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In Vitro Effects of Melatonin and Serotonin on Adrenal Steroidogenesis[1] (40000)

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Removal of the ovaries in rats results in increased adrenal secretion of the principal metabolites of corticosterone (5α-dihydrocorticosterone, DHB; and 3β,5α-tetrahydrocorticosterone, THB) with a proportionate fall in the production of corticosterone (1). We recently reported that the pineal gland modifies the elevated rate of DHB and THB formation observed after ovariectomy (2). Secretion of these metabolites declines after pinealectomy and proportionate output of corticosterone is restored *in vivo* and *in vitro*. Administration of melatonin *in vivo* reverses the effects of pinealectomy by stimulating the secretory rate of DHB and THB with a concomitant decline in proportionate output of corticosterone from adrenal effluent (3). These changes are mediated by changes in adrenal 5α-reductase activity. Pinealectomy suppresses reductase activity whereas melatonin stimulates activity of the enzyme. The indole is equally effective in hypophysectomized and pinealectomized rats (3). Thus, melatonin apparently does not require participation of the anterior pituitary gland or the pineal gland for its effects on adrenocortical function as has often been presumed (4). The present investigation defines more clearly the direct action of melatonin by incubating isolated adrenal tissue with the indole.

Materials and methods. Female Long-Evans rats were maintained under a constant photoperiod of 12-hr light: 12-hr dark. A temperature of 22 ± 0.5° was maintained. On Day 42 of age rats were rapidly decapitated between 0830 and 0930 hr, adrenals were removed and placed in ice-cold Krebs-Ringer bicarbonate solution (KRB). Adrenal 5α-reductase activity was assayed in whole adrenal homogenates. Adrenal tissue was incubated in air at 37° with 25 μg of corticosterone in a final volume of 0.25 ml for 60 min. Details of reaction mixture and other procedures have been presented (4). Rate of disappearance of added steroid was determined by the loss of fluorescence, shown previously to coincide with the formation of 5α-reduced metabolites (1, 6). Adrenal slices were incubated at 37° in 2 ml of KRB under an atmosphere of 95% O_2-5% CO_2. After a 30-min preincubation period, the medium was discarded. Fresh KRB was added for a final incubation of 60 min.

Melatonin and serotonin was added to incubation media to achieve a final concentration of 45 nmole/ml. A corresponding volume of saline vehicle was added to the control incubates. After incubation, media of both homogenates and slices were extracted with chloroform, and corticosterone was measured by acid fluorescence (7). Total corticosteroid production was evaluated by the blue tetrazolium reaction which is positive for steroids and an α-ketol side chain (8). In one experiment corticosterone and its principal metabolites, 5α-dihydrocorticosterone (DHB) and 3β,5α-tetrahydrocorticosterone (THB), were measured specifically by competitive protein binding assay (9) subsequent to isolation by paper chromatography (10). Identification and homogeneity of these compounds have been established by paper chromatography, thin-layer chromatography, infrared spectroscopy (1), and mass spectrometry (10).

Procedures of statistical analysis were selected according to comparisons planned for each experimental design (11). Bartlett's test was used to evaluate homogeneity of variance and log transformations were made when indicated. Multiple comparisons with control values were made by the Dunnett

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test, and single comparisons, by Student's *t* test. The Behrens–Fisher modification of the latter was used when heterogeneity of variance was encountered.

Results. Adrenal 5α-reductase activity (Table I) is stimulated by incubating adrenal homogenates in the presence of melatonin. Total steroid secretion by adrenal slices is also enhanced without a specific change in corticosterone secretion. Consequently proportionate secretion of corticosterone declined.

The next experiments were designed to ascertain whether serotonin mimicked the *in vitro* effects of melatonin. Table II indicates that serotonin has no effect on adrenal 5α-reductase activity but the stimulatory effect of melatonin is reproduced. Melatonin specifically enhances adrenal slice secretion of the principal reduced metabolites of corticosterone (DHB and THB) with a concomitant decline in corticosterone output (Table III). Proportionate secretion of corticosterone is likewise reduced. Serotonin is entirely ineffectual in altering these parameters.

Discussion. In 1970 Giordano *et al.* (12) found that the melatonin impaired adrenal homogenate production of Δ4-3-ketosteroids. Although the mechanism mediating this defect was unclear, they determined that it was not due to alterations in Δ5,3β-hydroxysteroid dehydrogenase activity. Our findings extend these observations to provide the mode of action. Melatonin enhances secretion of A ring-reduced metabolites by direct stimulation of 5α-reductase activity. This can account for the observed decline in Δ4-3-ketosteroids without corresponding alterations in Δ5,3β-hydroxysteroid dehydrogenase activity. However, in our hands the effect of melatonin on corticosterone production per se is variable; corticosterone output was not altered by melatonin in one experiment (Table I) but was greatly diminished in another (Table III). The basis for the variability is unclear. Nevertheless, presence of melatonin consistently resulted in decremental changes in proportionate output of corticosterone which are specifically associated with increased secretion of DHB and THB. The identical alterations in corticosteroidogenesis also occur when melatonin is administered *in vivo* to intact rats or subsequent to pinealectomy, ovariectomy, or hypophysectomy (3). Thus, melatonin exerts its effects directly on the adrenal cortex rather than being mediated by secondary influences. This implies that melatonin-specific receptors may exist in the adrenal. Kopin and co-workers (13) found the turnover rate of melatonin in the adrenal to be about one-half that of other organs which is consistent with existence of specific receptors. In addition 90–95% of the melatonin taken up by the adrenal is localized in the microsomal-cell sap fraction. Such conditions provide adequate opportunity for melatonin–reductase interaction since the enzyme is specifically localized in the microsomal fraction (14). The mechanism by which melatonin stimulates activity of this

TABLE II. *In Vitro* Effects of Melatonin and Serotonin on Adrenal 5α-Reductase Activity.[a]

	Reductase activity[b]
Saline (7)	0.06 ± 0.20
Melatonin (8)	2.01 ± 0.56*
Serotonin (8)	0.22 ±0.51

[a] Values are means ± SE. Number of animals indicated within parentheses.

[b] Micrograms of corticosterone reduced per 10 mg adrenal weight per hour.

* Mean differs from control value, $P < 0.05$.

TABLE I. *In Vitro* Effects of Melatonin on Adrenal Steroidogenesis[a]

	Reductase activity[b]	Corticosterone secretion[c]	Total steroid secretion[c]	Corticosterone/total steroids (%)
Saline (10)	0.55 ± 0.28	3.1 ± 0.6	6.9 ± 0.9	55.3 ± 3.3
Melatonin (10)	2.12 ± 0.40***	4.6 ± 0.5	12.4 ± 2.6*	35.3 ± 8.3**

[a] Values are means ± SE. Number of animals indicated within parentheses.

[b] Micrograms of corticosterone reduced per 10 mg adrenal weight per hour.

[c] Micrograms per mg adrenal weight per hour.

* Means differ from control value, $P < 0.05$.

** Means differ from control value, $P < 0.01$.

*** Means differ from control value, $P < 0.001$.

TABLE III. *In Vitro* Effects of Indoleamines on Steroidogenesis in Adrenal Slices.[a]

Treatment	Corticosterone secretion[b]	Dihydrocorticosterone secretion[b]	Tetrahydrocorticosterone secretion[b]	Corticosterone/total steroids (%)
Saline (8)	3.90 ± 0.81	2.72 ± 0.37	0.35 ± 0.03	47.7 ± 3.1
Melatonin (8)	1.95 ± 0.26**	3.51 ± 0.29*	0.50 ± 0.07*	33.1 ± 2.7***
Serotonin (8)	3.41 ± 0.81	2.11 ± 0.31	0.43 ± 0.06	54.8 ± 3.2

[a] Values are means ± SE. Number of animals indicated within parentheses.
[b] Micrograms per 100 mg adrenal weight per hour.
* Mean larger than control value, $P < 0.05$.
** Mean smaller than control value, $P < 0.025$.
*** Mean smaller than control value, $P < 0.005$.

enzyme is unknown. It probably involves enhancement of protein synthetic mechanisms since cycloheximide prevents the rise in reductase activity after hypophysectomy (14). Melatonin appears to be a potent stimulator of adrenal 5α-reductase activity. Its stimulatory effect stands in striking contrast with all other hormones so far studied. ACTH, prolactin, GH, testosterone, and estradiol inhibit 5α-reductase activity (1, 6, 15–18), whereas melatonin is the only hormone known to exert a stimulatory influence.

Summary. The effects of melatonin and serotonin on adrenal steroidogenesis were studied. Adrenals were obtained from 42-day-old intact female Long–Evans rats maintained under a photoperiod of 12-hr light:12-hr dark. Tissue slices and homogenates were incubated in the presence of 45 nmole/ml of melatonin or serotonin. Melatonin stimulates reductase activity. As a consequence secretion rates of dihydrocorticosterone and tetrahydrocorticosterone increase with a concomitant decline in proportionate secretion of corticosterone. Serotonin does not alter any of these parameters of adrenal steroidogenesis. ACTH, prolactin, GH, testosterone, and estradiol have long been known to inhibit 5α-reductase activity. The stimulatory influence of melatonin stands in striking contrast to the effects of these hormones.

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1. Kitay, J. I., Coyne, M. D., Swygert, N. L., and Gines, K. E., Endocrinology **89,** 565 (1971).
2. Ogle, T. F., and Kitay, J. I., Endocrinology **98,** 20 (1976).
3. Ogle, T. F., and Kitay, J. I., Neuroendocrinology **23,** 113 (1977).
4. Cardinali, D. P., Nagle, C. A., and Rosner, J. M., Gen. Comp. Endocrinol. **26,** 50 (1975).
5. Ogle, T. F., and Kitay, J. I., J. Endocrinol. **63,** 489 (1974).
6. Kitay, J. I., Coyne, M. D., and Swygert, N. L., Endocrinology **87,** 1257 (1970).
7. Guillemin, R., Clayton, G. W., Lipscomb, H. S., and Smith, J. D., J. Lab. Clin. Med. **53,** 830 (1959).
8. Nowaczynski, W., Goldner, M., and Genest, J., J. Lab. Clin. Med. **45,** 818 (1955).
9. Murphy, B. E. P., J. Clin. Endocrinol. **27,** 973 (1967).
10. Colby, H. D., and Kitay, J. I., Endocrinology **91,** 1523 (1972).
11. Bliss, C. E., "Statistics in Biology," Vol. 2. McGraw-Hill, New York (1967).
12. Giordano, G., Balestreri, R., Jacopino, G. E., Foppiani, E., and Bertolini, S., Ann. Endocrinol. (Paris) **31,** 1071 (1970).
13. Kopin, I. J., Pare, C. M. B., Axelrod, J., and Weissbach, H., J. Biol. Chem. **236,** 3072 (1961).
14. Caffrey, J. L., and Kitay, J. I., Fed. Proc. **32,** 255 (1973).
15. Kitay, J. I., Coyne, M. D., and Swygert, N. L., Proc. Soc. Exp. Biol. Med. **137,** 229 (1971).
16. Kitay, J. I., Coyne, M. D., Newsome, W., and Nelson, R., Endocrinology **77,** 902 (1965).
17. Kitay, J. I., Coyne, M. D., and Swygert, N. L., Endocrinology **89,** 432 (1971).
18. Witorsch, R. J., and Kitay, J. I., Endocrinology **91,** 764 (1972).

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