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SHORT COMMUNICATION

IN VITRO EFFECTS OF MELATONIN ON HCG STIMULATION OF STEROID ACCUMULATION BY RABBIT OVARIAN FOLLICLES

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SUMMARY

Isolated rabbit ovarian follicles were incubated with HCG and/or melatonin, serotonin, 5-hydroxy-DL-tryptophan or N-acetyl serotonin. Melatonin alone significantly decreased the accumulation of steroids elicited by HCG stimulation. Follicles did not bind labelled melatonin whereas the specific binding of [^{125}I]-HCG was 4.11 pmol per mg protein.

Melatonin, the hormone secreted by the pineal gland is generally accepted as an antigonadotropic substance exerting its influence at the level of the hypothalamus and pituitary [1, 2]. However, melatonin has been found to be concentrated in the rat and cat ovary [3] and to stimulate the production of progesterone by human corpora lutea and of androstenedione by stroma tissue [4]. Since the rabbit follicle is similar to the testes in terms of testosterone production [5] the present study was undertaken to test the hypothesis that melatonin would inhibit HCG induced testosterone production by the follicle.

The incubation procedure was as follows: follicles greater than 1 mm in diameter were incubated singly or in groups of 2 in minimum essential medium (MEM) containing L-glutamine and buffered with HEPES (N-2-hydroxyethylpiperazine-N^1-2-ethane-sulphonic acid). The volume of medium used each time was 200 μl. Incubation was carried out at 37°C in an atmosphere of 95% CO_2 5% O_2 for 2 h followed by a 3 h incubation in medium containing various test substances. Media and follicles were then frozen until assayed for steroids by radioimmunoassay and protein by the Lowry method.

The following substances were obtained from Sigma: human chorionic gonadotropin (HCG, Lot 27C-0036); melatonin (Lot 84C-0009); serotonin (Lot 44C-1634); N-acetyl serotonin (Lot 124C-01201); 5-hydroxy-DL-tryptophan (Lot 12C-3180): [^{125}I]-HCG, (Lot no. 296-234 specific activity 99.89 μCi/μg) and acetyl-5-methoxytryptamine, N-[2-aminoethyl-2-^{3}H], (Lot no. 1134-003, specific activity 31.5 Ci/mmol) were obtained from New England Nuclear.

For binding studies six follicles were used per incubation of 3 hr duration. Excess HCG or melatonin was added where appropriate to determine non-specific binding. For melatonin binding the follicles were digested in NCS® (Amersham-Searle Corp.) solubilizer prior to counting in toluene phosphor. Bound HCG was measured after washing the follicles five times with MEM, and counting in a gamma counter.

Statistical analysis was carried out by analysis of variance with the Duncan new multiple range test [6]. A p value of 0.05 or less was considered significant.

Steroid concentrations in the medium after 3 h incubation are shown in Table 1. Testosterone and progesterone accumulation were consistently elevated in the presence of HCG. Melatonin had no effect on androstenedione and testosterone accumulation except in the presence of HCG. Progesterone accumulation with HCG stimulation was depressed with 500 nM melatonin.

The uptake of ^{3}H-melatonin in ovarian follicles was less than 1% and did not significantly change in the presence of excess non-labelled melatonin. [^{125}I]-HCG, on the other hand, was bound by the follicles (4.11 ± 0.17 pmol mg protein) and this binding was not altered by the presence of melatonin but could be displaced with excess HCG.

In the present study, melatonin was found to inhibit the HCG induced accumulations of progesterone, androstenedione and testosterone in the isolated rabbit follicle.

Table 1. Steroid accumulation in medium after 3 h incubation with various test substances (data represent ng/mg in log transformation) $n = 4$

		Testosterone	Androstenedione	Progesterone
(a)	Minimum essential medium	0.69 ± 0.31	1.02 ± 0.03	0.91 ± 0.11
(b)	N-acetyl-serotonin (50 nM)	0.69 ± 0.14	0.82 ± 0.27	1.40 ± 0.01
(c)	5-Hydroxy-DL-tryptophan (50 nM)	0.73 ± 0.11	1.31 ± 0.13	1.19 ± 0.08
(d)	Melatonin (50 nM)	0.70 ± 0.11	0.91 ± 0.06	1.05 ± 0.21
(e)	Serotonin (50 nM)	0.61 ± 0.13	0.85 ± 0.16	0.96 ± 0.19
(f)	Serotonin (500 nM)	0.61 ± 0.20	0.97 ± 0.04	0.93 ± 0.14
(g)	HCG (200 I.U.)	2.14 ± 0.13†	1.58 ± 0.17†	2.04 ± 0.21†
(h)	Serotonin (50 nM) + HCG (200 I.U.)	2.11 ± 0.05†	1.74 ± 0.16†	1.99 ± 0.15†
(i)	Melatonin (5 nM) + HCG (200 I.U.)	1.81 ± 0.11†	1.38 ± 0.19	1.96 ± 0.20†
(j)	Melatonin (50 nM) + HCG (200 I.U.)	1.62 ± 0.06*†	1.04 ± 0.16*†	1.89 ± 0.18†
(k)	Melatonin (500 nM) + HCG (200 I.U.)	1.68 ± 0.08*†	1.02 ± 0.12*	1.52 ± 0.12*†

* Significantly different from HCG treatment.
† Significantly different from controls.

This is in contrast to the findings with human corpora lutea [4] but are similar to the direct effects of melatonin in inhibiting testicular steroidogenesis *in vitro* [7–9] and amplifies the close relationship between the rabbit follicle and testicular tissue as far as endocrine function is concerned.

Progesterone production by the follicles was also inhibited in the presence of 500 nM melatonin but not 5 or 50 nM. This effect is unlike that seen in the human corpus luteum where a significant stimulation of progesterone synthesis occurred *in vitro* [4]. Whether melatonin has any direct effects on the ovary *in vivo* remains to be determined.

The inhibition of testosterone production by melatonin did not seem to be due to decreased binding of HCG. Melatonin itself was not bound to the follicles which would indicate that there are no receptors for this indole in the follicle. It is likely that melatonin exerted its effect by inhibiting the secretion of testerone into the medium or on the controlling enzyme systems [8, 9].

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