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SHORT COMMUNICATION

BIOGENIC AMINES AND RABBIT FOLLICULAR TESTOSTERONE PRODUCTION

E. V. YOUNGLAI

Department of Obstetrics and Gynecology, Program in Reproductive Biology, McMaster University Medical Center, Hamilton, Ontario, Canada L8S 4J9

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SUMMARY

Incubation of isolated rabbit follicles with epinephrine, norepinephrine, isoproterenol, phentolamine and propanolol at various doses from 0.1 to 1.0 μg/ml, failed to stimulate testosterone production. When used in combination with LH (5 μg/ml) phentolamine (100 μg/ml) enhanced ($p < 0.05$) and propanolol (100 μg/ml) depressed ($p < 0.01$) the testosterone response to LH alone. These data suggest that α and β blockers may modify follicular response to LH.

It was reported that biogenic amines can increase adenylate cyclase activity [1] and progesterone synthesis [2] in ovarian tissue. In view of these studies and the fact that the isolated rabbit follicle can respond to various stimulants with a marked elevation in testosterone production [3] it was deemed important to study the effects of catecholamines in this system.

Follicles (1–2.5 mm dia.) were isolated from rabbit ovaries and incubated as single intact follicles in 0.2 ml minimum essential medium with Earle's salts, 5% normal rabbit serum and buffered with HEPES. At various times after incubation test substances were added. Briefly, for epinephrine and isoproterenol, varying concentrations were added to the follicles following a 1.5 h incubation period in medium alone. Medium was replaced completely at each change with the follicles being washed 3 times before the addition of new media. For the α and β blockers these solutions were kept with follicles for successive 1 h periods before, during and after exposure to LH for 1 h. Media were stored and analysed for testosterone by radioimmunoassay of ether extracts of suitable aliquots [3]. The antiserum used (Dr. G. E. Abraham's S741 No. 2) cross-reacts with 17β-hydroxy-5α-androstan-3-one which is not secreted by the ovary [4]. Results are therefore expressed in terms of testosterone equivalents.

Catecholamines and inhibitors were obtained from the following sources: isoproterenol and norepinephrine (Sigma), epinephrine (Parke-Davis), phentolamine hydrochloride (Rogitine®, Ciba) and propanolol (Inderal®, Ayerst). Ovine LH (NIH-LH-S18) was obtained from the Hormone Distribution Officer, NIAMDD. Norepinephrine and epinephrine were used at concentrations of 0.1, 0.5 and 1.0 μg/ml; isoproterenol at 0.1 and 0.5 μg/ml; phentolamine at 1, 10 and 100 μg/ml; propanolol at 1, 10 and 100 μg/ml and LH at 5 μg/ml. Number of incubations for each test substance varied from 5–10.

When the various catecholamines and inhibitors were added in the medium directly to follicles no change in testosterone production could be observed. Levels of testosterone in the presence and absence of test substance were all similar and in accord with what might be expected when follicles were incubated in medium alone [3].

When adrenergic blockers were used in conjunction with LH some effect on testosterone production could be observed (Fig. 1). In the absence of any test substance the usual decrease in steriodogenesis occurs. LH caused an

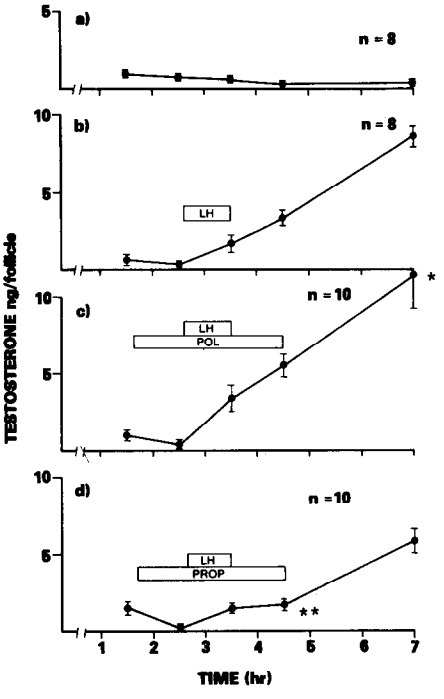

Fig. 1. Effects of adrenergic blockers on LH stimulated testosterone production by isolated rabbit follicles. Differences were compared between (b) and (c) or (d) at identical time intervals. LH = luteinizing hormone (5 μg/ml); POL = phentolamine (100 μg/ml); PROP = propanolol (100 μg/ml). $*p < 0.05$ $**p < 0.01$.

increase in testosterone production which is significant within 1 h of exposure ($p < 0.01$). In the presence of phentolamine there appeared to be an increase in steroidogenesis over that induced by LH alone but this was not significant until after LH and the β-blocker were removed. Propanolol, however, seemed to prevent testosterone production but when removed steroidogenesis proceeded as with LH alone. This inhibition of steroidogenesis for as

long as the inhibitor is present is similar to previous findings from this laboratory using protein synthesis inhibitors [5] and inhibitors of steroidogenic enzymes [6]. Common to all the data is the fact that resumption of steroidogenesis occurs after medium containing both LH and inhibitor are removed. The results of the present study suggest that catecholamines do not have a significant role in ovarian follicular steroidogenesis *in vitro* and are in agreement with recent findings that although corpora lutea tissue was sensitive to biogenic amines, follicles were not [7].

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