Journal of Steroid Biochemistry, 1973, Vol. 4, pp. 533–535. Pergamon Press. Printed in Great Britain

EFFECT OF ADENOSINE-3′,5′-CYCLIC MONOPHOSPHATE ON β-HYDROXY STEROID DEHYDROGENASE IN THE BOVINE CORPUS LUTEUM

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(*Received* 6 *April* 1973)

SUMMARY

Adenosine-3′,5′-cyclic monophosphate (cyclic-AMP) inhibits the enzyme 3β-hydroxy steroid dehydrogenase which converts pregnenolone to progesterone in a two step reaction along with 3-ketosteroid 5-ene-4-ene isomerase in the corpus luteum of pregnant and non-pregnant cows. The inhibition by cyclic AMP could be overcome by increasing the concentration of NAD^+.

INTRODUCTION

PREVIOUS studies with bovine adrenal [1], rat adrenal [2], rat ovaries [3] and mouse ovaries [4] demonstrated that adenosine-3′,5′-cyclic monophosphate (cyclic AMP) inhibited the conversion of pregnenolone to progesterone. The reaction was carried out in two stages with the participation of a 3β-hydroxy steroid dehydrogenase (EC 1.1.1.51) which requires NAD^+ and a 3-keto steroid 5-ene-4-ene isomerase (EC 5.3.3.1). On the other hand, it has been reported that cyclic AMP enhances progesterone synthesis *in vitro* in slices of bovine corpus luteum [5, 6], chopped rabbit ovarian tissue [7, 8] and corticoid synthesis in rat adrenal tissue [9]. The effect of cyclic AMP on the conversion of pregnenolone to progesterone has not been studied in homogenates of the bovine corpus luteum. This report compares the effect of cyclic AMP on 3β-hydroxy steroid dehydrogenase of bovine corpus luteum with those of bovine and rat adrenals and rat and mouse ovaries.

MATERIALS AND METHODS

Chemicals. Nicotinamide adenine dinucleotide (NAD^+) and Adenosine-3′,5′-cyclic monophosphoric acid (cyclic AMP) from Sigma (U.S.A.), pregnenolone and progesterone from Mann Research Laboratories (U.S.A.), ethyl acetate (spectroscopic grade) from Burdick and Jackson (U.S.A.).

All other chemicals and reagents were of reagent grade.

Preparation of corpus luteum tissue. Bovine corpus luteum was collected from a local slaughter house shortly after the animals were killed and kept in ice cold saline. They were processed within 2 h or kept frozen until use. Freezing the tissue did not result in any apparent loss of enzyme activity. A 5% homogenate was prepared in 0·1 M phosphate buffer, 0·9% saline mixture (pH 7·4) with an all glass homogenizer or with a Polytron homogenizer [Brinkmann Instruments, Inc.,

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Abbreviations and trivial names used: CAMP, cyclic AMP; adenosine-3′,5′-cyclic monophosphate; NAD^+, nicotinamide adenine dinucleotide; Pregnenolone, 3β-hydroxy-5-pregnen-20-one; Progesterone, 4-pregnene-3,20-dione.

Westbury, New York]. The homogenate was centrifuged at 2500 rev./min for 10 min in a refrigerated centrifuge.

Incubation and assay. Incubation and assay of the enzyme activity in the homogenates of bovine corpus luteum were carried out according to the procedure of Rubin *et al.* [10]. The incubation medium contained 0·1 ml pregnenolone in propylene glycol (100 μg), 0·01–1·0 μmol of NAD^+ in 0·2 ml deionized water, 0·2 ml homogenate (equivalent to 10 mg of wet tissue) and buffer was added to make a final vol of 1·9 ml. Blanks without pregnenolone were included with each incubation. Cyclic AMP, 10 μmol per incubation, was added to each concentration of NAD^+. Incubations were carried out in air for 20 min at 37°, and stopped by the addition of 10 ml of ethyl acetate. Ethyl acetate extracts were dehydrated with molecular sieve, type 3A, and evaporated to dryness under a current of nitrogen at 60°C. The spectra of samples were routinely taken between 220 and 260 nm in a Cary-14 spectrophotometer to analyze the purity of the product and progesterone was quantitated from the 240 nm absorption of the above scans. Recovery was over 98% when determined with standard progesterone samples. The proteins were assayed by the procedure of Lowry *et al.* [11].

RESULTS AND DISCUSSION

The effect of 10 μmol of cyclic AMP on the 3β-hydroxy steroid dehydrogenase in corpus luteum tissue taken from non-pregnant cows is shown in Table 1. It

Table 1. Effect of cyclic AMP on 3β-hydroxy steroid dehydrogenase activity in bovine corpora lutea (non-pregnant)

NAD added (μmol)	Progesterone formed (mg/g tissue)[a] Control	With cAMP	% Inhibition
1·0	2·29±0·16(8)[b]	1·93±0·13(5)	16
0·5	1·96±0·18(8)	1·49±0·09(5)	24
0·1	1·55±0·15(11)	1·09±0·09(9)*	30
0·05	1·31±0·12(10)	0·84±0·09(7)*	36
0·01	1·01±0·09(8)	0·63±0·07(6)*	38

[a]Values are mean ± S.E.
[b]Numbers within parenthesis represent number of experiments.
*P value $<0·05$.

was observed that by increasing the concentration of the cofactor NAD^+, higher activity was obtained. The enzyme activity was significantly inhibited by cyclic AMP, being larger at lower concentrations of NAD^+. Similar results were obtained with corpus luteum tissues removed from pregnant cows (Table 2).

Cyclic AMP was reported to increase the conversion of cholesterol to pregnenolone and to decrease the production of progesterone in both intact and solubilized rat ovarian mitochondria [12]. The inhibition of 3β-hydroxy steroid dehydrogenase by cyclic AMP in the mitochondria and microsomes of rat adrenals [2], rat ovaries [3] and mouse ovaries [4] has also been reported. It was observed in those cases that by increasing the concentration of NAD^+ the inhibition by cyclic AMP could be reversed [2, 3]. The action of cyclic AMP was similar to leutinizing hormone with slices of bovine corpus luteum *in vitro*. Incorporation of radioactive

Table 2. Effect of cyclic AMP on the 3β-hydroxy steroid dehydrogenase activity in bovine corpora lutea (pregnant)

NAD added (μmol)	Progesterone formed (mg/g tissue)[a] Control	With cAMP	% Inhibition
1·0	2·15±0·08(4)[b]	1·72±0·08(4)	20
0·5	1·98±0·19(4)	1·34±0·14(4)*	32
0·1	1·39±0·20(5)	1·03±0·16(4)	26
0·05	1·53±0·31(4)	0·94±0·17(4)	39
0·01	1·16±0·15(4)	0·82±0·21(5)	29

[a]Values are mean ± S.E.
[b]Numbers within parenthesis represent number of experiments.
*$P < 0·05$.

acetate[5] and cholesterol[6] into progesterone was increased when slices of bovine corpus luteum were incubated with cyclic AMP. The results reported here are the first observations with homogenates of corpus luteum and confirm the observations in rat adrenals[2], rat ovaries[3] and mouse ovaries[4] on the inhibition of the enzyme activity by cyclic AMP and the reversal thereof by NAD^+. These results contradict the earlier results with the slices of bovine corpus luteum reporting increased incorporation of active precursors into progesterone[5, 6]. Yeast glyceraldehyde-3-phosphate dehydrogenase[13], rat testicular NAD^+-isocitrate dehydrogenase, malate dehydrogenase[14] and 17β-hydroxy steroid dehydrogenase of rat testis[15] are also reported to be inhibited by cyclic AMP. These dehydrogenases are NAD^+-linked. The studies suggest that the cyclic AMP effect of these NAD^+-linked enzymes may be due in part to the structural similarity of the two compounds. The data presented in this communication lend support to the hypothesis that inhibition of NAD^+-linked dehydrogenases could be due to competition between NAD^+ and cyclic AMP for NAD^+ sites.

ACKNOWLEDGEMENTS

This work was supported in part by research grant number HD5724 from the National Institutes of Health.

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