and were homogenized with a motor-driven teflon pestle. The homogenates were centrifuged at 3000 *g* for 15 min at 0°C. The supernatant solution was removed and extracted three times with 3 ml ether saturated with H<sub>2</sub>O. The aqueous phase was first dried and then reconstituted with 300 µl acetate buffer at pH 6.2. cAMP and cGMP were assayed with the Schwarz-Mann radioimmunoassay kits for which the procedures are those described by Steiner, Kipnis, Utiger & Parker (1969) and Steiner, Pagliari, Chase & Kipnis (1972).

The antibodies were raised in rabbits against a succinyl-cAMP-albumin conjugate and against a succinyl-cGMP-albumin conjugate: the antibodies were specific for cAMP and cGMP respectively. The labelled antigens were cAMP (succinyl-cAMP-tyrosine methyl ester-<sup>125</sup>I) and cGMP (succinyl-cGMP-tyrosine methyl ester-<sup>125</sup>I) derivatives. Tests of cross-reactivity with the cAMP antiserum showed that reactivity equivalent to cAMP required a 10<sup>4</sup>-fold amount of cGMP, 10<sup>6</sup>-fold amount of AMP and greater than 10<sup>6</sup>-fold amount of ATP. A similar test with the cGMP antiserum required a 33 × 10<sup>3</sup>-fold greater amount of cAMP and a 10<sup>7</sup>-fold amount of ATP to attain the reactivity equivalent to cGMP. The sensitivity of the assays for cAMP and cGMP was 0.05–0.10 pmol for cAMP and 0.10–0.20 pmol for cGMP. The specificity of the assays allowed direct measurements of cAMP and cGMP in all preparations without separation of other nucleotides.

## Results

These are summarized in Table 1. The contents of both cAMP and cGMP were significantly ( $P < 0.001$ ) higher in Day-6 than in Day-5 blastocysts, but the ratio of the two nucleotides changed significantly ( $P < 0.05$ ).

**Table 1.** Mean  $\pm$  s.e.m. content of cAMP and cGMP (pmol/blastocyst) in rabbit blastocysts collected on Day 5 or Day 6 *post coitum*

	Age		Significance
	Day 5	Day 6	
cAMP	0.273 $\pm$ 0.030 (4)	2.592 $\pm$ 0.150 (5)	$P < 0.001^*$
cGMP	0.484 $\pm$ 0.004 (5)	1.773 $\pm$ 0.134 (4)	$P < 0.001^*$
cAMP/cGMP	0.522 $\pm$ 0.023	1.522 $\pm$ 0.055	$P < 0.05^\dagger$

The numbers in parentheses indicate the number of experiments: there were 25–40 Day-5 and 10–20 Day-6 blastocysts in each experiment.

\* Statistical analysis by Student's *t* test.

† Statistical analysis by Wilcoxon Rank Sum test.

## Discussion

Very little information is available about cAMP and cGMP in the mammalian preimplantation embryo, and more work is necessary to elucidate their possible role in the metabolism of the preimplantation embryo. Fisher & Gunaga (1975) found that in mouse preimplantation embryos the concentration of cAMP decreased during development from  $41.2 \pm 1.4$  pmol/1000 two-cell embryos to  $12.8 \pm 4.1$  pmol/1000 Day-4 blastocysts. However, our results demonstrate that the contents of both cAMP and cGMP in rabbit blastocysts increased significantly from Day 5 to Day 6 of pregnancy (Table 1). Increases in these nucleotides have been noted during early myogenesis (Moriyama *et al.*, 1976; Novak, Drummond, Skala & Hahn, 1972). It has been proposed that the increment in intracellular content of cGMP acts as a positive signal and that of cAMP as a negative signal in controlling cellular growth and differentiation (Hadden, Hadden, Haddox & Goldberg, 1972; Otten, Johnson & Pastan, 1972; Seifert & Rudland, 1974a, b). The changed ratio of cAMP to cGMP in rapidly growing rabbit blastocysts suggests that these nucleotides may be involved in the regulation of embryonic growth and differentiation.

The cAMP-cGMP ratio has also been related to steroidogenesis in the adrenal glands (Sharma *et al.*, 1974) and the ovaries (Ratner, 1976), but whether the inverse relationship between the contents of cAMP and cGMP plays any role in blastocyst steroidogenesis (Dickmann, Dey & Sen Gupta, 1976) has yet to be determined. (Supported by NIH (HD-08644) and the National Foundation (1-406).

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